

HEPATOLOGY

A clinical textbook

Mauss, Berg, Rockstroh, Sarrazin, Wedemeyer

10th Edition **2020**

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Hepatology – A clinical textbook

Tenth Edition, 2020

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Editors

Stefan Mauss

Thomas Berg

Jürgen Rockstroh

Christoph Sarrazin

Heiner Wedemeyer

Hepatology – A clinical textbook is now in its tenth edition and the editors as the book are more mature. The current edition has again been thoroughly updated to reflect the latest medical progress. Because of this regular revision process it remains an up-to-date reference for all aspects of clinical hepatology. This would not have been possible without the continuous contributions of all the authors who have dutifully revised and updated their chapters.

Again, the book is available in print and as a free download at
www.hepatologytextbook.com

Disclaimer

Hepatology is an ever-changing field. The editors and authors of *Hepatology – A Clinical Textbook* have made every effort to provide information that is accurate and complete as of the date of publication. However, in view of the rapid changes occurring in medical science, as well as the possibility of human error, this book may contain technical inaccuracies, typographical or other errors. Readers are advised to check the product information currently provided by the manufacturer of each drug to be administered to verify the recommended dose, the method and duration of administration, and contraindications. It is the responsibility of the treating physician who relies on experience and knowledge about the patient to determine dosages and the best treatment for the patient. The information contained herein is provided “as is” and without warranty of any kind. The editors disclaim responsibility for any errors or omissions or for results obtained from the use of information contained herein.

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Jürgen Rockstroh
Heiner Wedemeyer

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Preface of the first edition

Hepatology is a rapidly evolving field that will continue to grow and maintain excitement over the next few decades. Viral hepatitis is not unlike HIV 10 or 15 years ago. Today, hepatitis B viral replication can be suppressed by potent antiviral drugs, although there are risks regarding the emergence of resistance. Strategies to enhance the eradication rates of HBV infection still need to be developed. On the other hand, hepatitis C virus infection can be eradicated by treatment with pegylated interferon plus ribavirin, although the sustained virologic response rates are still suboptimal, particularly in those infected with genotype 1. Many new antiviral drugs, especially protease and polymerase inhibitors, are currently in clinical development, and the data from trials reported over the last few years provide optimism that the cure rates for patients with chronic hepatitis C will be enhanced with these new agents, and even that all-oral regimens are around the corner! In other areas of hepatology, e.g., hereditary and metabolic liver diseases, our knowledge is rapidly increasing and new therapeutic options are on the horizon.

In rapidly evolving areas such as hepatology, is the book format the right medium to gather and summarise the current knowledge? Are these books not likely to be outdated the very day they are published? This is indeed a challenge that can be convincingly overcome only by rapid internet-based publishing with regular updates. Another unmatched advantage of a web-based book is the free and unrestricted global access. Viral hepatitis and other liver diseases are a global burden and timely information is important for physicians, scientists, patients and health care officials all around the world.

The editors of this web-based book – Thomas Berg, Stefan Mauss, Jürgen Rockstroh, Christoph Sarrazin and Heiner Wedemeyer – are young, bright, and internationally renowned hepatologists who have created an excellent state-of-the-art textbook on clinical hepatology. The book is well-written and provides in-depth information without being lengthy or redundant. I am convinced that all five experts will remain very active in the field and will continue to update this book regularly as the science progresses. This e-book should rapidly become an international standard.

Stefan Zeuzem – Frankfurt, Germany, January 2009

Therapeutic options and diagnostic procedures in hepatology have quickly advanced during the last decade. In particular, the management of viral hepatitis has completely changed since the early nineties. Before nucleoside and nucleotide analogues were licensed to treat hepatitis B and before interferon α + ribavirin combination therapy were approved for the treatment of chronic hepatitis C, very few patients infected with HBV or HCV were treated successfully. The only option for most patients with end-stage liver disease or hepatocellular carcinoma was liver transplantation. And even if the patients were lucky enough to be successfully transplanted, reinfection of the transplanted organs remained major challenges. In the late eighties and early nineties discussions were held about rejecting patients with chronic hepatitis from the waiting list as posttransplant outcome was poor. Today, just 15 years later, hepatitis B represents one of the best indications for liver transplantations, as basically all reinfection can be prevented. In addition, the proportion of patients who need to be transplanted is declining – almost all HBV-infected patients can nowadays be treated successfully with complete suppression of HBV replication and some well-selected patients may even be able to clear HBsAg, the ultimate endpoint of any hepatitis B treatment.

Hepatitis C has also become a curable disease with a sustained response of 50–80% using pegylated interferons in combination with ribavirin. HCV treatment using direct HCV enzyme inhibitors has started to bear fruit (we draw your attention to the HCV chapters).

Major achievements for the patients do sometimes lead to significant challenges for the treating physician. Is the diagnostic work-up complete? Did I miss any recent development to evaluate the stage and grade of liver disease? What sensitivity is really necessary for assays to detect hepatitis viruses? When do I need to determine HBV polymerase variants, before and during treatment of hepatitis B? When can I safely stop treatment without risking a relapse? How to treat acute hepatitis B and C? When does a health care worker need a booster vaccination for hepatitis A and B? These are just some of many questions we have to ask ourselves frequently during our daily routine practice. With the increasing number of publications, guidelines and expert opinions it is getting more and more difficult to stay up-to-date and to make the best choices for the patients. That is why *Hepatology – A Clinical Textbook* is a very useful new tool that gives a state-of-the-art update on many aspects of HAV, HBV, HCV, HDV and HEV infections. The editors are internationally-known experts in the field of viral hepatitis; all have made significant contributions to understanding the pathogenesis of virus-induced liver disease, diagnosis and treatment of hepatitis virus infections.

Hepatology – A Clinical Textbook gives a comprehensive overview on the epidemiology, virology, and natural history of all hepatitis viruses

including hepatitis A, D and E. Subsequent chapters cover all major aspects of the management of hepatitis B and C including coinfections with HIV and liver transplantation. Importantly, complications of chronic liver disease such as hepatocellular carcinoma and recent developments in assessing the stage of liver disease are also covered. Finally, interesting chapters on autoimmune and metabolic non-viral liver diseases complete the book.

We are convinced that this new up-to-date book covering all clinically relevant aspects of viral hepatitis will be of use for every reader. The editors and authors must be congratulated for their efforts.

Michael P. Manns – Hannover, January 2009

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1. Hepatitis A

Sven Pischke and Heiner Wedemeyer

The virus

Hepatitis A is an inflammatory liver disease caused by infection with the hepatitis A virus (HAV). HAV is a single-stranded 27 nm non-enveloped, icosahedral RNA virus, which was first identified by immune electron microscopy in 1973 (Feinstone 1973). The virus belongs to the hepadnavirus genus of the *Picornaviridae*. Recent structure-based phylogenetic analysis placed HAV between typical picornavirus and insect picorna-like viruses (Wang 2015). Recent work suggests a rodent origin of HAV based on a large screening for hepatoviruses in more than 200 small mammal species (Drexler 2015). HAV uses host cell exosome membranes as an envelope which leads to protection from antibody mediated neutralisation (Feng 2013) but also facilitates detection of HAV by plasmacytoid dendritic cells which are main sources for type I interferon during infection (Feng 2015). Of note, only blood but not bile HAV shows host-derived membranes.

Seven different HAV genotypes have been described, of which four are able to infect humans (Lemon 1992).

The positive-sense single-stranded HAV RNA has a length of 7.5 kb and consists of a 5' non-coding region of 740 nucleotides, a coding region of 2225 nucleotides and a 3' non-coding region of approximately 60 nucleotides.

Acute hepatitis A is associated with a limited type I interferon response (Lanford 2011), which may be explained by cleavage of essential adaptor proteins by an HAV protease-polymerase precursor (Qu 2011). Recently HAV has been shown to interact with the mitochondrial antiviral signaling (MAVS) protein resulting in interferon-independent intrinsic hepatocellular apoptosis and hepatic inflammation (Hirai-Yuki 2016). A dominant role of CD4⁺ T cells to terminate HAV infection has been established in HAV infected chimpanzees (Zhou 2012). However, in humans strong HAV-specific CD8 T cells have also been described, potentially contributing to resolution of infection (Schulte 2011). A failure to maintain these HAV-specific T cell responses could increase the risk for relapsing HAV.

Epidemiology

HAV infections occur worldwide, either sporadically or in epidemic outbreaks. An estimated 1.4 million cases of HAV infections occur each

year. HAV is usually transmitted and spread via the faecal-oral route (Lemon 1985). Thus, infection with HAV occurs predominantly in areas of lower socioeconomic status and reduced hygienic standards, especially in low-income, tropical countries. Not surprisingly, a study investigating French children confirmed that travel to countries endemic for HAV is indeed a risk factor for the presence of anti-HAV antibodies (Faillon 2012). In high-income countries like the US or Germany the number of reported cases has decreased markedly in the past decades, according to official data published by the Centers for Disease Control and Prevention (CDC, Atlanta, USA) and the Robert Koch Institute (RKI, Berlin, Germany) (Figure 1). This decrease is mainly based on improved sanitary conditions as, e.g., recently demonstrated for Southern Italy (Zuin 2016). Moreover, vaccination programmes have also resulted in fewer HAV infections in various endemic countries including Argentina, Brazil, Italy, China, Russia, Ukraine, Spain, Belarus, Israel and Turkey (Hendrickx 2008).

Despite of the overall decrease in the frequency of hepatitis A in industrialised countries HAV outbreaks still occur. For example, HAV outbreaks have been described both in Europe and the US that were linked to frozen berries (Guzman Herrador 2014, Fitzgerald 2014) or imported pomegranate arils (Collier 2014). An outbreak of HAV was also described in Tel Aviv, Israel. Interestingly four of the patients (5%) had been previously vaccinated. In addition to the observed outbreak, HAV could be detected in sewage samples from various regions in Israel indicating the presence of this virus across Israel (Manor 2016).

Transmission

HAV is transmitted faecal-orally either by person-to-person contact or ingestion of contaminated food or water. Usually HAV is restricted to humans and is not considered to be a zoonosis. However, experimental HAV infection of pigs has been demonstrated (Song 2015). HAV transmission is also possible by blood transfusion but considered to be extremely rare (da Silva 2016).

Five days before clinical symptoms appear, the HAV can be isolated from the faeces of patients (Dienstag 1975). The virus stays detectable in the faeces up to two weeks after the onset of jaundice. Faecal excretion of HAV up to five months after infection can occur in children and immunocompromised persons. A recent study from Brazil evaluated the risk of household HAV transmission within a cohort of 97 persons from 30 families (Rodrigues-Lima 2013). Person-to-person transmission was seen in six cases indicating a relevant risk for relatives of patients with HAV. On the other hand, there was no evidence of HAV transmission in another

incident by an HAV-infected food handler in London (Hall 2014). Further studies are necessary to evaluate the use of HAV vaccination of relatives at risk in this setting.

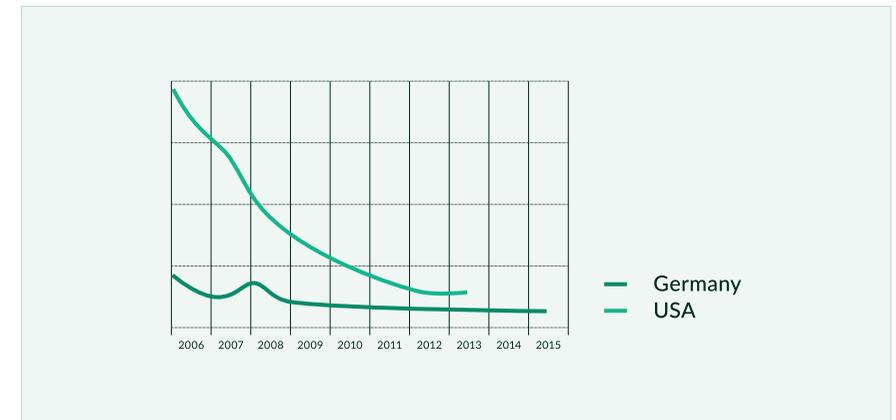


Figure 1. Number of reported cases of HAV infections in the US and Germany over the last decade (Sources: CDC through 2012 and Robert Koch Institute through 12/2015)

Risk groups for acquiring an HAV infection in high-income countries are health care providers, military personnel, psychiatric patients and men who have sex with men. Parenteral transmission by blood transfusion has been described but is a rare event. Mother-to-fetus transmission has not been reported (Tong 1981). Distinct genetic polymorphisms including variants in ABCB1, TGFB1, XRCC1 may be associated with a susceptibility to HAV (Zhang 2012).

Recently it was shown that the number of reported HAV infections in the USA decreased from 6 cases/ 100000 in 1999 to 0.4 cases/ 100000 in 2011, while the percentage of hospitalisations due to HAV increased from 7.3% to 24.5% indicating that HAV is becoming a rare condition but can still cause serious morbidity, especially in elderly and patients with underlying liver disorders (Ly 2015). In line with this report the overall immunity to HAV is declining in United States (Klevens 2015) suggesting that vaccination coverage needs to be improved.

Clinical course

The clinical course of HAV infection varies greatly, ranging from asymptomatic, subclinical infections to cholestatic hepatitis or fulminant liver failure (Figure 2).

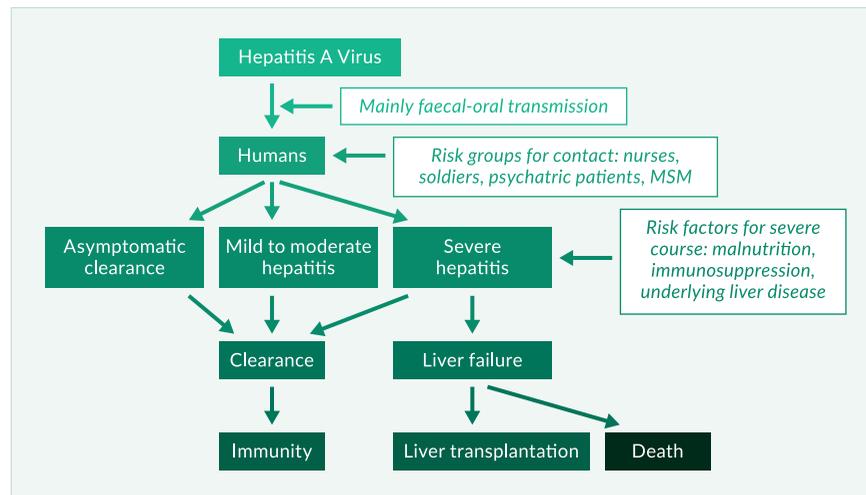


Figure 2. Possible courses of HAV infection

Most infections in children are either asymptomatic or unrecognised, while 70% of adults develop clinical symptoms of hepatitis with jaundice and hepatomegaly.

The incubation time ranges between 15 and 49 days with a mean of approximately 30 days (Koff 1992). Initial symptoms are usually non-specific and include weakness, nausea, vomiting, anorexia, fever, abdominal discomfort, and right upper quadrant pain (Lednar 1985). As the disease progresses, some patients develop jaundice, darkened urine, uncoloured stool and pruritus. The prodromal symptoms usually diminish when jaundice appears.

Approximately 10% of infections take a biphasic or relapsing course. In these cases the initial episode lasts about 3–5 weeks, followed by a period of biochemical remission with normal liver enzymes for 4–5 weeks. Relapse may mimic the initial episode of the acute hepatitis and complete normalisation of ALT and AST values may take several months (Tong 1995). A recent investigation in two HAV-infected chimpanzees demonstrated that the CD4 count decreased after clinical signs of HAV disappeared (Zhou 2012). Eventually, an intrahepatic reservoir of HAV genomes that decays slowly in combination with this CD4 response, may explain the second phase of disease, but further observations on human patients are required to verify this.

Cases of severe fulminant HAV leading to hepatic failure occur more often in patients with underlying liver disease. Conflicting data on the course of acute HAV have been reported for patients with chronic hepatitis C (HCV). While some studies showed a higher incidence of fulminant hepatitis (Vento 1998), other studies do not confirm these findings and even suggest that HAV superinfection may lead to clearance of HCV infection

(Deterding 2006). Other risk factors for more severe courses of acute HAV are age, malnutrition and immunosuppression. Severity of liver disease during acute HAV has recently been shown to be associated with a distinct polymorphism in TIM1, the gene encoding for the HAV receptor (Kim 2011). An insertion of six amino acids at position 157 of TIM1 leads to more efficient HAV binding and greater NKT lytic activity against HAV infected liver cells.

In contrast to hepatitis E, there are no precise data on the outcome of HAV infection during pregnancy. Some data suggest an increased risk of gestational complications and premature birth (Elinav 2006).

HAV has a lethal course in 0.1% of children, in 0.4% of persons aged 15–39 years, and in 1.1% in persons older than 40 years (Lemon 1985). In contrast to the other faecal-orally transmitted hepatitis (hepatitis E), no chronic courses of HAV infection have been reported so far.

Extrahepatic manifestations

Extrahepatic manifestations are uncommon in HAV (Pischke 2007). If they occur, they usually show an acute onset and disappear upon resolution of HAV infection in most cases. Possible extrahepatic manifestations of acute HAV infection are arthralgia, diarrhoea, renal failure, red cell aplasia, generalised lymphadenopathy, and pancreatitis. Arthralgia can be found in 11% of patients with hepatitis A.

Very uncommon are severe extrahepatic manifestations like pericarditis and/or renal failure. An association of hepatitis A with cryoglobulinaemia has been reported but is a rare event (Schiff 1992). Furthermore, cutaneous vasculitis can occur. In some cases, skin biopsies reveal anti-HAV-specific IgM antibodies and complements in the vessel walls (Schiff 1992). In contrast to hepatitis B or C, renal involvement is rare, and there are very few case reports showing acute renal failure associated with HAV infection (Pischke 2007). Recently it has been shown that approximately 8% of HAV cases are associated with acute kidney injury (Choi 2011).

Diagnosis

Diagnosis of acute HAV is based on the detection of anti-HAV IgM antibodies or HAV RNA. The presence of HAV IgG antibodies can indicate acute or previous HAV infection. HAV IgM and IgG antibodies also become positive early after vaccination, with IgG antibodies persisting for at least two to three decades after vaccination. Antibodies against HAV and HAV RNA can also be detected in saliva (Amado Leon 2015). Available serological tests show a very high sensitivity and specificity. Recently, a study from Taiwan

revealed that HIV-infected patients develop protective antibody titres after HAV vaccination less frequently than healthy controls (Tseng 2012). In addition a study examining the immune response to HAV vaccination in 282 HIV positive patients (Mena 2013) demonstrated that male sex or HCV coinfection were associated with lower response rates. Furthermore, it was shown that in people living with HIV, HAV vaccination with three doses results in an improved durability of antibodies in comparison with two-dose vaccination (Cheng 2016), while a Nicaraguan study on children demonstrated that one-dose vaccination resulted in an adequate long-term immune memory (Mayorga 2016).

A large study investigated 183 adolescents (age 15 to 16 years) who had been vaccinated with a two-dose HAV vaccination at an age of 6, 12 or 15 months. Seropositivity was lower in children who were vaccinated at 6 months as well as in children where maternal HAV antibodies were transferred (Spradling 2015). This study demonstrates that HAV vaccination should usually be performed after 12 months of age, which is in line with the current US recommendations. Delayed seroconversion may occur in immunocompromised individuals, and testing for HAV RNA should be considered in immunosuppressed individuals with unclear hepatitis. HAV RNA testing of blood and stool can determine if the patient is still infectious. However, it has to be kept in mind that various in-house HAV RNA assays may not be specific for all HAV genotypes and thus false negative results can occur.

Elevated results for serum aminotransferases and serum bilirubin can be found in symptomatic patients (Tong 1995). ALT levels are usually higher than serum aspartate aminotransferase (AST) in non-fulminant cases. Increased serum levels of alkaline phosphatase and gamma-glutamyl transferase indicate a cholestatic form of HAV infection. The increase and the peak of serum aminotransferases usually precede the increase of serum bilirubin. Laboratory markers of inflammation, like an elevated erythrocyte sedimentation rate and increased immunoglobulin levels, can also frequently be detected.

Recently within a small pilot study, examining 10 patients with acute HAV, saliva contained HAV RNA in 8/10 (80%) and anti HAV IgM in 10/10 (100%) (Armado Leon 2014). The relevance of this finding and the potential value of saliva testing needs to be studied in larger cohorts.

Treatment and prognosis

There is no specific antiviral therapy for treatment of HAV. Of note, recent work demonstrated that cyclosporine A and silibinin inhibits HAV replication *in vitro* (Esser-Nobis 2015). The clinical value of this observation

still needs to be determined.

Recently a study from the Netherlands investigated the use of post-exposure HAV vaccination or prophylaxis with immunoglobulins in patients with household contact with HAV. In this study, none of the patients who received immunoglobulins developed acute HAV in contrast to some patients who received the vaccine. The study revealed that HAV vaccination post-exposure might be a sufficient option in younger patients (<40 years) while older patients (>40 years) might benefit from immunoglobulins (Whelan 2013). The disease usually takes a mild to moderate course, which does not require hospitalisation, and only in fulminant cases is initiation of symptom focused therapy necessary. Prolonged or biphasic courses should be monitored closely. HAV may persist for some time in the liver even when HAV RNA becomes negative in blood and stool (Lanford 2011), which needs to be kept in mind for immunocompromised individuals. Acute hepatitis may rarely proceed to acute liver failure; liver transplantation is required in few cases. In the US, only 4% of all liver transplantations performed for acute liver failure were due to HAV (Ostapowicz 2002). In a cohort of acute liver failures at one transplant centre in Germany, approximately 1% of patients had HAV infection (Hadem 2008). The outcome of patients after liver transplantation for fulminant HAV is excellent. Timely referral to liver transplant centres is therefore recommended for patients with severe or fulminant HAV.

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2. Hepatitis B

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Introduction

Approximately one third of the world’s population has serological evidence of past or present infection with the hepatitis B virus (HBV). Despite the availability of HBV vaccines, the global prevalence of chronic HBV infection is estimated to be 3.7% (Lok 2016). The World Health Organization estimates that in 2015 257 million people were living with chronic HBV (defined as HBsAg positive) resulting in approximately 887.000 deaths mostly due to cirrhosis and hepatocellular carcinoma (WHO 2017, WHO 2019).

Since the discovery of HBV by Blumberg in 1965, progress has been impressive, with the availability of vaccines in the 1980s and the development of potent antiviral drugs two decades later. Nevertheless, the global burden of chronic HBV remains substantial and healing of chronic HBV still remains almost impossible.

There is a wide range of HBV prevalence rates in different parts of the world (from 0.1% up to 20%). Low prevalence areas (<2%) represent 12% of the global population and include Western Europe, the United States and Canada, Australia and New Zealand. In these regions, the lifetime risk of infection is less than 20%. Intermediate prevalence is defined as 2% to 7%, with a lifetime risk of infection of 20–60% and includes the Mediterranean countries, Japan, Central Asia, the Middle East, and Latin and South America, representing about 43% of the global population. High prevalence areas (≥8%) include Southeast Asia, China, and sub-Saharan Africa, where a lifetime likelihood of infection is greater than 60%. The diverse prevalence rates are probably related to differences in age at infection, which correlates with the risk of chronicity. The progression rate from acute to chronic HBV infection decreases with age. Approximately 90% of infections acquired perinatally will progress compared to 5% or less for adult infections (Stevens 1975, Wasley 2008, Pan 2016).

The incidence of new HBV infections has decreased in most high-income countries, most likely due to the implementation of vaccination strategies (Rantala 2008, Leroy 2015). However, exact data is difficult to generate as many cases remain undetected due to the asymptomatic nature of the infection. In Germany, 4.507 cases (acute or chronic) of HBV were documented in 2018, corresponding to an incidence rate of 5.4 per 100,000 inhabitants (RKI 2019). From 2001 until 2009 there was a stable decrease in

infections which then stagnated until 2014. Since 2015 there has been a rise in the number of infections mostly due to migration and boosted testing as well as a change in the definition of diagnosis (RKI 2019). Likewise, the incidence of acute HBV in the United States has decreased considerably in the last two decades (Wasley 2008, CDC 2012). Due to the persisting opioid crisis the number of acute HBV infections in the US slightly increased in 2017 (CDC 2017). Moreover, in Germany as well as in the US the major number of infections is found in foreign born people, presenting 2/3 of all reported cases in Germany (RKI 2019). Although estimates are difficult due to a continuously growing migration from high to low prevalence areas, a further drop in prevalence is expected due to the implementation of vaccination programmes (Belongia 2008). In Germany, 84,4% of all children starting school in 2017 were fully vaccinated against HBV, with a trend toward increasing coverage (RKI 2019).

Thus screening instruments are most important in diagnosing chronic HBV in migrants, especially facing an increased risk for HCC in those patients. (ECDC 2019).

Although the incidence of acute HBV infection is decreasing in most countries, overall HBV-related complications are still on the rise (Gomaa 2008, Hatzakis 2011, Zhang 2013). Reasons for this increase may be the delay of vaccination effects and the improved diagnosis rate of HBV cases. When looking at the age-adjusted rate ratios of HBV-related HCC incidence, a continuous decline can be observed following the launch of vaccination programmes. Recently published results of a large population-based controlled trial in Chinese newborns show that HCC incidence was significantly lower in the vaccinated group compared to the control group, with a hazard ratio of 0.16 (Qu 2014).

Transmission

The predominance of transmission modes varies considerably in different geographic areas. For example, in Western Europe (a low prevalence area), the main routes are unprotected sexual intercourse and intravenous drug use. In sub-Saharan Africa (a high prevalence area), perinatal infection is the predominant mode of transmission. Horizontal transmission, particularly in early childhood, is regarded as the major route of transmission in intermediate prevalence areas.

Sexual transmission

Sexual transmission of HBV in people who are unvaccinated largely occurs among heterosexual men or women who either have multiple sex partners or contact with sex workers, or among men who have sex with men (MSM). In low prevalence areas, sexual transmission is the major route of transmission. In the United States, heterosexual contacts amount up to 40% of newly diagnosed HBV infections, MSM approximately 25% (Wasley 2008), in Germany in 2018 27% of newly diagnosed HBV infections were due to sexual intercourse with 58% in heterosexual contacts and 42% in MSM (RKI 2019). Comparatively high rates of HIV/HBV coinfections are observed in German MSM, as less than half of HIV positive patients are vaccinated against HBV (Jansen 2015). However, as noted above, infection in adulthood leads to chronic hepatitis in less than 5% of cases. Measures to prevent sexual HBV transmission are vaccination – especially of risk groups – and safer sex practices.

Percutaneous inoculation

Percutaneous inoculation seems to be an effective mode of HBV transmission, with an estimated risk as up to 30% in individuals without post-exposure prophylaxis (PEP) or adequate vaccination (Deisenhammer 2006, Hofmann 2002). The most important percutaneous transmission route is sharing syringes and needles by people who inject drugs (PWID), representing about 15% of newly diagnosed HBV infections in low prevalence areas such as Europe and the United States (Wasley 2008). Sharing razors or toothbrushes are other potential ways of percutaneous transmission, although absolute risk remains unknown. In addition, practices like acupuncture, tattooing, and body piercing have been associated with transmission of HBV. Public health education and the use of disposable needles or equipment are important methods of prevention.

Perinatal transmission

Perinatal transmission is the major route of HBV transmission in many parts of the world, and an important factor in maintaining the reservoir of the infection, particularly in high prevalence areas. In the absence of prophylaxis, chronic HBV infection will develop in 80 to 90% of infants born to mothers who are positive for HBV e antigen (HBeAg) (Lee 2006). Neonatal vaccination has demonstrated high efficacy, indicating that transmission mostly occurs at or shortly before birth. On the other hand,

cesarean section seems less protective than for other vertically transmitted diseases such as HIV.

The risk of transmission from mother to infant is related to the mother's HBV replicative rate. There seems to be a direct correlation between maternal HBV DNA levels and the likelihood of transmission. In mothers with highly replicating HBV the risk of transmission may be up to 85 or 90%. This risk steadily drops at lower HBV DNA levels (Burk 1994, Zhang 2012). Some studies report that perinatal transmission is rare if the mother has HBV DNA $<10^5$ log copies/mL (Li 2004).

All women should be tested for HBsAg at the first prenatal visit/first trimester and this should be repeated later in pregnancy if appropriate (CDC 2011, EASL 2017). Newborns born to HBV positive mothers can be effectively protected by passive-active immunisation (>90% protection rate) (Del Canho 1997, Dienstag 2008, WHO 2015). HBV immunoglobulin for passive immunisation should be given as early as possible (within 12 hours), but can be given up to seven days after birth if replicative HBV infection of the mother is detected later. Active immunisation follows a standard regimen and is given at three time points (10 µg at day 0, month 1, and month 6). However, immunoprophylaxis fails in 10 to 30% of infants born to mothers with an HBV DNA level greater than 10^6 log copies/mL (Zou 2012). In a Chinese cohort study, no HBV infection was observed in infants born to HBeAg negative mothers who received HBV vaccine, independently of immunoglobulin administration (Zhang 2014).

Anti-HBV treatment of the mother with nucleoside analogues may be considered, especially in mothers with high HBV DNA levels. The use of telbivudine, lamivudine, and tenofovir appears to be safe in pregnancy with no increased adverse maternal or fetal outcome (Brown 2016). Adefovir and entecavir are not recommended in pregnancy (Cornberg 2011). Treatment of mothers with telbivudine prevented almost all cases of vertical transmission compared to a vertical transmission rate of about 10% in the arm receiving only active-passive immunisation (Han 2011, Wu 2014). Tenofovir starting from 30 weeks of gestation until postpartum week 4 combined with immunoprophylaxis demonstrated significantly lower transmission rates in HBeAg positive mothers with viral loads $>200,000$ IU/mL compared to immunoprophylaxis alone (Pan 2016). In women with lower viral load a recently study from Thailand reported that additional use of Tenofovir from 28 weeks of gestation on, did not result in a lower mother-to-child HBV transmission in HBeAg-positive mothers together with the administration of HBV immune globulin and HBV vaccine (Jourdain 2018). Lamivudine seems to be another safe, low cost and equally effective option to prevent vertical transmission in highly viraemic HBV-infected pregnant women (Jackson 2014, Zhang 2014). A meta-analysis showed that the use of any antiviral therapy reduced mother-to-child transmission, as defined by

infant HBV surface antigen seropositivity (risk ratio = 0.3, 95% CI 0.2–0.4) or infant HBV DNA seropositivity (risk ratio 5 0.3, 95% CI 0.2–0.5) at 6–12 months (Brown 2016).

As mentioned earlier, cesarean section should not be performed routinely. If the child is vaccinated, (s)he may be breastfed (Hill 2002). Taking lamivudine or tenofovir during breastfeeding results in lower exposure to drugs than due to in utero exposure during pregnancy and thus does not support contraindicating their use during breastfeeding (Ehrhardt 2014).

Horizontal transmission

Horizontal transmission includes household, intrafamilial and child-to-child transmission via minor breaks in the skin or mucous membranes. At least 50% of infections in children cannot be accounted for by mother-to-infant transmission and, in many endemic regions, before the introduction of neonatal vaccination, the prevalence peaked in children 7 to 14 years of age (Papatheodoridis 2008). HBV remains viable outside the human body for a prolonged period and is infectious in the environment for at least 7 days (Lok 2007). Although HBV DNA has been detected in various body fluids of HBV carriers, there is no firm evidence of HBV transmission via body fluids other than blood.

In one study, family members of inactive HBsAg carriers had a higher HBsAg positivity rate than the general population over a 10-year period. Despite negative HBV DNA levels, transmission risk was not negligible in these patients, and horizontal transmission seems to be independent of the HBV DNA level (Demirturk 2014).

Blood transfusion

Blood donors are routinely screened for HBV surface antigen (HBsAg). Therefore incidence of transfusion-related HBV has significantly decreased. The risk of acquiring post-transfusion HBV depends on factors like prevalence and donor testing strategies. In low prevalence areas it was estimated to be one to four per million blood components transfused (Dodd 2000, Polizzotto 2008). In high prevalence areas it is considerably higher (around 1 in 20,000) (Shang 2007, Vermeulen 2011).

There are different strategies for donor screening. Most countries use HBsAg screening of donors. Others, including the United States, use both HBsAg and anti-HBc. Routine screening of anti-HBc remains controversial, as the specificity is low and patients with cleared hepatitis have to be excluded. Some countries e.g. the USA, Germany, Spain and Singapore use

mini-pool nucleic acid testing for HBV DNA screening. Through this testing the risk of HBV transmission through transfusion has been lowered to 1 in 1 million (Busch 2019, Stramer 2013). With increasing implementation of NAT in high prevalence countries, the risk of transmission through blood transfusion will be extremely diminished in those countries.

Nosocomial infection

Nosocomial infection can occur from patient to patient, from patient to health care worker and vice versa. HBV is considered the most commonly transmitted blood-borne virus in the healthcare setting. Despite implementation of prevention strategies (including the use of disposable needles and equipment, sterilisation of surgical instruments, and vaccination of healthcare workers) documented cases of nosocomial infections occur (Williams 2004, Amini-Bavil-Olyaei 2012). However, the exact risk of nosocomial infection is unknown. The numbers of cases reported from this route is likely to be underestimated as many infections might be asymptomatic and only a fraction of exposed patients is recalled for testing.

The incidence of HBV infection in health care workers is lower than in the general population due to routine vaccination (Duseja 2002, Mahoney 1997). Therefore, transmission from healthcare workers to patients is rare, while the risk of transmission from an HBV positive patient to a health care worker seems to be higher.

Healthcare workers who are HBV positive are not generally prohibited from working. HBeAg negative healthcare workers are not considered to be infectious, whereas HBeAg positive healthcare workers should wear double gloves and not perform certain activities, to be defined on an individual basis (Gunson 2003, Cornberg 2011). However, cases of transmission from HBsAg positive, HBeAg negative surgeons to their patients have been reported (Teams 1997) and a precore stop codon mutation was found responsible for HBeAg non-expression despite active HBV replication (Borzooy 2015). Therefore, HBV DNA testing has been implemented in some settings, although this may not always be reliable due to fluctuating levels of HBV DNA. In most high-income countries, guidelines for HBV positive healthcare workers have been established and should be consulted (Cornberg 2011).

The risk of transmission of HBV through sharps injuries (when the patient is HBeAg positive) is estimated with 1:3 (Riddell 2015). Despite HBV being highly infectious, only 15 cases of occupational transmission by sharps injuries have been reported in Germany during 2018 (RKI 2019). This low number probably relates to the high percentage of healthcare workers

who are immunised against HBV. Based on vaccination history, previous response to vaccination, type of exposure, and the HBV status of the source patient a vaccine can be given shortly after exposure either as the first dose of a primary course or as a booster. The additional use of immunoglobulin aims to provide passive immunity if the source patient is known to be at high risk of HBV infection and the recipient has not been previously adequately immunised or is a known non-responder to the vaccine.

Organ transplantation

Transmission of HBV infection has been reported after transplantation of extrahepatic organs from HBsAg positive donors (e.g., kidney, cornea) (Dickson 1997). Organ donors are therefore routinely screened for HBsAg. The role of anti-HBc is controversial, as it is in screening of blood donors. Reasons are the possibility of false positive results, the potential loss of up to 5% of donors even in low endemic areas, and the uncertainty about the infectivity of organs, especially extrahepatic organs, from donors who have isolated anti-HBc (Dickson 1997). Although an increased risk of HBV infection for the recipient of anti-HBc positive organs has been postulated, no donor-derived HBV transmission has been observed in a recent case series of anti-HBc positive donors (Horan 2014, Niu 2014). Evidence exists that patients who have recovered from HBV may benefit from preemptive antiviral therapy in the case of profound immunosuppression (e.g., chemotherapy involving monoclonal antibodies such as rituximab or immunosuppressive treatment) because of the risks associated with a form of HBV reactivation referred to as reverse seroconversion (Di Bisceglie 2014).

Postexposure prophylaxis

In case of exposure to HBV in any of the circumstances mentioned above, post-exposure prophylaxis is recommended for all non-vaccinated persons. A passive-active immunisation is recommended. The first dose of passive and active immunisation should be given as early as possible. 12 hours after the exposure is usually considered the latest time point for effective post-exposure prophylaxis. One dose of HBV-immunoglobulin (HBIG) should be administered at the same time, if the source is known to be HBsAg positive. The other two doses of vaccine should be administered after 4 and 12–24 weeks.

Vaccinated individuals with a documented response do not need post-exposure prophylaxis. Individuals who have had no post-vaccination testing should be tested for anti-HBs titre as soon as possible. If this is not

possible, or the anti-HBs titre is insufficient (<100 IU/L), they will require a second course of vaccination.

Individuals who are documented non-responders will require two doses of HBIG given one month apart.

Natural history and clinical manifestations

The spectrum of clinical manifestations of HBV infection varies in both acute and chronic disease. During the acute phase, manifestations range from subclinical or anicteric hepatitis to icteric hepatitis, and in some cases fulminant hepatitis. During the chronic phase, manifestations range from an asymptomatic carrier state to chronic hepatitis, cirrhosis, and hepatocellular carcinoma. Extrahepatic manifestations can occur in both acute and chronic infection.

Acute hepatitis

After HBV infection, the incubation period lasts from one to four months. A prodromal phase may appear before acute hepatitis develops. During this period a serum sickness-like syndrome may develop. This syndrome manifests with fever, skin rash, arthralgia and arthritis. It will usually cease with the onset of hepatitis. At least 70% of patients then have subclinical or anicteric hepatitis, while less than 30% will develop icteric hepatitis. The most prominent clinical symptoms of hepatitis are right upper quadrant discomfort, nausea, jaundice and other unspecific constitutional symptoms. In case of coinfection with other hepatitis viruses or other underlying liver disease the clinical course may be more severe. Symptoms – including jaundice – generally disappear after one to three months, but some patients have prolonged fatigue even after normalisation of serum aminotransferase concentrations.

During the acute phase, alanine and aspartate aminotransferase levels (ALT and AST) may increase to 1000–2000 IU/L. ALT is typically higher than AST. Bilirubin levels may be normal in a substantial portion of patients. In patients who recover, normalisation of serum aminotransferases usually occurs within one to four months. Persistent elevation of serum ALT for more than six months indicates progression to chronic hepatitis.

The rate of progression from acute to chronic HBV is primarily determined by the age at infection (Ganem 2004, McMahon 1985). In adult-acquired infection the chronicity rate is 5% or less, whereas it is higher if acquired at younger ages. Approximately 90% for perinatal acquired infection (up to six months of age) become chronic, but this rate decreases

to 20–60% for infections acquired between the age of six months and five years (Caredda 1989, Smedile 1982).

For decades it was assumed that the virus is cleared in patients who recover from acute HBV. However, even in patients positive for anti-HBs and anti-HBc, HBV DNA may persist lifelong in the form of covalently closed circular DNA (cccDNA) and this latent infection maintains the T cell response that enables viral control (Yotsuyanagi 1998, Guner 2011, Gerlich 2013, Zhong 2014). It is now accepted that complete eradication rarely occurs. This is important, as immunosuppression can lead to reactivation of the virus, e.g., after organ transplant or during chemotherapy (Di Bisceglie 2014).

Fulminant hepatic failure is rare, only occurring in approximately 0.1–0.5% of patients. Reasons and risk factors for fulminant HBV are not well understood (Garfein 2004). This may correlate with substance use or coinfections with other viruses. Fulminant HBV is believed to be due to massive immune-mediated lysis of infected hepatocytes. This is why many patients with fulminant HBV have no evidence of HBV replication at presentation (Wright 1992).

Antiviral treatment of patients with acute HBV usually is not recommended (Cornberg 2011). In adults, the likelihood of fulminant HBV is less than 1%, and the likelihood of progression to chronic HBV is less than 5%. Therefore, treatment of acute HBV is mainly supportive in the majority of patients. Antiviral treatment with HBV polymerase inhibitors can be considered in certain subsets of patients, e.g., patients with a severe or prolonged course of HBV, patients coinfecting with other hepatitis viruses or underlying liver diseases, patients with immunosuppression, or patients with fulminant liver failure undergoing liver-transplantation (Kondili 2004, Tillmann 2006).

In addition, patient contacts should be tested for HBV and vaccinated if appropriate.

Chronic hepatitis

In adult-acquired infection, HBV chronicity is 5% or lower, as mentioned earlier. In perinatally acquired infection it is estimated to be approximately 90%, and 20–50% for infections between the age of one and five years (Ganem 2004, McMahon 1985). Most patients will not have a history of acute hepatitis.

Most patients with chronic HBV (CHB) are clinically asymptomatic. Some may have nonspecific symptoms such as fatigue. In most instances, significant clinical symptoms will develop only if liver disease progresses to decompensated cirrhosis. In addition, extrahepatic manifestations may

cause symptoms.

Accordingly, a physical exam will be normal in most instances. In advanced liver disease there may be clinical signs of chronic liver disease including splenomegaly, spider angioma, caput medusae, palmar erythema, testicular atrophy, gynecomastia. In patients with decompensated cirrhosis, jaundice, ascites, peripheral edema, and encephalopathy may be present.

Laboratory testing shows mild to moderate elevation in serum AST and ALT in most patients, whereas normal transaminases occur rarely. During exacerbation, serum ALT concentration may be as high as 50 times the upper limit of normal. Alpha-fetoprotein concentrations correlate with disease activity. In exacerbations of HBV, concentrations as high as 1000 ng/mL may be seen (Lok 1989).

The natural course of CHB infection is determined by the interplay of viral replication and the host immune response. Other factors that may play a role in the progression of HBV-related liver disease include gender, alcohol consumption, and concomitant infection with other hepatitis viruses. The outcome of CHB infection depends upon the severity of liver disease at the time HBV replication is suppressed (UpToDate 2020). Liver fibrosis is potentially reversible once HBV replication is controlled.

There are several typical patterns of CHB acquired in adult or later childhood:

First, infection with a wildtype HBV variant: There is the classic necroinflammatory state with high HBV DNA, HBeAg positive, high ALT and active liver disease.

Second, infection with a precore mutant, which has become much more common than wildtype virus in the recent years. After infection with a precore mutant HBeAg is negative despite considerable HBV DNA replication and elevated ALT.

Third, a low or non-replicative phase, where serum ALT is normal, HBeAg is negative and anti-HBe antibodies are usually present and HBV DNA is low or not detectable. This status is characterised by partial immune control of the HBV infection.

In perinatally acquired chronic HBV infection there are three different states: (i) an immune tolerance phase, (ii) an immune clearance phase, and (iii) a late non-replicative phase.

The immune tolerance phase, which usually lasts 10 to 30 years, is characterised by high levels of HBV replication, as manifested by the presence of HBeAg and high levels of HBV DNA in serum. However, there is no evidence of active liver disease as seen by normal serum ALT concentrations and minimal changes in liver biopsy. It is thought that this lack of liver disease despite high levels of HBV replication is due to immune tolerance to HBV (Dienstag 2008), although the exact mechanisms are unknown. This phenomenon of immune tolerance is believed to be the most

important reason for the poor response to interferon therapy in HBeAg positive patients with normal ALT levels. During this phase there is a very low rate of spontaneous HBeAg clearance. It is estimated that the rate of spontaneous HBeAg clearance is only 15% after 20 years of infection.

During the second to third decade, the immune tolerant phase may convert to immune clearance. The spontaneous HBeAg clearance rate increases – estimated to be 10 to 20% annually. If HBeAg seroconversion occurs, exacerbations of hepatitis with abrupt increases in serum ALT are very often observed. These exacerbations follow an increase in HBV DNA and might be due to a sudden increase in immune-mediated lysis of infected hepatocytes. Most often there are no clinical symptoms during exacerbation, and ALT increase is only detected by routine examinations. Some patients may develop symptoms mimicking acute hepatitis. Anti-HBc IgM titres and alpha-fetoprotein may increase. If such patients are not known to be HBV-infected, misdiagnosis of acute HBV can be made. HBeAg seroconversion and HBV DNA clearance from serum is not always achieved after exacerbation. In these patients, recurrent exacerbation with intermittent disappearance of serum HBV DNA with or without HBeAg loss may occur. The non-replicative phase is usually characterised by the absence of HBV DNA and normalisation of serum ALT, like in adult chronic HBV.

Very few patients with chronic HBV infection become HBsAg negative in the natural course of infection. The annual rate of HBsAg clearance has been estimated to be less than 2% in patients from high-income countries and even lower (0.1–0.8%) in patients of Asian origin (Liaw 1991) following an accelerated decrease in HBsAg levels during the three years before HBsAg seroclearance (Chen 2011). If loss of HBsAg occurs, prognosis is considered favourable. However, clearance of HBsAg does not exclude development of cirrhosis or hepatocellular carcinoma in some patients, although the exact rate of these complications is unknown. This phenomenon is thought to be linked to the fact that HBV DNA may still be present in hepatocytes despite HBsAg loss.

Prognosis and survival

There is a wide variation in clinical outcome and prognosis of chronic HBV infection. Recent data showed that in France about three-quarters of patients with chronic HBV who progressed to a liver-related complication had an additional liver-related risk factor (Mallet 2016). The risk of progression appears to be higher if immune activation occurs. Moreover, increased all-cause mortality in HBsAg positive patients was observed (Montuclard 2015). The lifetime risk of liver-related death has been

estimated to be 40 to 50% for men and 15% for women in a Chinese cohort (Beasley 1982). However, it has to be noted that these data are based from pre-nucleos(t)ide analogue era and prognosis of patients with CHB have markedly improved during the last decade.

Estimated five-year rates of progression (Fattovich 2008) are:

- Chronic hepatitis to cirrhosis – 10 to 20%
- Compensated cirrhosis to hepatic decompensation – 20 to 30%
- Compensated cirrhosis to hepatocellular carcinoma – 5 to 15%

Survival rates are:

- Compensated cirrhosis – 85% at five years
- Decompensated cirrhosis – 55 to 70% at one year and 15 to 35% at five years

Viral replication

Survival is consistently worse in patients with signs of substantial viral replication compared to patients who are HBV DNA negative or who have very low HBV DNA levels. During the natural course of chronic infection, the appearance of the precore stop codon and basal core promoter variants initiates the seroconversion from HBeAg to anti-HBe positivity and leads to the awakening of the immune response. However, variants may emerge and lead to HBeAg negative CHB with high viraemia levels. The prevalence of HBeAg negative CHB has been increasing over the last decades. Acute exacerbations accompanied by high viral replication, elevated ALT levels and histological activity are a common feature of HBeAg negative CHB leading to cirrhosis and HCC much faster than in HBeAg positive CHB patients (Alexopoulou 2014, Papatheodoridis 2001).

In recent years, HBV DNA levels have been linked to disease progression and has replaced HBeAg positivity as a marker for disease activity (Chen 2006). This is true both for progression to cirrhosis and risk of HCC. Therefore, most treatment guidelines are based on HBV viraemia. A reasonable cut-off to distinguish patients with a low compared to high risk of progression and indication for antiviral treatment is 10^4 log copies/mL (corresponding to approximately 2×10^3 IU/mL) (Cornberg 2011), although other cut-offs may be used.

Duration of viral replication is linked with the risk of development of cirrhosis and HCC. As necroinflammation may persist longer in patients with a prolonged replicative phase, the risk of disease progression is elevated. Conversely, even in patients with decompensated cirrhosis, suppression of HBV replication and delayed HBsAg clearance can improve liver disease (Fung 2008).

Alcohol use

Heavy alcohol use is associated with faster HBV progression to liver injury and an elevated risk of developing cirrhosis and HCC (Bedogni 2008, Marcellin 2008). Survival is reduced compared to heavy alcohol users who are HBV negative. However, there is no clear evidence that heavy alcohol use is associated with an enhanced risk of chronic HBV infection, although prevalence of HBV is estimated to be fourfold higher than in controls (Laskus 1992) with variation among regions and cohorts (Rosman 1996).

Hepatitis C (HCV) coinfection

In patients with HBV/HCV coinfection, HCV usually predominates. This may lead to lower levels of transaminases and HBV DNA (Jardi 2001). The rate of HBsAg seroconversion even appears to be increased, as there is a well-known entity of occult HBV infection (patients with negative HBsAg but detectable serum HBV DNA) in patients with chronic HCV (Cacciola 1999, Torbenson 2002, Raimondo 2005). Despite lower aminotransferases and HBV DNA levels, liver damage is worse in most cases. The risks of severe hepatitis and fulminant hepatic failure seem to be elevated if both infections occur simultaneously regardless of whether it is an acute coinfection of HBV and HCV or acute HCV in chronic HBV (Liaw 2004).

Hepatitis D coinfection

An acute HBV/HDV coinfection tends to be more severe than an acute HBV infection alone. It is more likely to result in fulminant hepatitis. If HDV superinfection occurs in patients with CHB, HDV usually dominates, and HBV replication is suppressed (Jardi 2001). Severity of liver disease is worse and progression to cirrhosis is accelerated (Fattovich 2000, Grabowski 2010, Heidrich 2012) (*see Chapter 10*).

Extrahepatic manifestations

The two major extrahepatic complications of chronic HBV are polyarteritis nodosa and renal impairment due to glomerular disease. They occur in up to 10% of patients with chronic HBV and are thought to be mediated by circulating immune complexes (Han 2004).

Polyarteritis nodosa

The clinical manifestations are similar to those in patients with

polyarteritis who are HBV negative. There may be some clinical benefit to antiviral therapy.

Nephropathy/Glomerulonephritis

HBV can induce both membranous nephropathy and, less often, membranoproliferative glomerulonephritis. Most cases occur in children. The clinical hallmark is proteinuria. In contrast to polyarteritis nodosa, there is no significant benefit of antiviral treatment.

For further details, please refer to extrahepatic manifestations in Chapter 13.

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3. Hepatitis C

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Epidemiology

Hepatitis C is a disease with significant global impact. According to the World Health Organization there are 71 million people chronically infected with the hepatitis C virus (HCV), corresponding to 3% of the world's population (WHO 2019). In Europe and the United States, HCV is the most common chronic liver disease and responsible for the majority of liver transplants.

Prevalence and genotype distribution vary considerably among different regions (WHO 2019; Polaris Observatory 2017) . The prevalence is highest in the Eastern Mediterranean Region (15 million, 2.3%) and the WHO European Region (14 million, 1.5%). In North America and north and western Europe lower prevalence rates around 1% are estimated. Certain groups are preferentially affected with the highest risk factor in most cases being injection drug use. But patients undergoing hemodialysis and persons who received blood transfusions before 1991 are also at high risk.

It is estimated that there had been 1.75 million (Incidence rate 23,7 per 100000) new HCV infections in 2015 globally (WHO 2019). But as acute infections remain asymptomatic in most cases, it is difficult to determine the actual number of new HCV infections. In addition, it is not generally possible to determine the duration of infection upon diagnosis. In 2017 an estimated 19% (13.1 million) of those infected globally were aware of their infection with only 5 million being referred to antiviral treatment by the end of 2017 (WHO 2019). Nevertheless, it had been assumed that the number of new infections has considerably decreased over the past decades. In the US, it is estimated that the number of new cases of HCV infection has decreased from approximately 230,000 per year in the 1980s to about 20,000 cases per year in the early 2000s (Wasley 2008)but began to increase again steadily with an estimated 30,500 cases in 2014 up to 44,700 cases in 2017 (CDC 2019).

Overall, epidemiology of HCV is rapidly changing due to a scale up in screening and prevention measures and high cure rates in the era of interferon free direct acting antiviral (DAA) treatment. Highly effective and well tolerated treatment options are now available even for those with advanced liver disease(European Association for the Study of the Liver. Electronic address 2018; Ghany 2019). The implementation of a routinely screening of donated blood for bloodborne viruses in the early

1990s and changes in injection practices as well as needle exchange and opioid substitution programs have already led to a significant reduction of transfusion associated HCV infections and infections in people who inject drugs (PWID). But a substantial increase can be observed in some regions especially in those being younger of age without reported injecting drug use, mainly driven by an increase in acute HCV infections and reinfections in HIV positive men who have sex with men (MSM) (Boesecke 2012; Boesecke 2015). And, since the introduction of a pre-exposure prophylaxis (PrEP) for HIV, increasing numbers of acute HCV infections in HIV negative MSM are documented as well (Hoornenborg 2019; Boerekamps 2018).

Transmission

Parenteral exposure to HCV is the most efficient means of transmission. The majority of patients infected with HCV in Europe and the US acquired the disease through intravenous drug use. While infection via transfusion of blood products has become rare since routine testing of the blood supply for HCV began in the early 1990s other routes of transmission have been acknowledged. Routes of transmission vary in specific regions and subgroups in the world.

Possible routes of transmission are:

- Injection drug use
- Nosocomial/Unsafe medical procedures and injections
- Blood transfusion and organ transplantation
- Having been in jail more than three days
- Sex with a person who injects drugs/Men who have sex with men
- Heterosexual and household contacts
- Perinatal

Injection drug use

Injection drug use has been the most commonly identified source of acute HCV infection. It is estimated that most newly acquired infections occur in individuals who have injected illicit drugs. The seroprevalence of anti-HCV antibodies in groups of PWID may be up to 70% with considerable variation depending on factors such as region, risk behaviour, socioeconomic status etc. underscoring the efficiency of transmission via direct blood contact (Sutton 2008). HCV infection has also been associated with a history of injecting recreational drugs such as methamphetamine in a sexual context

or intranasal cocaine use, presumably due to blood on shared straws or other sniffing paraphernalia. This may explain partly the recent increase in cases of acute HCV in HIV positive MSM (Schmidt 2011, Boesecke 2015). Both WHO and CDC now recognise sex as an HCV transmission route.

Nosocomial/Unsafe medical procedures and injections

Unsafe medical procedures: Until 2000 about 40% of new HCV infections globally were acquired through unsafe medical injections reusing unsterilized devices. Largest numbers of unsafe injections and procedures were reported in the eastern Mediterranean Region and South East Asia. Since 2000 great efforts have been made resulting in a substantial risk reduction in many regions (WHO 2017).

Haemodialysis: Patients who participate in haemodialysis programmes are at increased risk for HCV. The prevalence of HCV antibodies in such patients reaches 15%, although it has declined in recent years (Fissell 2004). A number of risk factors have been identified for HCV infection among dialysis patients. These include blood transfusions, duration of hemodialysis, HCV prevalence in the dialysis unit, and type of dialysis. The risk is higher with in-hospital haemodialysis as opposed to peritoneal dialysis.

Needle-stick injuries: There is some risk of HCV transmission for healthcare workers after unintentional needle stick injury or exposure to other sharp objects. The incidence of seroconversion after exposure to an HCV positive source is generally estimated to be less than 2% (Service 2001). However, data are divergent and figures ranging from 0 to 10% can be found. Exposure of HCV to intact skin has not been associated with HCV transmission.

Blood transfusion

Historically, blood transfusion or use of other blood products was a major risk factor for transmission of HCV. In some historic cohorts 10% or more of patients who received blood transfusions were infected with hepatitis C (Alter 1989). However, blood donor screening for HCV since the early 1990s has nearly eliminated this transmission route. Blood donors are screened for anti-HCV antibodies and HCV RNA – at least in high-income countries. The risk is now estimated to be between 1:500,000 and 1:1,000,000 units (Pomper 2003).

Before screening was introduced, over 90% of patients in cohorts of multiply transfused patients such as hemophiliacs, were infected with HCV

(Francois 1993). Since the use of recombinant clotting factors, new cases of HCV have become uncommon in these patients.

Organ transplantation

Transplant recipients who receive organs from HCV positive donors have a high risk of acquiring HCV infection. Transmission rates in different cohorts vary from 30 to 80% (Pereira 1991; Roth 1994). Therefore, most transplant organisations have developed strategies for screening and selective utilisation of organs from HCV positive donors. The introduction of second generation DAAs in 2014 offered a high possibility of cure for HCV even in those patients being referred to as difficult- to -treat due to end-stage kidney or liver disease and in patients awaiting transplantation. Thus, DAA treatment can be offered before or after solid organ transplantation with beneficial effects on liver function and post-transplant survival (European Association for the Study of the Liver. Electronic address and European Association for the Study of the 2018). In the US the willingness of transplant recipients to accept an organ of an anti-HCV + donor as well as the use of anti-HCV (+) organs for transplantation increased in the last few years. Short term data concerning allograft survival seems to be promising in the era of DAA treatment (Wang 2020).

People in prison

Prevalence and incidence rates for HCV are higher for people in prison comparing to the general population. Main risk factors for the transmission of HCV in prison are injecting drug use associated with needle and syringe sharing as well as unprotected sex and unsafe tattooing (Mason 2019; Falla 2018). Opioid substitution programs as well as needle exchange programs and a scale up of screening for HCV and subsequent DAA treatment before release constitute a high possibility to reduce transmission of HCV rapidly in this well-defined subgroup (Dalgic 2019).

Men who have sex with men

In the last two decades sexual transmission of HCV in men who have sex with men, especially in those being HIV positive has come into focus. Whereas HCV transmission by sexual contact is uncommon between heterosexual couples, there is no doubt that sexual transmission of hepatitis C is possible particularly where sexual practices associated with traumas

are combined with stimulants such as methamphetamine, mephendrone or crystal meth being injected in a sexual context. Outbreaks of cases of acute HCV in several cities in Europe and the United States among MSM have focused attention on sexual transmission of HCV (Boesecke 2015; Boesecke 2012). As there is evidence that HCV can be transmitted sexually, condoms might reduce this risk. Anal sex without condoms, fisting, having many sex partners in a short time period, a concomitant sexually transmitted disease including HIV and use of recreational drugs have been identified as risk factors (Danta 2007; Schmidt 2011; Vanhommerig 2015). Mucosal damage might also be a prerequisite for HCV transmission. According to these observations, the seroprevalence of HCV in MSM ranges from about 4 to 8%, which is higher than the HCV prevalence reported for general populations in European countries. Furthermore, since the introduction of PrEP an increase of acute HCV infections in HIV negative MSM has been described, emphasizing the need for further prevention strategies and behavioral interventions.

Heterosexual or household contact

Common household contacts do not pose a risk of HCV transmission.

The exact risk of HCV transmission in monogamous heterosexual relationships has been difficult to determine. It appears that the risk in long-term partnerships is very low. In prospective cohorts of monogamous, heterosexual couples, there was a long-term transmission risk of 0.01% or lower (Vandelli 2004). Factors that may increase the risk of HCV infection include greater numbers of sex partners, history of sexually transmitted diseases, sexual practices associated with higher risk of trauma and bleeding and not using a condom (Tohme 2010). Whether underlying HIV infection increases the risk of heterosexual HCV transmission to an uninfected partner is unclear. Very often it is difficult to rule out the possibility that transmission results from risk factors other than sexual exposure.

Nevertheless, patients with acute or chronic HCV should be advised that transmission through sexual contact is possible, although the risk is extremely low in heterosexual relationships. It is likely that the use of condoms will lower the risk of sexual transmission further. In most countries, there are no firm recommendations to use barrier precautions in stable monogamous heterosexual partnerships. The transmission risk in MSM as discussed in the section above, is considerably higher and – as for risk of other sexually transmitted diseases – safer sex practices and counselling regarding the risk of injecting recreational drugs are advised for this group.

Perinatal transmission

The risk of perinatal transmission of HCV in HCV RNA positive mothers is estimated to be 5% or less (Ohto 1994). In mothers coinfecting with HCV and HIV this risk correlates with immunosuppression and has been described in up to 20%. To date, there are no specific recommendations for prevention of perinatal transmission (Pembrey 2005). Caesarean section has not been shown to reduce the transmission risk. There is no evidence that breastfeeding is a risk for infection among infants born to women with HCV. Early diagnosis of infection in new-borns requires HCV RNA testing since HCV antibodies are passively transferred from the mother.

Other rare transmission routes

Procedures involved in traditional medicine (e.g., scarification, cupping), tattooing, and body piercing bear the potential of transmitting HCV (Haley 2001). Possible transmissions through these procedures are very likely due to unsterile equipment (Tohme 2012). However, in most instances it is not clear if the risk is due to the procedure itself, or whether there are possible contacts with persons involved who are HCV positive. In addition, transmission via these routes is so rare that persons with exposure are not at increased risk for acquiring HCV.

Clinical manifestations

The spectrum of clinical manifestations of HCV infection varies in acute versus chronic disease. Acute HCV is most often asymptomatic (Vogel 2009) and leads to chronic infection in about 75% of cases. The manifestations of chronic HCV range from an asymptomatic state to cirrhosis and hepatocellular carcinoma. HCV is usually slowly progressive. Thus, it may not result in clinically apparent liver disease in many patients if the infection is acquired later in life. Approximately 20 to 30% of chronically infected individuals develop cirrhosis over a period of 20 to 30 years (WHO 2016).

Acute HCV

After inoculation of HCV, there is a variable incubation period. HCV RNA in blood (or liver) can be detected by PCR within several days to eight weeks. Aminotransferases become elevated approximately 6–12 weeks after

exposure (range 1–26 weeks). The elevation of aminotransferases varies considerably among individuals but tends to be more than 10–30 times the upper limit of normal (typically around 800 U/L). HCV antibodies can be found first around 8 weeks after exposure although in some patients it may take several months by ELISA testing.

However, the majority of newly infected patients will be asymptomatic and have a clinically non-apparent or mild course. Jaundice as a clinical feature of acute hepatitis C will be present in less than 25% of infected patients. Therefore, acute hepatitis C will not be noticed in most patients (Vogel et al. 2009). Periodic screening for infection may be warranted in certain groups of patients who are at high risk for infection, e.g., HIV positive MSM. If acute HCV is suspected HCV-RNA testing by PCR is recommended as HCV antibodies might not be present yet; particularly in HIV coinfecting individuals HCV seroconversion can be delayed.

Other symptoms that may occur are similar to those in other forms of acute viral hepatitis, including malaise, nausea, and right upper quadrant pain. In patients who experience such symptoms of acute hepatitis, the illness typically lasts for 2–12 weeks. Along with clinical resolution of symptoms, aminotransferase levels will normalise in about 40% of patients. Loss of HCV RNA, which indicates cure from hepatitis C, occurs in fewer than 20% of patients regardless of normalisation of aminotransferases. Implementation of highly efficacious DAA treatment led to several changes in management and treatment of acute HCV with varying recommendations in international guidelines (European Association for the Study of the Liver [EASL] 2018; Ghany 2019; EACS 2019). Early treatment initiation 4 weeks after diagnosis after spontaneous clearance has been ruled out as recommended by EACS has been shown to be beneficial for patients' outcome, to reduce transmission and to be cost effective.

Fulminant hepatic failure due to acute HCV infection is very rare. It may be more common in patients with underlying chronic hepatitis B virus infection (Chu 1999).

Chronic HCV

The risk of chronic HCV infection is high. 75–100% of patients remain HCV RNA positive after acute hepatitis C (Alter 1999, Vogel 2009). Most of these will have persistently elevated liver enzymes in further follow-up. HCV is defined as chronic after viral persistence for more than six months after presumed infection. Once chronic infection is established, there is a very low rate of spontaneous clearance.

It is unclear why HCV results in chronic infection in most cases. Genetic diversity of the virus and its rapid mutation rate may allow HCV to escape

immune recognition. Host factors may also be involved in the ability to spontaneously clear the virus. Factors that have been associated with successful HCV clearance are HCV-specific CD4 T cell and NK cell responses, high titres of neutralising antibodies against HCV structural proteins, IL28B gene polymorphisms and specific HLA-DRB1 and -DQB1 alleles (Lauer 2001, Thomas 2009, Rauch 2010). HCV infection during childhood appears to be associated with a lower risk of chronic infection, approximately 50 to 60% (Vogt 1999). Finally, there seem to be ethnic differences with lower risk of chronicity in certain populations, which may in part be explained by different distribution of host genotypes such as IL28B (Ge 2009).

Most patients with chronic infection are asymptomatic or have only mild non-specific symptoms as long as liver cirrhosis is not present (Merican 1993, Lauer 2001). The most frequent complaint is fatigue. Less common manifestations are nausea, weakness, myalgia, arthralgia, and weight loss. HCV has also been associated with cognitive impairment. All of these symptoms are non-specific and do not reflect disease activity or severity (Merican 1993). Very often symptoms may be caused by underlying diseases (e.g., depression), and it can be difficult to distinguish between different diseases. Fatigue as the most common symptom may be present in many other situations (including healthy control groups within clinical studies). HCV is rarely incapacitating.

Aminotransferase levels can vary considerably over the natural history of chronic HCV. Most patients have only slight elevations of transaminases. Up to one third of patients have normal serum ALT (Martinot-Peignoux 2001, Puoti 2002). About 25% of patients have serum ALT concentration of between 2 and 5 times above the upper limit of normal. Elevations of 10 times the upper limit of normal are very rarely seen.

There is a poor correlation between concentrations of aminotransferases and liver histology. Even patients with normal serum ALT show histologic evidence of chronic inflammation in the majority of cases (Mathurin 1998). The degree of injury is typically minimal or mild in these patients. Accordingly, normalisation of aminotransferases after interferon therapy does not necessarily reflect histologic improvement.

Extrahepatic manifestations

Around 30 to 40% of patients with chronic HCV have an extrahepatic manifestation of HCV (Zignego 2008). There are a wide variety of extrahepatic manifestations described as being associated with HCV:

- Hematologic manifestations (essential mixed cryoglobulinaemia, lymphoma)
- Autoimmune disorders (thyroiditis, presence of various autoantibodies)
- Renal disease (membranoproliferative glomerulonephritis)
- Dermatologic disease (porphyria cutanea tarda, lichen planus)
- Diabetes mellitus

For further details, refer to Chapter 13.

Natural history

The risk of developing cirrhosis within 20 years is estimated to be around 10 to 20%, with some studies showing estimates up to 50% (Poynard 1997, Wiese 2000, Sangiovanni 2006, de Ledinghen 2007). Due to the long course of HCV, the exact risk is very difficult to determine, and figures are divergent for different studies and populations. In fact, chronic HCV is not necessarily progressive in all affected patients. In several cohorts it has been shown that a substantial number of patients will not develop cirrhosis over a given time. It is estimated that about 30% of patients will not develop cirrhosis for at least 50 years (Poynard 1997).

Therefore, studies with short observation periods fail to show HCV increases mortality. In addition, survival is generally not impaired until cirrhosis has developed. On the other hand, there is no doubt that patients with chronic HCV have a high risk of cirrhosis, decompensation, and hepatocellular carcinoma in long-term follow-up. For example, in a cohort of patients with posttransfusion HCV evaluated more than 20 years after transfusion, 23% had chronic active hepatitis, 51% cirrhosis, and 5% hepatocellular carcinoma (Tong 1995). It is not completely understood why there are such differences in disease progression. An influence of host and viral factors has to be assumed, particularly other liver comorbidities such as high alcohol consumption and/or non-alcoholic fatty liver disease.

Cirrhosis and hepatic decompensation

Complications of HCV occur almost exclusively in patients who have developed cirrhosis. Interestingly, non-liver related mortality is higher in cirrhotic patients as well. However, cirrhosis may be very difficult to diagnose clinically, as most cirrhotic patients will be asymptomatic as long as hepatic decompensation does not occur. Findings that can be associated with cirrhosis are hepatomegaly and/or splenomegaly on physical examination, elevated serum bilirubin concentration, hyperalbuminaemia, or low platelets. Other clinical findings associated with chronic liver disease may be found such as spider angioma, caput medusae, palmar erythema, testicular atrophy, or gynecomastia. Most of these findings are found in less than half of cirrhotic patients, and therefore none is sufficient to establish a diagnosis of cirrhosis. Therefore, regular screening for liver fibrosis/cirrhosis, e.g. with transient elastography, is recommended by current guidelines (Ghany et al. 2019).

Hepatic decompensation can occur in several forms. Most common is ascites, followed by variceal bleeding, encephalopathy and jaundice. As mentioned earlier, hepatic decompensation will develop only in cirrhotic patients. However, not all patients with cirrhosis actually show signs of decompensation over time. The risk for decompensation is estimated to be close to 5% per year in cirrhotics (Poynard, Bedossa, and Opolon 1997). Once decompensation has developed the 5-year survival rate is roughly 50% (Planas 2004). For this group of patients, liver transplantation is the only effective therapy. Nevertheless, DAA treatment seems to have a clinically relevant impact on disease progression, development of hepatocellular carcinoma (HCC) and liver transplantation (Park 2019; Belli 2018).

HCC develops mostly in patients with cirrhosis. The risk for HCC has been estimated to be less than 3% per year once cirrhosis has developed (Di Bisceglie 1997; Fattovich 1997). However, HCV-associated HCC has significant impact on survival (*see chapter 18*).

Elevated concentrations of α -fetoprotein (AFP) do not necessarily indicate HCC. AFP may be mildly elevated in chronic HCV infection (i.e., 10 to 100 ng/mL) and are higher in patients with considerable fibrotic activity in the liver. Levels above 400 ng/mL as well as a continuous rise in AFP over time are suggestive of HCC.

Disease progression

Chronic HCV has different courses among individuals. It is not completely understood why there are differences in disease progression. Several factors have been identified that may be associated with such

differences. However, other factors not yet identified may also be important.

Age and gender: Acquisition of HCV infection after the age of 40 to 55 may be associated with a more rapid progression of liver injury, as well as male gender (Svrtlih 2007). Children appear to have a lower risk of disease progression (Pawlowska 2015). In one cohort, for example, of 77 patients with chronic HCV, 60% of HCV-RNA positive patients had abnormal ALT and 5% had developed cirrhosis after 2–3 decades of observation (Cesaro 2010).

Ethnic background: Disease progression appears to be slower and changes in liver histology less severe in African-Americans (Sterling 2004).

HCV-specific cellular immune response: The severity of liver injury is influenced by the cellular immune response to HCV-specific targets. Inflammatory responses are regulated by complex mechanisms and probably depend on genetic determinants such as HLA expression and chemokines such as interferon-gamma-inducible protein-10 (IP-10) (Hraber 2007, Larrubia 2008).

Alcohol intake: Alcohol increases HCV replication, enhances the progression of chronic HCV, and accelerates liver injury (Gitto 2009). Even moderate amounts of alcohol appear to increase the risk of fibrosis. Accordingly, in alcoholic patients with cirrhosis and liver failure a high prevalence of anti-HCV antibodies has been described. Alcohol intake should be avoided in all patients with chronic HCV. A safe level of alcohol intake has not been established.

Daily use of marijuana: Daily use of marijuana has been associated with more rapid fibrosis progression, possibly through stimulation of endogenous hepatic cannabinoid receptors.

Other host factors: Genetic polymorphisms of certain genes might influence the fibrosis progression rate (Jonsson 2008). For example, transforming growth factor β 1 (TGF β 1) phenotype or PNPLA3 (adiponutrin) are correlated with fibrosis stage (Zimmer 2011). Patients with moderate to severe steatosis (e.g. non-alcoholic fatty liver disease/non-alcoholic steatohepatitis) are at higher risk for developing hepatic fibrosis.

Viral coinfection: Progression of HCV is clearly accelerated in HIV positive patients (*see section on coinfection*). Acute hepatitis B (HBV) in a patient with chronic HCV may be more severe. Chronic HBV may be associated with decreased HCV replication as opposed to HCV-monoinfected patients, although HCV usually predominates. Nevertheless, liver damage is usually worse and progression faster in patients with dual HBV/HCV infections. Around one third of patients coinfecting with HBV and HCV lack markers of HBV infection (i.e., HBsAg) although HBV DNA is detectable.

Geography and environmental factors: There are some obvious geographic differences (Lim 2008). For example, hepatocellular carcinoma

is observed more often in Japan than in the United States. The reason for this is not clear.

Use of steroids: It is well known that use of steroids increases HCV viral load, while the effect on aminotransferases is variable. They tend to decrease in most patients, although increases in transaminases and bilirubin have also been described (Romero-Gutierrez 2014). Reducing dosage of corticosteroids returns HCV viral load to baseline. However, the clinical consequences of corticosteroid use are largely unknown. It seems reasonable to assume that short-term use of corticosteroids is not associated with significant changes in long-term prognosis.

Viral factors: The influence of viral factors on disease progression is unclear. Overall, there seems to be no significant role of different genotypes and viral quasispecies on fibrosis progression or outcome. However, coinfection with several genotypes may have a worse outcome as compared to mono-infection (Lin 2014).

It is very difficult to predict the individual course of HCV due to the many factors influencing disease progression. Today, assessment of liver fibrosis by non-invasive techniques such as transient elastography (FibroScan®) or by the more traditional liver biopsy is the best predictor of disease progression (Geba 2002, Caviglia 2014). The grade of inflammation and stage of fibrosis are useful in predicting further clinical course. In patients with severe inflammation or bridging fibrosis virtually all will develop cirrhosis within ten years. In contrast, patients with mild inflammation and no fibrosis have an annual progression risk to cirrhosis of around 1%.

Several predictive models of disease progression that include clinical parameters (e.g., hepatic decompensation) and laboratory parameters (e.g., bilirubin, INR) have been evaluated, but none of these models is routinely used in the clinic at present. In patients with cirrhosis, the MELD score (Model for End-Stage Liver Disease) and the Child score (Table 1) are used to stage disease and to describe the prognosis (see Chapters 19 & 20). The MELD Score is used especially to estimate relative disease severity and likely survival of patients awaiting liver transplant. It is calculated as: MELD Score = 10 x (0.957 x ln(creatinine)) + (0.378 x ln(bilirubin)) + (1.12 x ln(INR)) + 6.43. An online calculator and further information can be found at the website of the United Network for Organ Sharing (UNOS) (<http://www.unos.org>).

However, the best way to slow liver fibrosis and the risk for hepatic decompensation in cirrhotics is successful HCV treatment (van der Meer 2012, Anderson 2013). The new directly acting antivirals (DAAs) with their high efficacy and very favourable safety profiles are already largely contributing to lowering the disease burden caused by chronic HCV infection.

Table 1. Child-Pugh classification of severity of liver disease (Child 1964)*

	Points assigned		
	1	2	3
Ascites	Absent	Slight	Moderate
Bilirubin, mg/dL	<2	2–3	>3
Albumin, g/dL	>3.5	2.8–3.5	<2.8
Prothrombin time			
• Seconds over control	<4	4–6	>6
• INR	<1.7	1.7–2.3	>2.3
Encephalopathy	None	Grade 1–2	Grade 3–4

*A total score of 5–6 is considered stage A (well-compensated disease); 7–9 is stage B (significant functional compromise); and 10–15 is stage C (decompensated disease). These grades correlate with one- and two-year patient survival (stage A: 100 and 85 percent; stage B: 80 and 60 percent; stage C: 45 and 35 percent).

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4. Hepatitis E: a relevant disease with many aspects

Sven Pischke and Heiner Wedemeyer

Introduction

Hepatitis E is an inflammatory liver disease caused by the hepatitis E virus (HEV): This infection has been described to be endemic in many tropical countries with reduced sanitary conditions in the 1980ies. For more than two decades it has been considered to be a travel-associated, acute, self-limiting liver disease that only causes fulminant hepatic failure in specific, high-risk groups (Pischke 2013b). It has recently been estimated that HEV infection causes approximately 56,000 deaths each year worldwide (WHO 2014). Within the last decade sporadic cases of HEV infections have emerged also in industrialised countries, mostly caused by HEV genotype 3, for which zoonotic transmission has been described (Wedemeyer 2012, Pischke 2013).

In immunocompetent individuals infection with HEV usually leads to a clinically silent seroconversion or to an acute self-limited inflammation of the liver. In pregnant women and patients with pre-existing chronic liver diseases cases of fulminant liver failure by HEV infection are reported (Wedemeyer 2012).

Moreover, cases of chronic HEV infection associated with progressive liver disease have been described in several cohorts of immunocompromised individuals. In this context, diagnosis of HEV infection should rely on detection of HEV RNA, as testing for HEV-specific antibodies may lack sensitivity (Pischke 2010b).

To study the *in vitro* replication of HEV and possible inhibitors a stem cell derived cell culture system has been established and the *in vitro* antiviral effect of ribavirin and interferon has been demonstrated (Helsen 2015). Furthermore human liver chimeric mice have been established as a new model of chronic hepatitis E virus infection for preclinical drug evaluation (Allweis 2016, Sayed 2016). Furthermore, there is increasing evidence that HEV-specific T cell responses contribute to the control of HEV infection (Suneetha Hepatology 2012). Very recently, HEV-specific T cell responses have been characterised targeting the entire HEV genome without distinct immunodominant regions (Brown 2016).

Therapeutic options for chronic hepatitis E include reduction of immunosuppressive medication (Kamar 2011a), treatment with interferon

α (Haagsma 2010, Kamar 2010a) or therapy with ribavirin (Kamar 2010b, Mallet 2010, Pischke 2013a, Kamar 2014). Recently the direct acting antiviral (DAA) sofosbuvir, which has been developed for the treatment of hepatitis C has been shown to be effective against HEV *in vitro* as well as in some single patients, while other patients did not respond to sofosbuvir treatment (Dao 2016, van der Valk 2017, Donnelly 2017, de Martin 2016).

In 2012 a recombinant HEV vaccine was approved for use in China. This vaccine showed an efficacy of >90% in preventing acute symptomatic hepatitis E (Zhu 2010). It is unknown yet if and when this vaccine might become available in other countries.

In 2018 the European Association for the Study of the Liver (EASL) released their clinical practice guidelines on hepatitis E (EASL 2018).

Genetic characteristics of HEV

The hepatitis E virus is a non-enveloped, single-stranded RNA virus (Wedemeyer 2012). HEV has been classified into the species *Orthohepevirus A* in the virus family *Hepeviridae*. Other species within this family (*Orthohepevirus B-D*) infect a wide range of mammalian species including rodents and bats. The relevance of these species for humans is still under debate.

Previously 4 different classical HEV-genotypes (HEV GT 1-4) and 24 subtypes (1a-1e, 2a, 2b, 3a-3j, 4a-4g) have been separated (Meng 1999). However, basing on the identification of HEV-strains from rabbits, wild boars and camels a novel classification separated 8 HEV-genotypes and various subtypes have been identified (Smith 2016). The HEV genome includes two short non-coding regions surrounding three open reading frames (ORF 1 to 3). These ORFs contain the genetic information for various proteins that are necessary for capsid formation, virus replication and infectivity of HEV. Recently a novel viral protein named ORF 4 was identified which is specific to HEV GT 1 (Nair 2016).

HEV genotype 1 is responsible for endemic and epidemic infections by HEV in Asia and Africa, while genotype 2 is endemic in Western Africa and Mexico (Figure 1). These genotypes are usually transmitted fecal-orally by contaminated drinking water under conditions of poor sanitation. Only one study has described the possibility of HEV genotype 1 of infecting swine (Caron 2006). There is no known further report on zoonotic transmission for this genotype.

In contrast, HEV genotype 3 can be found in humans and animals in Europe, the US and Asia (Wedemeyer 2012). For this genotype, zoonotic transmission, foodborne transmission or via contact with infected animals has been well described.

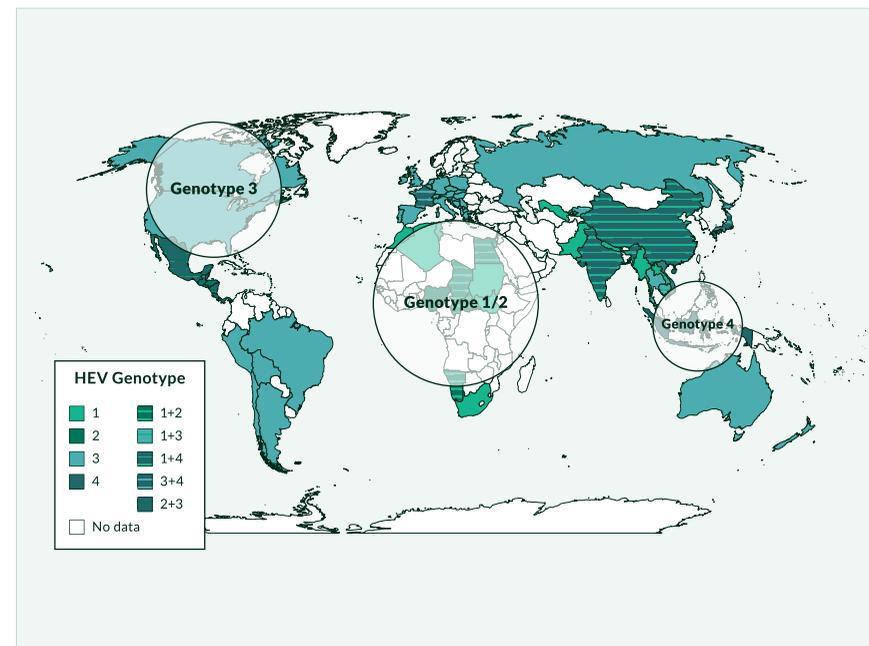


Figure 1. Worldwide distribution of the four classical humanopathogenic HEV genotypes (GT1-4)

Hepatitis E diagnosis

In immunocompetent patients the diagnosis of hepatitis E usually relies on the detection of HEV-specific antibodies. While IgG antibodies indicate acute and past HEV infections, IgM antibodies can only be found in patients with recent infections (Wedemeyer 2012), while HEV-specific IgG antibodies can be detected in patients with previous contact with HEV and ongoing HEV. There are different commercial assays available for detection of HEV-specific IgM and IgG antibodies. Comparison of six anti HEV IgM assays reveals a wide variation of diagnostic sensitivities and specificities as well as interassay disagreements (Drobeniuc 2010). A large European meta-analysis studying 73 studies demonstrated the large inter-assay variability and showed large differences in IgG seroprevalence rates between different European countries (Hartl 2016). The country with the highest seroprevalence rate was France, while the lowest anti-HEV frequency was described for Great Britain (Hartl 2016). Recently a further meta-analysis compared the anti HEV IgG seroprevalence in North and South America (Horvatits 2018). Hepatitis E virus is common in the USA, while the risk of HEV exposure was lower in many poorer South American countries. Thus a higher socioeconomic status does not protect populations from hepatitis E virus exposure. In addition the study demonstrated that

anti HEV IgG seroprevalence did not differ significantly between Europe and the USA. Hence, hepatitis E virus is not limited to countries with low sanitary standards, and a higher socioeconomic status does not protect populations from hepatitis E virus exposure.

In addition to serological tests detection of HEV RNA by PCR has been established, to prove ongoing infection. Numerous assays using different primers have been developed (Meng 1999, Zhao 2007). Furthermore, quantitative PCR assays have been described (Ahn 2006, Enouf 2006). Recently a novel WHO-approved RNA standard assay has been developed (Baylis 2011).

In immunocompromised individuals, diagnosis of HEV infection may only be based on the detection of HEV RNA as seroassays lack sensitivity especially in the early phase of infection (Pischke 2010b). HEV RNA can not only be detected in serum samples but also in stool (Wedemeyer 2012), and thus infectivity of HEV infected persons can be determined by investigating stool for HEV RNA. Furthermore HEV RNA and HEV antigen could be detected in urine of patients with acute and chronic hepatitis E as well as in experimentally infected monkeys (Geng 2016), but the clinical relevance of this observation still needs to be determined. An HEV antigen assay for detection of HEV has been recently described (Gupta 2013). HEV antigen and HEV RNA show significant correlations but the sensitivity of HEV antigen testing might be lower (Zhao 2015). Analysis of a small outbreak of hepatitis E affecting 5/24 travelers to India showed that none of them tested positive for the antigen assay, while all of them were HEV RNA positive (Pischke 2017). This indicates the poor sensitivity for this assay for the detection of genotype 1 infections.

Worldwide distribution of HEV infections

Hepatitis E causes more than 70,000 deaths each year worldwide (Rein 2011). Most of these cases occur in the tropics, in areas with reduced hygienic standards, due to poor sanitation. Outbreaks in refugee camps are of major relevance, as reported in 2013 from the Sudan (CDC 2013).

However, the disease is not limited to developing countries. In the last few years an increasing frequency of diagnosed cases of HEV infections has been reported from various industrialised countries (Wedemeyer 2012, Adlhoch 2016). The presence of HEV RNA in urban sewage samples from Spain, the US and France has been shown, suggesting that HEV may be more prevalent in industrialised countries than previously assumed (Clemente-Casares 2003). In each of these three countries it was possible to discover HEV contamination in sewage samples in a notably high frequency. These findings may partially explain the huge gap between seroprevalence rates

and the rather low numbers of diagnosed and reported cases of acute hepatitis E in western countries. The mismatch between high seroprevalence rates and the low number of symptomatic cases has also been investigated in a recent study from Egypt. 919 anti-HEV seronegative individuals from rural Egypt were followed and, interestingly, 3.7% (n=34) of these individuals seroconverted to anti-HEV within 11 months of follow up (Stoszek 2006). However, none of these 34 individuals suffered from symptomatic hepatitis E. This finding corresponds with data from a recently published large vaccine study performed in China where very few of the patients in the placebo group who seroconverted during a follow-up period developed symptomatic acute hepatitis E (Zhu 2010). Overall, these data suggest that far less than 5% of all contacts with HEV lead to symptomatic hepatitis E (Wedemeyer 2011). In contrast to these findings small or large outbreaks may occur. E.g. an outbreak including five symptomatic, viraemic patients could be observed within a group of 24 German travelers to India (Pischke 2017). This demonstrates that some strains of HEV might lead to a higher clinical manifestation rate under special circumstances.

A rapid increase in reported HEV infections has been recognised in several industrialised countries over the last decade (Adlhoch 2016). To investigate the potential underlying reasons for this phenomenon, we analysed the time trend of the anti-HEV seroprevalence in healthy German individuals versus the number of reported cases of acute hepatitis E. Even though the number of reported cases has increased more than 5-fold in the last ten years (Figure 2), the anti-HEV IgG seroprevalence rate remained rather stable over the last 15 years (Pischke 2011a). In contrast, the number of scientific articles on HEV infections published in PubMed increased sharply during the same period (Figure 2). These findings may indicate that the increase of reported HEV cases in Germany and other industrialised countries is based on an increased awareness associated with more frequent diagnosis of hepatitis E but not a true increase in incidence rates (Pischke 2011a). In contrast to this observation, in the Netherlands the number of HEV positive blood products significantly increased between January 2013 and December 2014 indicating that new HEV transmission routes resulting an higher exposure of the general population of the Netherlands might exist (Hogea 2015). However, this observation needs to be verified in further studies.

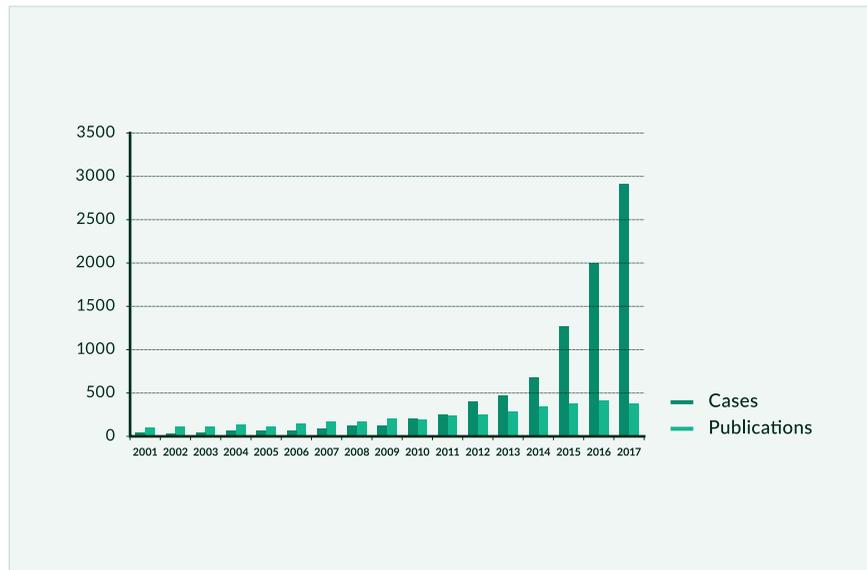


Figure 2. Number of reported HEV infections in Germany over the last decade and number of publications on HEV over the same time period.

Transmission of HEV

The vast majority of HEV infections worldwide is transmitted by the fecal-oral route. Patient-to-patient transmission is very rare but has been described from a large outbreak in Northern Uganda (Teshale 2011) and from hematology wards in Europe (Wedemeyer 2012). Blood borne transmission of HEV was suggested already in the late nineties (Fainboim 1999). Subsequent studies from Hong Kong, Japan, Great Britain and France confirmed blood transfusions as a possible source of HEV transmission (Wedemeyer 2012). A study from Germany investigating 1019 blood donors determined, that 0.35% seroconverted within 1 year (Juhl 2013). Another large study in 18737 German blood donors demonstrated a rate of 0.1% to be HEV RNA positive (Westhölter 2018). Consumption of uncooked pig meat could be identified as source of infection in the majority of the viremic donors. A study from the Netherlands revealed that 13 out of 40,176 blood donors were HEV-viremic (Slot 2013). These data correspond to one HEV positive blood donation per day in the Netherlands. A large study from England investigating 225,000 blood products confirmed blood transfusions as a possible source for HEV transmission with 0.035% of blood products being viremic for HEV (Hewitt 2014). Post-transfusion infections were associated with viral load in the blood product and absence of HEV antibodies. In the United States, a study identified two HEV RNA positive samples among 18,829 tested donations (Stramer 2015).

A study from the Netherlands estimated a viraemia duration of 68 days in apparently healthy blood donors with subclinical HEV infections (Hogema 2015). In line with these findings a study from Germany on 27 HEV viremic blood donors recently reported a median time span for confirmed HEV RNA viral clearance of 55 days (Kraef 2018). Three of these donors experienced prolonged viraemia of more than 100 days. At donation, serological testing failed to identify viremic donors as 70.4% of viremic donors had no detectable antibody response. The median time until first detection of anti-HEV IgM or IgG in antibody-naïve donors was 53 and 57 days respectively.

In contrast to blood-borne HEV infection, only three cases of HEV transmission by transplantation of a graft (liver or kidneys) from a patient with occult hepatitis E have been reported (Schlosser 2011, Pourbaix 2016).

Zoonotic transmission of HEV has been assumed to be the main source of HEV infections in industrialised countries (Figure 3). Both direct contact with HEV-infected domestic animals and foodborne transmission are possible (Wedemeyer 2012). Commercial food products such as pig meat may be contaminated with HEV as shown in studies from the Netherlands, France and Germany (Colson 2010, Melenhorst 2007, Wenzel 2011). Meat should be cooked higher than 70°C to prevent foodborne HEV infections (Emerson 2005, Johne 2016). Interestingly an HEV infection transmitted by camel meat leading to chronic hepatitis E in a liver transplant recipient has been demonstrated (Lee 2015). Although this is surely of limited relevance in European countries and the USA it highlights a novel mode of transmission in Arabian countries.

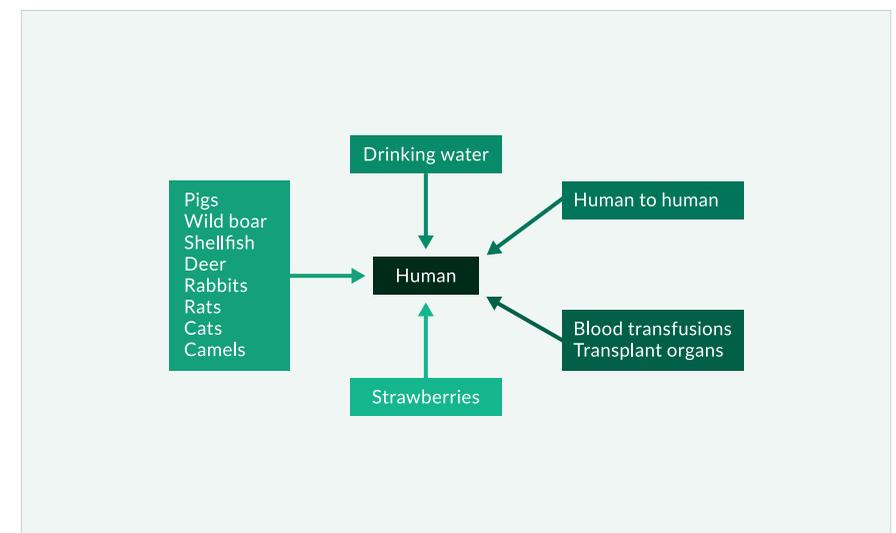


Figure 3. Possible sources of HEV infection

Acute hepatitis E in immunocompetent individuals

In the vast majority of cases, contact with HEV takes an asymptomatic course (Stoszek 2006, Wedemeyer 2012, Wedemeyer 2013), especially if the contact happens during childhood (Buti 2008). Immunocompetent individuals should be able to clear the virus spontaneously. In symptomatic cases the incubation period of HEV infections ranges from three to eight weeks with a mean of 40 days (Wedemeyer 2012). Recently a study focusing on 85 travel-related HEV genotype 1 infections found a median incubation period of 30 days (Azman 2018). The peak of HEV viraemia can be detected in the early phase of infection while the peak of ALT elevation usually occurs around 6 weeks after infection (Wedemeyer 2012).

Initial symptoms in acute hepatitis E are typically unspecific and can include flu-like myalgia, arthralgia, weakness and vomiting. In some patients jaundice, itching, uncoloured stool and darkened urine occur accompanied by elevation of liver transaminases, bilirubin, alkaline phosphatase and gamma-glutamyl transferase.

HEV infection can lead to **more severe acute liver disease in pregnant women** or patients with underlying chronic liver diseases progressing to fulminant hepatic failure in individual cases (Wedemeyer 2012). Possible explanations for the more severe course in pregnant women are hormonal and immunological changes during pregnancy (Navaneethan 2008). Recently an association between reduced expression of the progesterone receptor and fatal outcome of hepatitis E in pregnant women has been reported (Bose 2011).

Single cases of **prolonged courses of HEV infection** in immunocompetent individuals with up to two years of viraemia have been described from France (Mallet 2010), Spain (Gonzalez Tallon 2011) and China (Liu 2011). However, no case of HEV-associated liver cirrhosis or development of hepatocellular carcinoma has been reported in immunocompetent individuals. Prolonged HEV viraemia may indicate a previously undiagnosed disturbance of the immune system in otherwise healthy individuals (Höner zu Siederdisen 2014).

Acute and chronic HEV infections in organ transplant recipients

Chronic courses of HEV infection have been described in European liver or kidney transplant recipients since 2008 (Gerolami 2008, Haagsma 2009,

Kamar 2008, Pischke 2010b, Behrendt 2014). 14 cases of acute hepatitis E were initially reported in kidney- and liver-transplanted patients from southwest France (Kamar 2008). Eight of them developed a chronic course leading to persistently elevated ALT levels, significant histological activity and fibrosis after a follow-up of more than 12 months (range 10 to 18). Subsequently, additional cases of chronic HEV infections have been reported in transplant patients by several groups (Wedemeyer 2012), clearly demonstrating that chronic hepatitis E can be associated with progressive liver disease in patients after organ transplantation (Kamar 2011c).

A study from Germany examined 226 liver-transplant patients and 129 patients with chronic liver disease to evaluate the frequency of chronic HEV infections in liver transplant recipients in a low endemic country (Pischke 2010b). All patients were tested for HEV RNA and anti-HEV IgG. Two cases of chronic HEV infections in liver transplant patients were identified. One of them developed significant liver fibrosis (ISHAK F3) within less than 2 years. Both patients were infected with HEV genotype 3. The possibility of reverse zoonotic transmission was experimentally confirmed by infecting pigs with a patient's blood. HEV RNA was detectable in various organs of the pigs including muscle. Thus, these findings further support the recommendations that eating uncooked meat should be avoided by organ transplant recipients as this may represent a source for acquiring HEV infection.

Retrospective data on hepatitis E in transplant recipients were summarised from 17 centres. Overall, 85 cases of HEV infection were described, 56 (66%) of whom developed chronic hepatitis E. Of note, chronicity was associated with the use of tacrolimus and with low platelet count (Kamar 2011c). However it has to be considered that the vast majority of patients had been recruited by one centre and experiences from other regions and transplant centres need to be reported.

Chronic courses of HEV infection have also been reported in heart transplant recipients (de Man 2011, Pischke 2012b). A study from Germany investigating heart transplant recipients and non-transplant cardiac patients revealed that the seroprevalence of HEV-specific antibodies is increased 5-fold in these patient groups in comparison to healthy controls (Pischke 2012b). It has been assumed that medical procedures, especially blood products, could explain this difference in seroprevalence rates.

Chronic HEV infections have also been described in lung transplant recipients from the Netherlands (Rizebos-Brilman 2013) and Germany (Pischke 2014).

Overall, all recipients of solid organ transplant with elevated liver enzymes should be tested for HEV RNA unless other obvious reasons already explain the hepatitis. In immunosuppressed patients, testing for HEV RNA should be applied as antibody testing may lack sensitivity.

Distinct immunosuppressive drugs may indirectly or directly affect HEV replication, which needs to be considered in the management of organ transplant recipients (Behrendt 2014).

In contrast to solid organ transplant recipients, studies from Germany (Koenecke 2012) and France (Abravanel 2012) did not observe any case of chronicity in stem cell transplant recipients, leading to the assumption that this phenomenon is rare in this patient population. However, a large study from the Netherlands, investigating 328 stem cell transplant recipients, identified 8 cases (2.4%) of chronic HEV viraemia. Four of these patients died after development of hepatitis, while the other four patients cleared HEV infection after a median period of 6.3 months. These data demonstrate that chronic HEV infections in stem cell transplant recipients are indeed relevant (Versluis 2013).

Hepatitis E in patients with HIV infection or other immunological deficiencies

Chronic hepatitis E was described for the first time in a patient with underlying HIV infection in 2009 (Dalton 2009). This patient had a CD4 T cell count of less than 200 cells and high HIV RNA levels (>100,000 copies/mL). However, subsequent studies from Spain (n=93) (Madejon 2009), Germany (n=123) (Pischke 2010a) and England (n=138) (Keane 2012) could not identify cases of chronic hepatitis in HIV-infected individuals. HEV RNA was detected for more than 10 months in only one out of 184 HIV positive individuals in France (Kaba 2010). This patient had particularly low CD4 counts (<50 cells/mm) while two additional patients with higher CD4 levels were able to clear HEV spontaneously. Thus, persistent HEV infection is rarely observed in HIV-infected patients. However, it has been demonstrated that HEV may still persist in single HIV infected patients despite improvement of their immune system (Kuniholm 2015, Ingiliz 2016).

In addition to HIV positive patients, chronic HEV infections in patients with different underlying conditions of immunosuppression including lupus erythematoses, granulomatosis, retroperitoneal fibrosis or CD4 deficiency have been reported (Grewal 2013, Höner zu Siederdisen 2014). In contrast to these diseases there was no case of chronic HEV infection within a German cohort of 73 patients with common variable immunodeficiency (CVID). It has been hypothesised that eventually regular immunoglobulin infusions in these patients may have protected them from infection (Pischke 2012a).

Extrahepatic manifestations of hepatitis E

Several symptoms have been assumed to be extrahepatic manifestations of acute or chronic or previous HEV infections (Pischke 2016). Neurological symptoms associated with acute or chronic HEV infection have been described in single cases in the past few years (Kamar 2011b). More recently, HEV infections were linked with neuralgic amyotrophy (van Eijk 2014) and Guillain-Barré syndrome (Van den Berg 2014). Especially the association of HEV and neuralgic amyotrophy seems to be proven. Within a large multicentric study 57 patients with neuralgic amyotrophy and simultaneous HEV infection have been compared with 61 neuralgic amyotrophy cases without HEV infection (van Eijk 2017). Those patients with HEV infection showed significantly more frequently bilateral involvement, damage outside the brachial plexus and involvement of phrenic nerve and lumbosacral plexus injury (van Eijk 2017). Furthermore a study on patients presenting with various non-traumatic neurological symptoms (n=464) identified that 2% had current/recent HEV infection, including patients with neuralgic amyotrophy, cerebral ischaemia or encephalitis (Dalton 2017). In addition to this observation a Chinese study found 5% of Myasthenia gravis patients (n=188) to be anti HEV IgM positive and 2% were viremic (Wang 2018). Thus there is an association of HEV infections with various neurological diseases.

Various additional case reports describing associations of HEV infection with cases of pancreatitis, thyroiditis and haematological disorders were published (Kamar 2015). The underlying mechanisms and the clinical relevance of these associations require further investigation. Possible explanations may be distinct features of heterologous immunity of HEV and HEV replication in non-liver tissues (Wedemeyer 2016).

In addition, an increased anti-HEV seroprevalence rate in patients with autoimmune hepatitis has been reported, indicating a possible role of previous HEV infections in later development of autoimmune hepatitis (Pischke 2014).

It still needs to be determined if extrahepatic manifestations are caused by direct effects of the virus or if, indirectly, immunological mechanisms are responsible (Pischke 2016). A possible link between HEV and cryoglobulinaemia has been suggested (Pischke 2014, Kamar 2012).

Treatment of chronic hepatitis E

Treatment options for chronic hepatitis E include reduction of immunosuppression, administration of pegylated interferon α or use of ribavirin. The first step in the treatment of chronic HEV infection should

be to evaluate if it is possible to reduce the immunosuppressive medication (Wedemeyer 2012). Reduction of immunosuppression in 16 solid organ transplant recipients with chronic hepatitis E led to clearance of HEV in 4 cases (25%) (Kamar 2011a). A second possible treatment option is the use of PEG-IFN α (Haagsma 2010, Kamar 2010a). Treatment durations varied between 3 and 12 months. Overall, 4 out of 5 patients were successfully treated with sustained clearance of HEV RNA. However, the use of interferon can be associated with significant side effects and may cause rejection in organ transplant recipients. Interferon α is therefore not recommended in heart or kidney transplant recipients. The antiviral efficacy of ribavirin monotherapy has been evaluated by two French groups (Kamar 2010b, Mallet 2010). A sustained virologic response was observed in 2/2 and 4/6 treated patients, respectively. Ribavirin has also been used in a non-transplanted patient with severe acute hepatitis E who showed rapid improvement of symptoms and liver function tests during treatment (Gerolami 2011).

A study from France demonstrated the safe use of ribavirin in non-transplant individuals with acute HEV genotype 3 infections (Peron 2015). Furthermore the use of ribavirin has been demonstrated in one single case with severe HEV genotype 1 infection (Pischke 2013a). Starting and stopping rules for ribavirin treatment of acute hepatitis E still need to be defined. In contrast to immunocompetent individuals, in solid organ transplant recipients with chronic HEV infection ribavirin remains a frequently used therapeutic option. A multicentre French study confirmed that treatment of chronic HEV infections in transplant recipients with ribavirin is safe and efficient (Kamar 2014). However, ribavirin treatment failures have been described (Pischke 2012b, Pischke 2013a) that may be linked to selection of a distinct HEV polymerase variant (G1634R) with increased replication fitness (Debing 2014). Still, the role of the G1634 variant for treatment response requires further investigation if it increases the risk of ribavirin treatment failure (Lhomme 2015). The G1634R variant has been detected as a minor viral population already before therapy in patients with subsequent treatment failure (Todt 2016). Of note, ribavirin induces HEV mutagenesis *in vivo* and additional HEV variants may emerge during treatment (Todt 2016).

Sofosbuvir displays activity against HEV *in vitro* (Dao 2016). It has been debated that the dose required to induce antiviral effects might be much higher than steady state concentrations achieved in patients with the standard sofosbuvir dose of 400 mg qd (Wang 2016). Nevertheless, a decline in HEV RNA was observed in a patient who failed to clear HEV with ribavirin therapy who received sofosbuvir but viral relapse occurred after the end of therapy (Van der Walk 2017). Another patient who has been co-infected with HCV and HEV and who was treated with sofosbuvir and daclatasvir did not show a virological response concerning HEV. Thus, further research is required to answer the question if sofosbuvir, could have a role in the

therapy of chronic hepatitis E. Currently there is a registered ongoing study (Sof-E) evaluating the effect of sofosbuvir in chronically HEV infected patients who failed to achieve HEV clearance under ribavirin treatment.

An *in vitro* study demonstrated that silvestrol, a natural compound isolated from the plant *Aglaia foveolata* is a potent inhibitor of the release of HEV infectious viral particles (Biedenkopf 2017). The clinical relevance of this finding still needs to be studied.

Vaccination

A vaccine developed by GSK and the Walter Reed Army Institute that was successfully tested in a phase 2 study (Shrestha 2007) has not been further developed. A group from China reported data from a very large successful phase 3 vaccine trial (Zhu 2010). This trial included almost 110,000 individuals who received either a recombinant HEV vaccine (“Hecolin”) or placebo. The vaccine efficacy after three doses was 100% concerning prevention of symptomatic acute hepatitis. This vaccine was approved in China in early 2012. It is currently not known if and when this vaccine will become available outside China. Moreover, the efficacy of this vaccine needs to be evaluated in special risks groups such as patients with end-stage liver disease or immunosuppressed individuals. It is also unknown if HEV 239 also protects from HEV genotype 3 infection (Wedemeyer 2011). However, it was demonstrated that either the vaccine or naturally acquired, post-infectious antibodies are able to prevent symptomatic hepatitis E, but not asymptomatic infection (Zhang 2013). Furthermore it was shown that this vaccine could be safely used in pregnant women (Wu 2012). However, it is important to note that the vaccine does not induce sterilising immunity and that asymptomatic infection occurred in vaccinated individuals.

The use of this vaccine in developing countries needs to be discussed and investigated. Eventually this vaccine may help to prevent the morbidity and mortality caused by hepatitis E.

Conclusions and recommendations

In general, HEV infection has a self-limiting course associated with the clinical picture of acute hepatitis in immunocompetent populations. Special populations like pregnant women may be more likely to develop hepatic failure. In patients with immunosuppression of different etiologies, chronic cases have been reported.

In organ transplant recipients the diagnosis of HEV infection should not be based on serological assays alone as these assays may lack sensitivity.

Detection of HEV RNA by PCR in serum or stool represents the gold standard for diagnosis of HEV infection.

The prevalence of chronic HEV infection in solid organ transplant recipients depends on the general prevalence in the population and is low in most industrialised countries. However, chronic hepatitis E occurs and needs to be considered in the differential diagnosis of graft hepatitis, as persistent HEV infection can be associated with progressive graft hepatitis and the development of liver cirrhosis. Currently, all reported cases of chronic HEV infections in transplant recipients have been due to HEV genotype 3 or 4. It is not known if chronic hepatitis E can also be caused by the genotypes 1 or 2.

Organ transplant recipients and other immunocompromised individuals should avoid eating uncooked meats to avoid infection with HEV.

First results indicate that ribavirin treatment of chronic hepatitis E (3 to 5 months duration) is effective to achieve sustained virologic response in immunocompromised persons. In contrast, in immunocompetent individuals with acute HEV infection this treatment is only required in few cases to avoid liver failure.

The relevance of extrahepatic manifestations associated with acute or chronic HEV infection needs further exploration, especially the association between positive anti-HEV serostatus and autoimmune hepatitis, cryoglobulinaemia or neurological symptoms.

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5. HBV virology

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Introduction

The human hepatitis B virus (HBV) is a small-enveloped DNA virus causing acute and chronic hepatitis. Despite the availability of a safe and effective vaccine, HBV infection still represents a major global health burden, with about 240 million people chronically infected worldwide (Cornberg 2019). Many epidemiological and molecular studies have shown that chronic HBV infection represents the main risk factor for hepatocellular carcinoma development (Pollicino 2011, Dandri 2016, Levrero 2016, WHO 2018). The rate for chronicity is approximately 5% in adult infections, but it reaches 90% in neonatal infections. HBV transmission occurs vertically and horizontally via exchange of body fluids. In serum, up to 10¹² HBV genome equivalents per mL serum can be found. Although HBV does not induce direct cytopathic effects under normal infection conditions (Wieland 2004, Thimme 2003), liver damage and chronic inflammation are believed to be induced mainly by the ongoing attempts of the immune system to counteract the infection (McMahon 2009, Dandri 2012).

HBV is the prototype member of the *Hepadnaviridae* family, which are the smallest known DNA-containing, enveloped animal viruses. Characteristic of HBV is its high tissue- and species-specificity, as well as a unique genomic organization with asymmetric mechanism of replication (Nassal 2015). Since all hepadnaviruses use a reverse transcriptase to replicate their genome, they are considered distantly related to retroviruses. Despite the tremendous progresses made in understanding the molecular virology of HBV, some key steps of the infection and interrelations between HBV and host components are still poorly understood. Nevertheless, the discovery of the cellular receptor (Yan 2012) and the establishment of innovative infection models and molecular techniques have opened up new possibilities to investigate specific steps of the lifecycle as well as the organisation and activity of the covalently closed circular DNA (cccDNA), the viral minichromosome that serves as the template of HBV transcription in the nucleus of the infected hepatocytes, enabling maintenance of chronic HBV infection (Allweiss 2017).

Taxonomic classification and genotypes

The *Hepadnaviridae* form their own taxonomic group as their biological characteristics are not observed in any other viral family. Based on host and phylogenetic differences, the family of *Hepadnaviridae* contains two genera: the *orthohepadnaviruses* infecting mammals, and the *avihepadnaviruses* that infect birds. To date, *orthohepadnaviruses* have been found in human (HBV), woodchuck (WHV) (Korba 1989), ground squirrel (GSHV) and woolly monkey (WMHBV) (Lanford 1998). *Avihepadnaviruses* include duck HBV (DHBV) (Mason 1980), heron HBV (HHBV) (Sprengel 1988), snow goose HBV (SGHBV), stork HBV (STHBV) (Pult 2001) and crane HBV (CHBV) (Roggendorf 2007, Funk 2007, Schaefer 2007). Moreover, new hepadnavirus species antigenically related to human HBV and capable of infecting human hepatocytes were also identified in bats (Drexel 2013). The relatedness of these viruses to HBV suggests that bats might constitute ancestral sources of primate hepadnaviruses.

Due to the lack of proofreading activity of the viral polymerase, nucleotide mutations occur during viral replication. This has led to the emergence of eight HBV genotypes, A-H, which differ in more than 8% of the genome, as well as different subgenotypes, which differ by at least 4% (Fung and Lok 2004, Guirgis 2010). The HBV genotypes have different geographic distribution (Liaw 2010), with predominance of genotype A in northwestern Europe, North and South America, genotype B and C in Asia and genotype D in eastern Europe and in the Mediterranean basin. The less diffuse remaining genotypes are mostly found in West and South Africa (genotype E), in Central and South America (genotypes F and H), while genotype G has been detected in France and in the US (Pujol 2009).

HBV structure and genomic organisation

Different types of viral particles can be visualised in the infectious serum by electron microscopy: the infectious virions and the subviral particles. The infectious virus particles are the so-called Dane particles (Dane 1970), have a spherical, double-shelled structure of 42–44 nm containing a single copy of the viral DNA genome, covalently linked to the terminal protein of the virus. A hallmark of HBV infection is the presence of two additional types of non-infectious subviral particles, the spheres and the filaments, which are composed of hepatitis B surface proteins and host-derived lipids, but do not contain the capsid and the HBV genome (Glebe 2007). The spherical structures measure around 22 nm in diameter, while the filaments are of similar width, but of variable lengths (Figure 1).

The viral membrane contains three viral surface proteins and is acquired

by the virus during budding into the endoplasmic reticulum (ER), whereas HBV egress appears to occur via multivesicular bodies (MVBs) (Hoffmann 2013). The surface proteins are named L (large or preS1), M (middle or preS2) and S (small). The surface proteins are produced in quantities largely exceeding the amount needed for the assembly of HBV virions and because of their self-assembly abilities, they are secreted abundantly as empty subviral particles (SVPs). As with nearly all enveloped viruses, HBV particles and SVPs also contain proteins of host origin (Glebe 2007, Urban 2010).

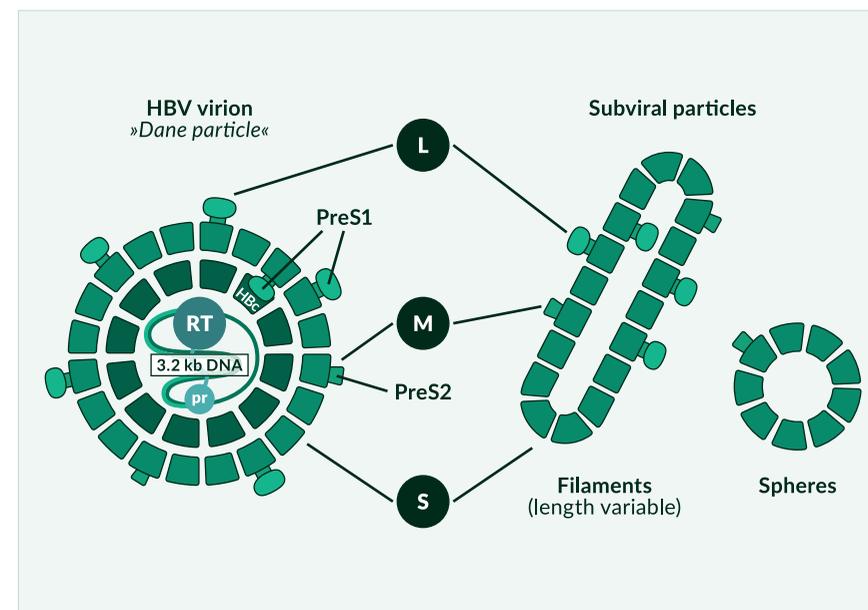


Figure 1. Schematic representation of the HBV virion and non-infectious empty subviral particles (filaments and spheres). Within the nucleocapsid (HBcAg, shown in black) is depicted the partial double-stranded viral genome (rcDNA) covalently linked to the terminal protein of reverse transcriptase. The presence and distribution of the three surface proteins L (preS1 or large), M (preS2 or middle) and S (small) are shown both on HBV and subviral particles (adapted from Glebe 2007).

The HBV genome consists of a partially double-stranded relaxed circular DNA of approximately 3200 nucleotides in length, varying slightly from genotype to genotype, that in concert with the core protein (HBcAg) forms the nucleocapsids (Nassal 2015). Within the Dane particle the negative strand of the viral DNA is present in full-length, carrying the complete genetic information. In contrast, the positive strand spans only approximately two-thirds of the genome in length, whilst its 3' end is variable in size (Summers 1988, Lutwick 1977). The viral polymerase is covalently bound to the negative strand by a phosphotyrosine bond. At the

5' end of the positive strand a short RNA oligomer originating from the pre-genomic (pg) RNA residually remains bound covalently after the viral DNA synthesis. The negative strand also contains a small redundancy of 8–9 nucleotides in length on both the 5' end and the 3' end, named the R region. These redundant structures are essential for viral replication (Seeger 1986, Nassal 2015).

The HBV genome displays four major open reading frames (ORFs) that are organised in a unique and highly condensed way (Block 2007). As shown in Figure 2, all ORFs are in an identical orientation, partially overlap and are encoded by the negative strand. On the genome, 6 start codons, four promoters and two transcription-enhancing elements have been identified. The four major ORFs are: I) the preS/S, encoding the three viral surface proteins; II) the precore/core, encoding both the core protein, essential for the formation of the nucleocapsid, and the non-structural pre-core protein, also known as the secreted e-antigen (HBeAg); III) the pol ORF of the viral polymerase, which possesses reverse transcriptase, DNA polymerase and RNase H activities, and the terminal protein; and IV) the X ORF, coding for the small regulatory X protein, which is essential to establish productive viral infection (Zoulim 1994, Lucifora 2011). Characteristic of the 4 major HBV ORFs is that they utilise a single common polyadenylation signal (Nassal 2015). In addition, splicing of HBV RNA has been observed both in experimental in vitro systems and in liver of chronic HBV patients. Although the biological relevance of this alternative splicing regulation remains elusive, recent studies indicated that the expression of HBV splicing and splicing generated proteins (HBSP) may contribute to hinder the recruitment of innate immune cells through downregulation of chemokine expression in hepatocytes (Duriez 2017). Moreover, splicing efficiency appears to be cell-type dependent, thus hinting at a possible contribution of HBSP as restriction factors of HBV productive infection (Ito 2019).

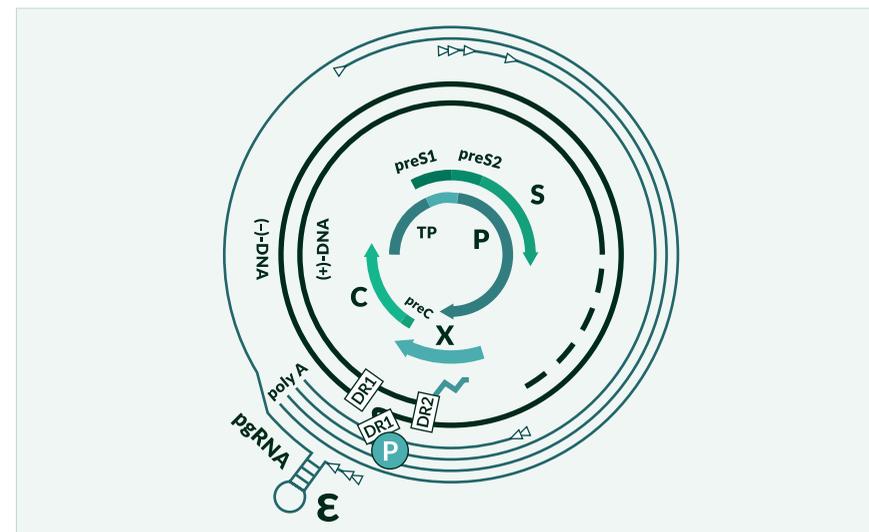


Figure 2. Genome organisation and transcripts of the human hepatitis B virus. The outer thin lines represent the viral transcripts that initiate at different sites, under the control of distinct promoters, but are all terminated after a common polyadenylation site. The RNA signal on the terminally redundant pgRNA is indicated as a hairpin. The thick lines represent the rcDNA form of the genome as present in infectious virions. The 5' end of the minus-strand DNA is covalently linked to the terminal protein of the polymerase. The 5' end of the incomplete plus-strand DNA is constituted by an RNA oligo derived from the 5' end of pgRNA. DR1 and DR2 indicate the direct repeats. The inner arrows indicate the open reading frames (adapted from Nassal 2015).

HBV structural and non-structural proteins

The three surface proteins (L, M, and S) are encoded from one open reading frame (PreS/S) which contains three start codons (one for the large, one for the middle and one for the small protein) but promotes the transcription of 2 mRNAs of 2.4 and 2.1 Kb, named preS and S RNAs (Urban 2014). Notably, the preS/S ORF entirely overlaps with the polymerase open reading frame (Nassal 2015). The three HBV envelope proteins share the C-terminal domain of the S-protein, while the M- and L-protein display progressive N-terminal extensions of 55 and, genotype-dependent, 107 or 118 amino acids (preS2 and preS1). The small envelope protein contains the hepatitis B surface antigen (HBsAg). In virions the stoichiometric ratio of L, M and S is about 1:1:4, while the more abundantly secreted non-infectious subviral particles (SVPs) contain only traces of L-protein (Urban 2014). The envelope proteins are co-translationally inserted into the ER membrane, where they aggregate, bud into the ER lumen, and are secreted by the cell, either as 22 nm subviral envelope particles (SVPs) or as 42 nm infectious virions (Dane particles), after having enveloped the DNA-containing nucleocapsids. The surface proteins of mammalian *Hepadnaviridae* have been

shown to be N- and O-glycosylated (Schmitt 2004). These glycosylations have been shown to be responsible for proper secretion of progeny viral particles. During synthesis, the preS1 domain of L is myristoylated and translocated through the ER. This modification and the integrity of the first 77 amino acids of preS1 have been shown to be essential for infectivity (Glebe 2005, Schulze 2010). In particular the region located within the amino acids 21-47 of the large surface protein contains the binding site of the cellular receptor, the sodium-taurocholate co-transporting polypeptide (NTCP). Both spherical and filamentous SVPs are secreted into the blood of infected individuals in a large excess relative to the infectious particles. The biological function of the excess of SVPs in patients is not clear. It was suggested that SVPs might absorb the neutralising antibodies produced by the host and hence increase the ability of the infectious particles to reach the hepatocytes. Of note, most of the anti-HBs antibodies that are developed by vaccination recognize a region located within the first loop (amino acid 124-137) and the second loop (amino acids 139-147) of HBsAg. Different lines of evidence indicate that the high amounts of SVPs and circulating viral antigens contribute to create a state of immune tolerance on both innate and adaptive immunity against HBV (Dandri 2012, Dembek 2018).

In the cytoplasm, the core protein dimerises and self-assembles to form an icosahedral nucleocapsid. The full-length core protein is 183 amino acids in length and consists of an assembly domain and a nucleic acid-binding domain, which plays an active role in binding and packaging of the pregenomic RNA together with the viral polymerase, and thus enables the RT polymerase/RNA complex to initiate reverse transcription within the newly forming nucleocapsids (Kann 1999, Daub 2002, Nassal 2015). The core protein can be phosphorylated by several kinases. This step along with the presence of the viral polymerase is important for the specific packaging of the pgRNA (Kann 1999, Porterfield 2010).

The viral polymerase is the single enzyme encoded by the HBV genome and is an RNA-dependent DNA polymerase with RNase H activity. The HBV polymerase consists of three functional domains and a so-called spacer region; the terminal protein (TP) is located at its N-terminal domain, and serves as a primer for reverse transcription of the pgRNA into a negative-strand DNA (Zoulim 1994). The spacer domain separates the terminal protein from the polymerase domains (Nassal 2015).

Despite the occurrence of nucleotide mutations due to the lack of proofreading capacity of the HBV polymerase, the peculiar genomic organisation of HBV, where most of the genes overlap, imposes stronger constraints on the amino acid sequence, which significantly reduces the occurrence of mutations in the absence of strong selective pressures. Nevertheless, it has been shown that antiviral therapy with nucleoside analogues can promote the selection of nucleotide mutations within

conserved domains of the reverse transcriptase, which leads to mutations on the amino acid sequence of the envelope proteins. Changes on the HBsAg structure may lead to reduced binding of anti-HBs antibodies, and hence, they may favour the selection of antibody escape mutants (Harrison 2006).

Besides the production of large amounts of empty SVPs, HBV produces and secretes a non-particulate form of the nucleoprotein, the precore protein, or HBeAg, which is not required for viral infection or replication, but appears to act as a decoy for the immune system, and hence, has tolerogenic functions in promoting viral persistence in the neonates of viremic mothers (Chen 2005, Visvanathan 2006). The precore and core proteins are translated from two distinct RNA species that have different 5' initiation sites: the precore RNA and the pgRNA. Indeed, the precore transcript, which also contains the full core gene, encodes a signal sequence that directs the precore protein to the lumen of the endoplasmic reticulum, where it is post-translationally processed. Here, the precore protein undergoes N- and C-terminal cleavage to produce the mature HBeAg form (p17), which is then secreted as a monomeric protein. Interestingly, 20 to 30% of the mature protein is retained in the cytoplasm, where it may antagonise TLR signalling pathways and so contribute to the suppression of the host innate immune responses (Lang 2011). As an important marker for active viral replication, the HBeAg is widely used in molecular diagnostics (Chen 2005, Hadziyannis 2006).

The X protein is a multifunctional regulatory protein with transactivating and pro-apoptotic potential, which can modify several cellular pathways (Bouchard 2004) and act as a carcinogenic cofactor (Kim 1991, Slagle 1996, Bouchard 2004). Numerous DNA transfection experiments have shown that over-expression of the X protein (HBx) causes transactivation of a wide range of viral elements and cellular promoters (Bouchard 2004). *In vitro* studies have shown that HBx can affect various cytoplasmic signal transduction pathways by activating the Src kinase, Ras/Raf/MAP kinase, members of the protein kinase C, as well as Jak1/STAT (Bouchard 2001, Bouchard 2004). Furthermore, *in vitro* binding studies show that HBx can regulate the proteasome function, and thus affecting the degradation of cellular and viral proteins (Zhang 2004), as well as mitochondrial function, by altering its transmembrane potential, and that HBx can modulate calcium homeostasis (Bouchard 2001, Yang 2011). In addition, several independent studies obtained using the woodchuck model (Zoulim 1994), human liver chimeric mice (Tsuge 2010) and HepaRG™ cells (Lucifora 2011), have shown that HBx is required to initiate HBV replication and to maintain virion productivity. Notably, these studies indicated that despite the establishment of comparable cccDNA amounts, transcription of HBV RNAs was dramatically impaired in cells inoculated with HBV X-minus mutants, indicating that HBx is essential to promote cccDNA-driven viral transcription. These findings are also in agreement with data showing

that HBx is recruited to the cccDNA minichromosome, where it was shown to participate in epigenetic control of cccDNA-driven HBV transcription (Belloni 2009, Levrero 2009). Of note, HBx was shown to mediate the degradation of the 'structural maintenance of chromosomes' (Smc) complex Smc5/6 (Decorsiere 2016, Murphy 2016). The study of Decorsiere et al. shows that HBx uses the damaged DNA binding protein 1 (DDB1) as an adaptor protein to interact with an E3 ubiquitin ligase enzyme named CRL4, which is a component of the ubiquitin-proteasome system. Several viruses are known to exploit the ubiquitin-proteasome system to ensure productive infection. Being involved in chromosome organisation and DNA repair, the smc5/6 complex probably binds to the cccDNA acting as a host factor suppressing viral transcription. Thus, ubiquitination and degradation of the Smc5/6 complex by the cell's proteasome machinery, which was demonstrated to occur both in HBV infected human hepatocytes *in vitro* and in humanised mice *in vivo*, represents a new mechanism by which HBx can contribute to initiate and maintain active cccDNA transcription.

Most HBV-related HCC show the integration of HBV DNA sequences including the X gene (Brechot 2004, Pollicino 2011, Lupberger 2007). Although HBV integrated forms are frequently rearranged and hence not compatible with the expression of functional proteins, HBx sequences deleted in the C-terminal portion have been frequently detected in tumoural cells (Iavarone 2003). Intriguingly, such HBx deletion variants were shown to retain their ability to support cccDNA transcription when expressed *in vitro* (Riviere 2019), thus suggesting that not only HBx wildtype, but also such HBx variant forms may participate in cell transformation.

In virus-associated cancers, viral proteins have been shown to participate in epigenetic alterations by disturbing the host DNA methylation system. Interestingly, HBx appears to act as an epigenetic modifying factor in the human liver, which can modulate the transcription of DNA methyltransferases required for normal levels of genomic methylation and maintenance of hypomethylation of tumour suppressor genes (TSGs) (Park 2007). HBx-promoted hypermethylation of TSGs suggests a novel mechanism by which this promiscuous transactivating protein may accelerate hepatocarcinogenesis.

The HBV replication cycle

The generation of various HBV-transfected human hepatoma cell lines and the use of related HBV viruses – including the duck hepatitis B virus (DHBV) and the woodchuck hepatitis virus (WHV) – have significantly contributed to elucidate many steps of the hepadnavirus replication cycle (Dandri 2013). Nevertheless, the limited availability of robust *in vitro*

infection systems and accessible animal models of HBV infection has significantly hindered the identification of cellular factors mediating the early steps of HBV infection in human hepatocytes. The first step in HBV infection involves a non-cell-type specific primary attachment to the cell-associated heparan sulfate proteoglycans (Schulze 2007). This first reversible attachment step is then followed by an irreversible binding of the virus to a specific hepatocyte-specific receptor (Urban 2014). Using mutational analysis, important determinants for infectivity were identified within the HBV envelope proteins. These include 75 amino acids of the preS1 domain of the HBV L-protein, its myristoylation and the integrity of a region in the antigenic loop of the S domain (Gripon 2005, Engelke 2006, Meier 2013). Of note, HBV and HDV infection can be blocked by a small myristoylated lipopeptide (Myrcludex-B) containing the same amino acid sequence of the preS1 domain of the HBV-L protein (Petersen 2008, Lütgehetmann 2012). Although cell polarisation, in addition to the differentiation status of the hepatocytes, was shown to play an essential role in the infection process (Schulze 2011), the identity of the receptor has remained a mystery for many years. By using a method called zero-length photo cross-linking and tandem affinity purification, the preS1 peptide was seen to specifically interact with a sodium taurocholate cotransporting polypeptide (NTCP), a multiple transmembrane transporter localised to the basolateral membrane of highly differentiated primary hepatocytes (Yan 2012). NTCP mediates the transport of conjugated bile acids and some drugs from portal blood to the liver. Based on the discovery that NTCP functions as viral entry receptor by interacting with the large surface protein of HBV, cell lines susceptible to HBV infection could be established demonstrating that both HBV and HDV infection can be established in human hepatoma cell lines (Yan 2012, Nkongolo 2013). Although large amounts of input viruses (MOI >1000) are generally used to achieve efficient HBV infection in these culture systems, the availability of *in vitro* assays permitting investigation of the early steps of infection as well as rapid screening of new anti-HBV agents has opened new opportunities in HBV research. Such *in vitro* studies showed for instance that HBV entry is inhibited by cyclosporins and oxysterols, which are known to bind to NTCP, in hNTCP-transfected hepatoma cells (Nkongolo 2013, Watashi 2013). In addition, binding of HBV or of Myrcludex-B to the cellular receptor NTCP was shown to limit its function, thus altering the hepatocellular uptake of bile salts and the expression profile of genes of the bile acid metabolism (Oehler 2014).

Despite the importance of having discovered the functional cellular receptor mediating HBV entry, additional hepatocyte-specific and species-specific factors appear to be involved in the HBV infection process, as infection rates and virion productivity are generally low in NTCP expressing human cell lines. Intriguingly, establishment of transient HDV infection could be

described in murine cells engineered to express the human NTCP, whereas HBV infection establishment failed in mouse hepatocytes expressing the human NTCP (Li 2014, He 2015). Since HBV and HDV utilise the same envelope proteins for cell entry, additional downstream species-specific factors appear responsible for these discrepancies. As a consequence, no transgenic mice permissive for HBV infection are currently available.

Upon binding to the cell membrane, two possible entry pathways have been proposed. Experimental evidence suggests that HBV can be either involved in an endocytosis process, followed by the release of the nucleocapsid from endocytic vesicles, or HBV may enter the hepatocytes after fusion of the viral envelope at the plasma membrane. As soon as the viral nucleocapsids are released into the cytoplasm, the relaxed circular partially double-stranded DNA (rcDNA) with its covalently linked polymerase needs to enter the cell nucleus in order to convert the rcDNA genome into a covalently closed circular form (cccDNA) (Nassal 2015). Previous studies indicated that the viral capsids are transported via microtubules to the nuclear periphery (Rabe 2006). The accumulation of the capsids at the nuclear envelope would then facilitate interactions with nuclear transport receptors and adaptor proteins of the nuclear pore complex (Kann 1999). Although immature capsids may remain trapped within the nuclear baskets by the pore complexes, the mature capsids eventually disintegrate, permitting the release of both core capsid subunits and of the viral DNA polymerase complexes, which diffuse into the nucleoplasm (Schmitz 2010).

Although the mechanism of cccDNA formation is still not defined, the establishment of productive HBV infection requires the removal of the covalently attached viral polymerase and completion of the positive-strand by the cellular replicative machinery to form the supercoiled cccDNA molecule, which is then incorporated into the host chromatin and serves as the template of viral transcription and replication (Nassal 2015, Newbold 1995). Because of similarities between rcDNA and cellular topoisomerase-DNA adducts that are repaired by tyrosyl-DNA-phosphodiesterase (TDP) 1 or TDP2, recent studies have provided evidence that HBV indeed uses these cellular enzymes to release the P protein from the rcDNA and thus initiates cccDNA biogenesis (Königer 2014). Unlike the provirus DNA of retroviruses, the cccDNA does not need to be incorporated into the host genome. Nevertheless, integration of HBV DNA sequences does occur, particularly in the course of hepatocyte turnover and in the presence of DNA damage (Petersen 1998, Summers 2004, Mason 2005, Allweiss 2018).

Disguised as a stable minichromosome (Bock 1994, Bock 2001, Levrero 2009, Tropberger 2015), the cccDNA uses the cellular transcriptional machinery to produce all viral RNAs necessary for protein production and viral replication, which takes place in the cytoplasm after reverse transcription of an over-length pregenomic RNA (pgRNA) (Figure 3).

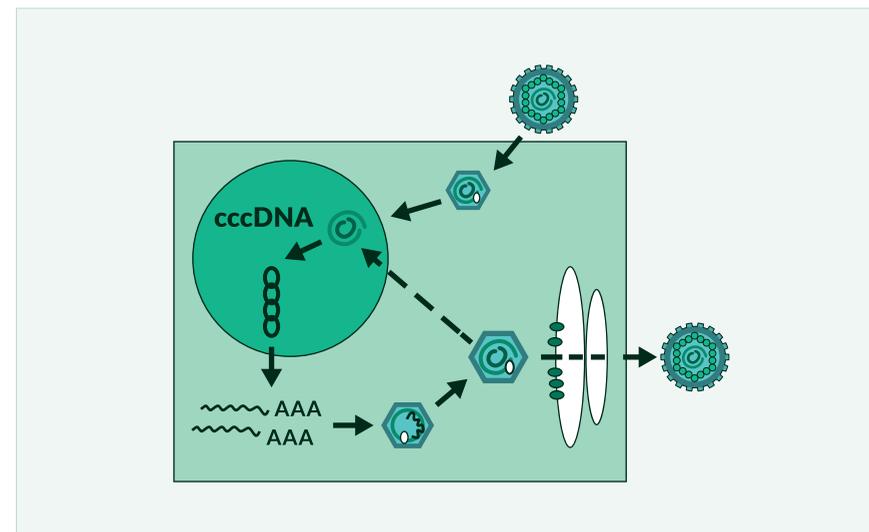


Figure 3. The HBV lifecycle. Upon hepatocyte infection the nucleocapsid is released into the cytoplasm and the rcDNA transferred to the cell nucleus where it is converted into the cccDNA minichromosome. After transcription of the viral RNAs, the pgRNA is encapsidated and reverse-transcribed by the HBV polymerase. Through Golgi and ER apparatus the core particles acquire the envelope and are secreted. Via viral entry and retransporting of the newly synthesised HBV DNA into the cell nucleus, the cccDNA pool can be amplified.

Experimental DHBV infection studies indicate that the cccDNA can be formed not only from incoming virions, but also from newly synthesised nucleocapsids, which instead of being enveloped and secreted into the blood, are transported into the nucleus to ensure accumulation, and later maintenance, of the cccDNA pool (Zoulim 2005b, Nassal 2015). According to this scenario, multiple rounds of infection are not needed to establish a cccDNA pool in infected duck hepatocytes. Moreover, expression of the DHBV viral large surface (LS) protein was shown to induce a negative-feedback mechanism, whereby the accumulation of the LS protein would be fundamental to shut off the cccDNA amplification pathway and redirect the newly synthesised rcDNA-containing nucleocapsids to envelopment and extracellular secretion (Kock 2010). Although this peculiar nuclear re-entry mechanism has been clearly demonstrated for the duck HBV (Summers 1991, Wu 1990) and a high copy number of cccDNA molecules is generally detected in chronically infected ducks and woodchucks (up to 50 copies/cell) (Zhang 2003, Moraleda 1997, Dandri 2000), lower cccDNA intrahepatic loads are generally determined in human liver biopsies obtained from chronically HBV-infected patients (median 0.1 to 5 cccDNA copy/cell) (Werle-Lapostolle 2004, Wong 2004, Laras 2006, Volz 2007, Wursthorn 2006, Lutgehetmann 2008) and in chronically HBV-infected human-liver chimeric mice (Petersen 2008, Lutgehetmann 2011, Allweiss 2018), suggesting that different viral and host mechanisms may control cccDNA dynamics and cccDNA pool size in

human infected hepatocytes. One elegant study showed that HBV converts the rcDNA into cccDNA less efficiently than DHBV in the same human cell background (Kock 2010).

Although the formation of the cccDNA minichromosome is essential to establish productive infection, studies performed in humanised mice indicated that several weeks are necessary for HBV to spread among human hepatocytes *in vivo*, even in the absence of adaptive immune responses, whereas the increase of the amount of cccDNA molecules per cell seems to remain low (Volz 2013, Allweiss 2018).

HBV polymerase inhibitors do not directly affect cccDNA activity and various *in vitro* and *in vivo* studies support the notion that the cccDNA minichromosome is very stable in quiescent hepatocytes, while cell division severely impact its stability (Dandri 2000, Allweiss 2018). Thus, the significant decrease in cccDNA levels (approximately 1 log₁₀ reduction) generally determined after one year of therapy with polymerase inhibitors (Werle-Lapostolle 2004) is supposed to derive from the lack of sufficient recycling of viral nucleocapsids to the nucleus, due to the strong inhibition of viral DNA synthesis in the cytoplasm, and less incoming viruses from the blood. Nevertheless, cccDNA depletion is expected to require many years of nucleos(t)ide drug administration. Thus, despite the absence of detectable viraemia, the persistence of the cccDNA minichromosome within the infected liver is responsible for the failure of viral clearance and the relapse of viral activity after cessation of antiviral therapy with polymerase inhibitors in chronically infected individuals. Furthermore, if viral suppression is not complete, the selection of resistant variants escaping antiviral therapy is likely to occur (Zoulim 2009). Resistant HBV genomes can be archived in infected hepatocytes when nucleocapsids produced in the cytoplasm by reverse transcription and containing resistant mutants are transported into the nucleus and added to the cccDNA pool. Under antiviral pressure, these variants will coexist with wild-type cccDNA molecules and function as templates for the production and possibly further selection of replication-competent resistant mutants, which will spread to other hepatocytes and, eventually may even replace the wild-type cccDNA molecules in the liver (Zoulim 2006, Zoulim 2009, Allweiss 2017).

During chronic HBV infection immune-mediated cell injury and compensatory hepatocyte proliferation appear to favour cccDNA decline and selection of cccDNA-free cells (Mason 2005, Allweiss 2018). Notably, studies with the duck model showed that antiviral therapy with polymerase inhibitors induced a greater cccDNA reduction in animals displaying higher hepatocyte proliferation rates (Addison 2002). Furthermore, the identification of uninfected cccDNA negative cell clones containing traces of infection in the form of viral integration indicates that cccDNA clearance without cell destruction can occur in chronically infected woodchucks

(Mason 2005). Thus, killing of hepatocytes may be instrumental not only to eliminate HBV infected cells but also to induce hepatocyte proliferation, an event that was shown to promote a clear reduction of cccDNA amounts per cell, and even its loss (Allweiss 2018). On the other hand, studies have shown that very low levels of cccDNA can persist indefinitely, possibly explaining lifelong immune responses to HBV despite clinical resolution of HBV infection (Rehermann 1996).

As mentioned previously, the cccDNA acts chemically and structurally as an episomal DNA with a plasmid-like structure, which is organised as a minichromosome by histone and non-histone proteins (Bock 1994, Bock 2001, Newbold 1995). Hence its function is regulated, similarly to the cellular chromatin, by the activity of various nuclear transcription factors, including transcriptional coactivators, repressors and chromatin-modifying enzymes (Levrero 2009, Belloni 2012, Tropberger 2015). Congruent with the fact that HBV infects hepatocytes, the cccDNA presents a broad panel of binding sites for liver-specific transcription factors (Levrero 2009, Quasdorff 2008).

The different HBV transcripts are transported into the cytoplasm, where they are respectively translated or used as the template for progeny genome production. Thus, the transcription of the pgRNA is the critical step for genome amplification and determines the rate of HBV replication. Of note, antiviral cytokines such as IFN α were shown to have the capacity to repress cccDNA transcription (Belloni 2012), as well as to promote its partial degradation (Lucifora 2014). Such findings point out the important role that immune modulating factors may play in reducing cccDNA loads and activity. Thus, identification of the factors affecting stability and transcriptional activity of the cccDNA in the course of infection and under antiviral therapy may assist in the design of new therapeutic strategies aimed at silencing and eventually depleting the cccDNA reservoir (Nassal 2015).

The next crucial step in HBV replication is the specific packaging of pgRNA plus the reverse transcriptase into new capsids. The pgRNA bears a secondary structure – named the ϵ structure – that is present at both the 5' and the 3' ends. The ϵ hairpin loop at the 5' end is recognised by the viral polymerase and acts as the initial packaging signal (Bartenschlager 1992). Binding of polymerase to the RNA stem-loop structure ϵ initiates packaging of one pgRNA molecule and its reverse transcription. The first product is single-stranded (ss)DNA of minus polarity; due to its unique protein priming mechanism, its 5' end remains covalently linked to the polymerase. The pgRNA is concomitantly degraded, except for its 5' terminal (approximately 15 to 18 nucleotides which serve as primer for plus-strand DNA synthesis), resulting in rcDNA. The heterogeneous lengths of the plus-strand DNAs generated by capsid-assisted reverse transcription may result from a non-identical supply of dNTPs inside individual nucleocapsids at the moment of their enclosure by the dNTP impermeable envelope. This predicts that

intracellular cores produced in the absence of envelopment should contain further extended positive DNAs. Alternatively, space restrictions in the capsid lumen could prevent plus-strand DNA completion; in this view, further plus-strand elongation after infection of a new cell might destabilise the nucleocapsid and thus be involved in genome uncoating (Nassal 2015).

The final replication step, the assembly and release of HBV Dane particles, is also not fully understood. The envelopment of the DNA-containing nucleocapsids requires a balanced coexpression of the S and L proteins in order to recruit the nucleocapsid to the budding site. Moreover, the release of infectious viral particles was shown to occur via multivesicular bodies (MVBs), whereas the release of subviral particles (SVPs) proceeds via the general secretory pathway (Hoffmann 2013). Although the role of the envelope proteins in regulating the amplification of cccDNA in HBV is not well-characterised, studies indicate that the lack of expression of the envelope proteins increase cccDNA levels, while co-expression of the envelope proteins not only favours the secretion of viral particles, but also limits the completion of the plus-strand (Lentz 2011).

Notably, in addition to HBV DNA, pregenomic RNA encapsidated and enveloped in virus-like particles is also found in the serum of chronically HBV-infected patients (van Bömmel 2015; Wang 2016) and such release of pgRNA-containing particles seems to accompany that of DNA-containing virions under normal conditions, whereas the amount of pgRNA-containing particles is not diminished after blocking the reverse transcription activity of the HBV polymerase with nucleotide/nucleoside analogues (NUCs) (Wang 2016). In contrast to NUC therapy, a study in HBV-infected human liver chimeric mice indicated that administration of peg-IFN α decreased the levels of both serum HBV DNA and pgRNA (Giersch 2016). Moreover, this study showed that levels of serum pgRNA correlated with levels of pgRNA and cccDNA determined intrahepatically, thus suggesting that measurements of serum pgRNA may serve as a suitable serological marker to determine the persistence of active cccDNA molecules in the liver of infected patients (Giersch 2016).

Animal models of HBV infection

Because of the narrow host range of infection, the study of HBV biology has been limited. Consequently researchers have attempted to establish animal models and cell culture systems that are permissive for HBV replication and at least partially reproduce some stages of HBV infection and can be used, e.g., for the preclinical testing of novel antiviral drugs.

Major fundamental progresses in HBV research were based on infection studies performed with HBV-related animal viruses: DHBV, which infects

Peking ducks (Mason 1980) and WHV (Summers 1978), which infects the Eastern American woodchuck (*Marmota monax*).

One of the major advantages of the DHBV model was that DHBV-permissive primary hepatocytes from ducklings or embryos were easily accessible and showed very high infectivity rates *in vitro* and *in vivo* with high levels of DHBV replication and antigen expression (Jilbert 1996). However, in contrast to mammalian hepadnaviruses, DHBV infection is cleared within a few days postinfection if the virus is not transmitted vertically and the DHBV genome shares little primary nucleotide sequence homology (40%) with HBV. Furthermore, DHBV infection is usually not associated with liver disease and development of hepatocellular carcinoma (HCC). Nevertheless, the duck model has contributed substantially to elucidate the hepadnaviral replication scheme (Mason 1982, Summers 1988, Delmas 2002) and has been also used for preclinical studies (Zimmerman 2008, Reaiche 2010, Chayama 2011).

WHV is more similar to HBV in terms of genomic organisation than the avian hepadnaviruses. Consequently, *in vitro* and *in vivo* studies with woodchuck hepatitis B virus (WHV) have been largely used for the preclinical evaluation of antiviral drugs now in use for treatment of HBV infection (Moraleda 1997, Tennant 1998, Mason 1998, Block 1998, Dandri 2000, Korba 2004, Menne 2005, Fletcher 2015). Moreover, experimental infection of newborn woodchucks almost invariably leads to chronic infection, whereas most animals infected at older ages develop acute hepatitis that results in an efficient immune response leading to viral clearance.

Since acute and chronic WHV infections in woodchucks show serological profiles similar to those of HBV infection in humans, the woodchuck system has provided important information about factors involved in the establishment of virus infection, replication and viral persistence (Lu 2001). Of note, virtually all WHV chronic carrier woodchucks succumb to HCC 2–4 years post infection and regenerative hepatocellular nodules and hepatocellular adenomas are characteristically observed in WHV-infected woodchuck livers (Korba 2004). Proto-oncogene activation by WHV DNA integration has been observed frequently and is thought to play a key role in driving hepatocarcinogenesis in woodchucks, often activating a member of the *myc* family by various mechanisms (Tennant 2004). Interestingly, WHV viral integration was used as a genetic marker to follow the fate of infected hepatocytes during resolution of transient infection in woodchucks (Summers 2003) and to estimate the amount of cell turnover occurring in the course of chronic infection (Mason 2005). Experimental infection studies in woodchucks also demonstrated that WHV mutants that lacked the X gene were unable or severely impaired to replicate *in vivo* (Chen 1993, Zoulim 1994, Zhang 2001). The woodchuck model of virally induced HCC has been used to test chemoprevention of HCC using long-term antiviral nucleoside therapy

and for the development of new imaging agents for the detection of hepatic neoplasms by ultrasound and magnetic resonance imaging (Tennant 2004). Nevertheless, one main difference between human and rodent hepatitis B resides in the absence of associated cirrhosis in woodchuck and squirrel livers, even after prolonged viral infection (Buendia 1998). It is possible that the rapid onset of hepatocyte proliferation following liver damage in rodents does account for this discrepancy. One general disadvantage for using woodchucks is that they are genetically heterogeneous animals, difficult to breed in captivity and to handle in a laboratory setting. Nevertheless, the woodchuck model has greatly contributed in advancing our understanding of the pathogenesis of HBV infection.

Although HBV infects humans exclusively, it can be used to infect chimpanzees experimentally and, to a certain extent, tupaia, the Asian tree shrew (Baumert 2005). Chimpanzees were the first animals found to be susceptible to HBV infection (Barker 1973) and played an important role in the development of vaccines and in the evaluation of the efficacy of therapeutic antibodies (Ogata 1999, Dagan 2003). Though chimpanzees are not prone to develop chronic liver disease (Gagneux 2004), they provide an ideal model for the analysis of early immunological events of HBV acute infection and pathogenesis (Guidotti 1999). Infection experiments with chimpanzees showed that the majority of viral DNA is eliminated from the liver by non-cytolytic mechanisms that precede the peak of T cell infiltration (Guidotti 1999). T cell depletion studies in chimpanzees also indicate that the absence of CD8 positive cells greatly delays the onset of viral clearance (Thimme 2003). Chimpanzees have been used for preclinical testing of preventive and therapeutic vaccines (Will 1982, Guidotti 1999, Kim 2008, Murray 2005). Nonetheless, the large size, the strong ethical constraints and the high costs of chimpanzees severely limit their use for research purposes.

The tree shrew species *Tupaia belangeri* has been analysed for the study of HBV both *in vitro* and *in vivo*, taking advantage of the adaptability of these non-rodent mammals to the laboratory environment (Baumert 2005, von Weizsacker 2004). Inoculation of tree shrews with HBV positive human serum was shown to result in viral DNA replication in their livers, HBsAg secretion into the serum, and production of antibodies to HBsAg and HBeAg (Walter 1996). Although experimental infection of tree shrew with HBV infectious serum is not highly efficient, productive HBV infection was successfully passed through five generations of tree shrews and was specifically blocked by immunisation with hepatitis B vaccine (Yan 1996). Whereas experimental infection of tree shrews causes only a mild, transient infection with low viral titres, primary hepatocytes isolated from them turned out to be a valuable alternative source of HBV-permissive cells (von Weizsacker 2004). Interestingly, the woolly monkey hepatitis B virus (WMHV), which was isolated from the endangered new world primate

woolly monkey (*Lagothrix lagotricha*) (Lanford 1998), was shown to infect primary tupaia hepatocytes very efficiently (Kock 2001, Dandri 2005a), thus providing a useful and more accessible alternative system for studying the early steps of hepadnaviral infection *in vitro* (Schulze 2011, Yan 2012).

Because of the different limitations encountered using chimpanzees and models based on HBV-related viruses, recent developments have focused on using the natural target of HBV infection: the human hepatocyte. However, primary human hepatocytes are not easy to handle, cannot be propagated *in vitro* and their susceptibility to HBV infection is generally low and highly variable. Furthermore, cultured cells may respond differently to the infection than hepatocytes in the liver. The generation of mice harbouring human chimeric livers offered new possibilities to overcome some of these limitations (Dandri 2001).

Two major models are currently available: the urokinase-type plasminogen activator (uPA) transgenic mouse (Rhim 1994) and the knockout fumarylacetoacetate hydrolase (FAH) mouse (Azuma 2007). In both systems, the absence of adaptive immune responses permits the engraftment of transplanted xenogenic hepatocytes, while the presence of transgene-induced hepatocyte damage creates the space and the regenerative stimulus necessary for the transplanted cells to repopulate the mouse liver. Both models permit the establishment of HBV infection, which can then persist for the lifespan of the chimeric mice (Dandri 2001, Bissig 2010). While mouse hepatocytes do not support HBV infection, human chimeric mice can be efficiently infected by injecting infectious serum derived from either patients or chimeric mice. Furthermore, genetically engineered viruses created in cell culture can be used to investigate phenotype and *in vivo* fitness of distinct HBV genotypes and variants (Tsuge 2005). Within the mouse liver human hepatocytes maintain a functional innate immune system and respond to stimuli induced by exogenously applied human IFN α (Belloni 2012, Allweiss 2014). The lack of an adaptive immune system and the undetectable responsiveness of mouse liver cells to human IFN α make the model ideal to exploit the capacities of HBV to interfere with pathways of the innate antiviral response in human hepatocytes (Lutgehetmann 2011), as well as to assess the efficacy of new therapeutic approaches (Petersen 2008, Volz 2013, Klumpp 2018). Moreover, humanised chimeric mice can be superinfected or simultaneously infected with different human hepatotropic viruses, such as HDV (Lütgehetmann 2012, Giersch 2014, Giersch 2019) and HCV (Hiraga 2009) to investigate the mechanisms of viral interference and response to antiviral treatment in the setting of coinfection. Moreover, different chimeric mouse models with a dual reconstitution of both components of the human immune system and human hepatocytes are emerging and their potential for the study of immune responses in HBV infection or immunotherapeutic strategies is currently explored (Dusséaux 2017).

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6. HCV virology

Bernd Kupfer

Hepatitis C virus (HCV) is a major cause of progressive liver disease with an estimated 185 million people infected worldwide, 350,000 of whom die each year from liver damage associated with the infection. HCV infection leads to chronic infection in up to 80% of infected individuals. The main complications of HCV are severe liver fibrosis and cirrhosis, and 30–50% of individuals with cirrhosis go on to develop hepatocellular carcinoma (Tong 1995, Poynard 1997). As a consequence, chronic HCV infection is the major reason for liver transplantation in high-income countries.

History

Until 1975, only two hepatitis viruses had been identified, the “infectious hepatitis virus” (hepatitis A virus, HAV) and the “serum hepatitis virus” (hepatitis B virus, HBV). However, as HAV and HBV were excluded from being the cause of approximately 65% of posttransfusion hepatitis, these cases were termed “non-A, non-B hepatitis” (NANBH) (Feinstone 1975). Inoculation of chimpanzees (Pan troglodytes) with blood products derived from humans with NANBH hepatitis led to persistent increases of serum alanine aminotransferase (ALT) indicating that an infectious agent was the cause of the disease (Alter 1978, Hollinger 1978). Subsequently, it was demonstrated that the NANBH agent could be inactivated by chloroform (Feinstone 1983). Moreover, it was reported that the infectious agent was able to pass through 80 nm membrane filters (Bradley 1985). Taken together these findings suggested that the NANBH causing agent would be a small virus with a lipid envelope. However, the lack of a suitable cell culture system for cultivation of NANBH and the limited availability of chimpanzees prevented further characterisation of the causative agent of NANBH for several years. In 1989, using a newly developed cloning strategy for nucleic acids derived from plasma of NANBH infected chimpanzees, the genome of the major causative agent for NANBH was characterised (Choo 1989). cDNA clone 5-1-1 encoded immunological epitopes that interacted with sera from individuals with NANBH (Choo 1989, Kuo 1989). The corresponding infectious virus causing the majority of NANBH was subsequently termed hepatitis C virus (HCV).

Before HCV was identified, a limited number of patients with NANBH were successfully treated by long-term administration of interferon α .

However, it was only after the molecular characterisation of HCV that it became possible to develop target-specific therapeutics as well as laboratory tests for diagnosis and monitoring of both HCV infection and treatment response.

Taxonomy and genotypes

HCV is a small-enveloped virus with one single-stranded positive-sense RNA molecule of approximately 9.6 kb. It is a member of the genus hepacivirus within the Flaviviridae family. This viral family contains four genera, flavivirus, pestivirus, hepacivirus, and pegivirus (Stapleton 2011). Novel hepaciviruses have been described from primates, bats, bank voles, horses, and dogs enabling researchers to possibly develop new model systems for the analysis of the molecular biology and the pathogenesis of HCV (Kapoor 2013, Drexler 2013, Lauck 2013).

Comparisons of HCV nucleotide sequences derived from individuals from different geographical regions revealed the presence of at least seven major HCV genotypes with a large number of subtypes within each genotype (Smith 2014). HCV strains belonging to the major genotypes 1, 2, 4, and 5 are found in sub-Saharan Africa whereas genotypes 3 and 6 are detected with extremely high diversity in South East Asia. This suggests that these geographical areas could be the origin of the different HCV genotypes. The emergence of different HCV genotypes in North America and Europe and other non-tropical countries appears to represent more recent epidemics introduced from the sites of the original HCV endemics (Simmonds 2001, Ndjomou 2003). In a recent study more than 1300 (nearly) complete HCV coding region sequences were analysed in order to validate new genotype and subtype assignments (Smith 2014). This revealed the presence of at least 7 different HCV genotypes and 67 subtypes. Genomes assigned to the newly described HCV genotype 7 could be detected in human subjects from Central Africa (Murphy 2015). The fast growing number of full-length HCV genome sequences will probably lead to even higher numbers of HCV genotypes. Moreover, it has been reported that inter-subtype as well as inter-genotype HCV recombinants occur (Shi 2012). Although these recombination variants still appear to be rare, this phenomenon may be relevant in patients treated with genotype-specific regimen.

Viral structure

Structural analyses of HCV virions are very limited since the virus is difficult to cultivate in cell culture systems, a prerequisite for yielding

sufficient virions for electron microscopy. Moreover, serum-derived virus particles are associated with serum low-density lipoproteins (Thomssen 1992), which makes it difficult to isolate virions from serum/plasma of infected subjects by ultracentrifugation. Visualisation of HCV virus-like particles via electron microscopy succeeds only rarely (Kaito 1994, Shimizu 1996a, Prince 1996) and it was a point of controversy if the detected structures really were HCV virions. Nevertheless, these studies suggested that HCV has a diameter of 55–65 nm confirming the prediction of the NANBH agent by ultra-filtration (Bradley 1985). In a recent study with highly purified HCV, heterogeneous viral particles with diameters between 50 and 80 nm were observed (Catanese 2013). Various forms of HCV virions appear to exist in the blood of infected individuals: virions bound to very low density lipoproteins (VLDL), virions bound to low density lipoproteins (LDL), virions complexed with immunoglobulins, and free circulating virions (Bradley 1991, Thomssen 1992, Thomssen 1993, Agnello 1999, Andre 2002). The reasons for the close association of a major portion of circulating virions with LDL and VLDL remain unexplained. One hypothesis is that HCV enters hepatocytes via the LDL receptor (Agnello 1999, Nahmias 2006). However, in a more recent study it was demonstrated that involvement of the LDL receptor led to non-productive HCV infection (Albecka 2012).

The design and optimisation of subgenomic and genomic HCV replicons in the human hepatoma cell line Huh7 offered for the first time the possibility to investigate HCV RNA replication in a standardised manner (Lohmann 1999, Ikeda 2002, Blight 2002). However, despite the high level of HCV gene expression, no infectious viral particles are produced with that replication system. Therefore, it cannot be used for structural analysis of cell-free virions.

Infectious HCV particles have been achieved in cell culture by using recombinant systems (Heller 2005, Lindenbach 2005, Wakita 2005, Zhong 2005, Yu 2007). However, even in these *in vitro* systems the limited production of viral particles prevents 3D structural analysis (Yu 2007). Nevertheless, it has been shown by cryoelectron microscopy (cryoEM) and negative-stain transmission electron microscopy that HCV virions isolated from cell culture have a spherical shape with a diameter of approximately 50 to 55 nm (Heller 2005, Wakita 2005, Yu 2007) confirming earlier results that measured the size of putative native HCV particles from the serum of infected individuals (Prince 1996). The outer surface of the viral envelope seems to be smooth. Size and morphology are therefore very similar to other members of the Flaviviridae family such as the dengue virus and the West Nile virus (Yu 2007). Modifying a baculovirus system (Jeong 2004, Qiao 2004) the same authors were able to produce large quantities of HCV-like particles (HCV-LP) in insect cells (Yu 2007). Analysing the HCV-LPs by cryoEM it was demonstrated that the HCV E1 protein is present in the

outer surface of the LPs. In a recent study, analysing viral particles derived from cultivated primary hepatocytes spike projections were observed in the outer surface of HCV (Catanese 2013). These spikes could be the key structures for viral adsorption and entry of HCV to the host hepatocytes.

Using 3D modelling of the HCV-LPs together with genomic comparison of HCV and well-characterised flaviviruses it was assumed that 90 copies of a block of two heterodimers of HCV proteins E1 and E2 form the outer layer of the virions with a diameter of approximately 50 nm (Yu 2007). This outer layer surrounds the lipid bilayer that contains the viral nucleocapsid consisting of the HCV core (C) protein. An inner spherical structure with a diameter of approximately 30–35 nm has been observed (Wakita 2005) representing the nucleocapsid that harbours the genomic viral RNA (Takahashi 1992).

Association of HCV particles with a set of lipoproteins in human sera suggests the existence of so-called lipoviral particles (LVP) *in vivo* (Lindenbach 2013).

Genome organisation

The genome of the hepatitis C virus consists of one 9.6 kb single-stranded RNA molecule with positive polarity. Similar to other positive-strand RNA viruses, the genomic RNA of hepatitis C virus serves as messenger RNA (mRNA) for the translation of viral proteins. The linear molecule contains a single open reading frame (ORF) coding for a precursor polyprotein of approximately 3000 amino acid residues (Figure 1). During viral replication the polyprotein is cleaved by viral as well as host enzymes into three structural proteins (core, E1, E2) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B). An additional protein (termed F [frameshift] or ARF [alternate reading frame]) is predicted as a result of ribosomal frameshifting during translation within the core region of the genomic RNA (Xu 2001, Walewski 2001, Varaklioti 2002, Branch 2005). Detection of anti-F protein antibodies in the serum of HCV positive subjects indicates that the protein is indeed expressed during infection *in vivo* (Walewski 2001, Komurian-Pradel 2004).

The structural genes encoding the viral core protein and the viral envelope proteins E1 and E2 are located at the 5' terminus of the open reading frame followed downstream by the coding regions for the non-structural proteins p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B (Figure 1). The structural proteins are essential components of the HCV virions, whereas the non-structural proteins are not associated with virions but are involved in RNA replication and virion morphogenesis.

The ORF is flanked by 5' and 3' non-translated regions (NTR; also

called untranslated regions, UTR or noncoding regions, NCR) containing nucleotide sequences relevant for the regulation of viral replication. Both NTRs harbour highly conserved regions compared to the protein encoding regions of the HCV genome. The high grade of conservation of the NTRs makes them candidates i) for improved molecular diagnostics, ii) as targets for antiviral therapeutics, and iii) as targets for an anti-HCV vaccine.

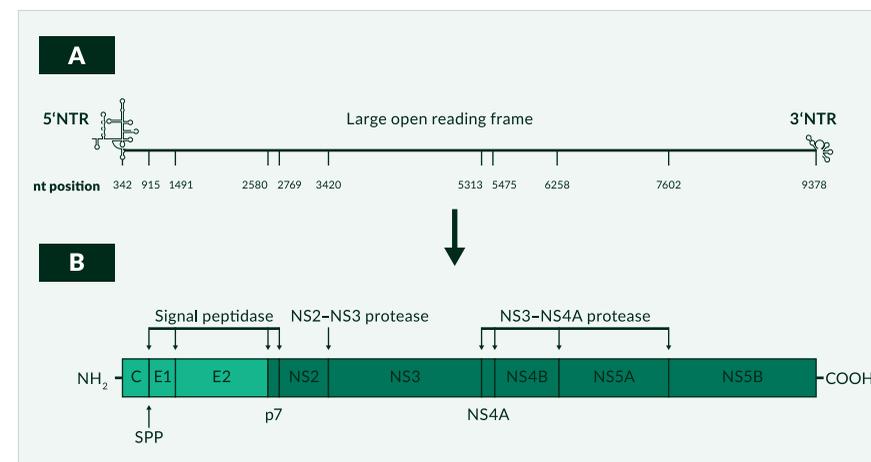


Figure 1. Genome organisation and polyprotein processing. A) Nucleotide positions correspond to the HCV strain H77 genotype 1a, accession number NC_004102. nt, nucleotide; NTR, non-translated region. B) Cleavage sites within the HCV precursor polyprotein for the signal peptide peptidase (SPP) and the viral proteases NS2/NS3 and NS3/NS4A, respectively

The 5'NTR is approximately 340 nucleotides long with a complex secondary structure of four distinct domains (I-IV) (Fukushi 1994, Honda 1999). The first 125 nucleotides of the 5'NTR spanning domains I and II have been shown to be essential for viral RNA replication (Friebe 2001, Kim 2002). Domains II-IV build an internal ribosome entry site (IRES) involved in ribosome binding and subsequent cap-independent initiation of translation (Tsukiyama-Kohara 1992, Wang 1993).

The 3'NTR consists of three functionally distinct regions: a variable region, a poly U/UC tract of variable length, and the highly conserved X tail at the 3' terminus of the HCV genome (Tanaka 1995, Kolykhalov 1996, Blight 1997). The variable region of approximately 40 nucleotides is not essential for RNA replication. However, deletion of this sequence led to significantly decreased replication efficiency (Yanagi 1999, Friebe 2002). The length of the poly U/UC region varies in different HCV strains ranging from 30 to 80 nucleotides (Kolykhalov 1996). The minimal length of that region for active RNA replication has been reported to be a homouridine stretch of 26 nucleotides in cell culture (Friebe 2002). The highly conserved 98-nucleotide X tail consists of three stem-loops (SL1-SL3) (Tanaka 1996,

Ito 1997, Blight 1997) and deletions or nucleotide substitutions within that region are most often lethal (Yanagi 1999, Kolykhalov 2000, Friebe 2002, Yi 2003). Another so-called “kissing-loop” interaction of the 3'X tail SL2 and a complementary portion of the NS5B encoding region has been described (Friebe 2005). This interaction induces a tertiary RNA structure of the HCV genome that is essential for HCV replication in cell culture systems (Friebe 2005, You 2008). Finally, both NTRs appear to work together in a long-range RNA-RNA interaction possibly resulting in temporary genome circularisation (Song 2006).

Genes and proteins

As described above, translation of the HCV polyprotein is initiated through involvement of some domains in NTRs of the genomic HCV RNA. The resulting polyprotein consists of ten proteins that are co-translationally or post-translationally cleaved from the polyprotein (Figure 1B). The N-terminal proteins C, E1, E2, and p7 are processed by a cellular signal peptidase (SP) (Hijikata 1991). The resulting immature core protein still contains the E1 signal sequence at its C terminus. Subsequent cleavage of this sequence by a signal peptide peptidase (SPP) leads to the mature core protein (McLauchlan 2002). The non-structural proteins NS2 to NS5B of the HCV polyprotein are processed by two virus-encoded proteases (NS2/NS3 and NS3) with the NS2/NS3 cysteine protease cleaving at the junction of NS2 and NS3 (Santolini 1995) and the NS3 serine protease cleaving the remaining functional proteins (Bartenschlager 1993, Eckart 1993, Grakoui 1993a, Tomei 1993).

The positions of viral nucleotide and amino acid residues correspond to the HCV strain H77 genotype 1a, accession number NC_004102. Some parameters characterising HCV proteins are summarised in Table 1.

Core. The core-encoding sequence starts at codon AUG at nt position 342 of the H77 genome, the start codon for translation of the entire HCV polyprotein. During translation the polyprotein is transferred to the endoplasmic reticulum (ER) where the core protein (aa 191) is excised by a cellular signal peptidase (SP). The C terminus of the resulting core precursor still contains the signal sequence for ER membrane translocation of the E1 ectodomain (aa 174–191). This protein region is further processed by the cellular intramembrane signal peptide peptidase (SPP) leading to removal of the E1 signal peptide sequence (Hüssy 1996, McLauchlan 2002, Weihofen 2002).

The multifunctional core protein has a molecular weight of 21 kilodalton (kd). *In vivo*, the mature core molecules are believed to form homo-multimers located mainly at the ER membrane (Matsumoto 1996). They have a

structural function since they form the viral capsid that contains the HCV genome. In addition, the core protein has regulatory functions including particle assembly, viral RNA binding, and regulation of RNA translation (Ait-Goughoulte 2006, Santolini 1994). Moreover, protein expression analyses indicate that the core protein may be involved in many other cellular reactions such as cell signalling, apoptosis, lipid metabolism, and carcinogenesis (Tellinghuisen 2002). However, these preliminary findings need to be analysed further.

Table 1. Overview of the size of HCV proteins*

Protein	No. of aa	aa position in ref. seq.	MW of protein
Core immature	191	1–191	23 kd
Core mature	174	1–174	21 kd
F protein or ARF protein	126–161		~ 16–17 kd
E1	192	192–383	35 kd
E2	363	384–746	70 kd
p7	63	747–809	7 kd
NS2	217	810–1026	21 kd
NS3	631	1027–1657	70 kd
NS4A	54	1658–1711	4 kd
NS4B	261	1712–1972	27 kd
NS5A	448	1973–2420	56 kd
NS5B	591	2421–3011	66 kd

* aa, amino acid; MW, molecular weight; kd, kilodalton; ref. seq., reference sequence (HCV strain H77; accession number NC_004102)

E1 and E2. Downstream of the core coding region of the HCV RNA genome two envelope glycoproteins are encoded, E1 (gp35, aa 192) and E2 (gp70, aa 363). During translation at the ER both proteins are cleaved from the precursor polyprotein by a cellular SP. Inside the lumen of the ER both polypeptides experience post-translational N-linked glycosylation (Duvet 2002). The glycoproteins E1 and E2 harbour 6 and 11 putative N-glycosylation sites, respectively. Recent findings suggest that HCV E2 contains further 6–7 putative sites for O-linked glycosylation (Bräutigam 2012).

E1 and E2 are type I transmembrane proteins with large hydrophilic ectodomains and short transmembrane domains (TMD) of 30 aa. The TMD is responsible for anchoring of the envelope proteins in the membrane of the ER and ER retention (Cocquerel 1998, Duvet 1998, Cocquerel 1999, Cocquerel 2001). Moreover, the same domains have been reported to contribute to the formation of E1-E2 heterodimers (Op de Beeck 2000). The E1-E2 complex is involved in adsorption of the virus to its putative receptors tetraspanin

CD81 and low-density lipoprotein (LDL) receptor inducing fusion of the viral envelope with the host cell plasma membrane (Agnello 1999, Flint 1999, Wunschmann 2000). However, the precise mechanism of host cell entry is still not understood completely. Several other host factors have been identified as involved in viral entry. These candidates include the scavenger receptor B type I (Scarselli 2002, Kapadia 2007), the tight junction proteins claudin-1 (Evans 2007) and occludin (Ploss 2009), the C-type lectins L-SIGN and DC-SIGN (Gardner 2003, Lozach 2003, Pöhlmann 2003) and heparan sulfate (Barth 2003).

Three hypervariable regions have been identified within the coding region of E2. These regions, termed hypervariable region 1 (HVR1), 2 (HVR2) and 3 (HVR3), have a sequence variability of up to 80% in their amino acid sequences (Weiner 1991, Kato 2001, Troesch 2006). The high variability of the HVRs reflects exposure of these domains to HCV-specific antibodies. In fact, E2-HVR1 has been shown to be the most important target for neutralising antibodies (Farci 1996, Shimizu 1996b). However, the combination of viral mutation with the selective pressure of the humoral immune response leads to viral escape via epitope alterations (Pantua 2013). Moreover, association of virions with lipoproteins and the presence of a glycan shield on the surface of the viral glycoproteins reduce the effectivity of neutralising antibodies, respectively (Voisset 2006, Helle 2010). This makes the development of vaccines that induce effective neutralising antibodies challenging.

The p7 protein. The small p7 protein (63 aa) is located between the E2 and NS2 regions of the polyprotein precursor. During translation the cellular SP cleaves the E2/p7 as well as the p7/NS2 junction. The functional p7 is a membrane protein localised in the endoplasmic reticulum where it forms an ion channel (Haqshenas 2007, Pavlovic 2003, Griffin 2003). The p7 protein is not essential for RNA replication since replicons lacking the p7 gene replicate efficiently (Lohmann 1999, Blight 2000), however it has been suggested that p7 plays an essential role for virus assembly, formation of infectious virions, and secretion (Sakai 2003, Haqshenas 2007, Gentzsch 2013).

NS2. The non-structural protein 2 (p21, 217 aa), together with the N-terminal portion of the NS3 protein, form the NS2/NS3 cysteine protease which autocatalyses the cleavage of the polyprotein precursor between NS2 and NS3 (Grakoui 1993b, Santolini 1995). The N-terminus of the functional NS2 arises from the cleavage of the p7/NS2 junction by the cellular SP. After cleavage from the NS3, the protease domain of NS2 seems to play an essential role in the early stage of virion assembly and morphogenesis (Jones 2007), probably through physical interactions with the E1-E2 glycoprotein and NS3/NS4A complexes (Stapleford 2011). Moreover, it was demonstrated that NS2 interacts with different host factors. The binding of NS2 to the liver-specific pro-apoptotic CIDE-B protein (Erdtmann 2003) leads to inhibition of CIDE-B-induced apoptosis. Furthermore, the HCV NS2 protein seems

to inhibit cell growth and induces cell cycle arrest in the S phase through down-regulation of cyclin A expression (Yang 2006). Finally, it seems that HCV NS2 is involved in the inhibition of cellular IFN β production (Kaukinen 2013), weakening the unspecific antiviral cellular response.

NS3. The non-structural protein 3 (p70; 631 aa) is cleaved at its N terminus by the NS2/NS3 autoprotease. The C terminal portion of NS3 (442 aa) has ATPase/helicase activity, i.e., it catalyses the binding and unwinding of the viral RNA genome during viral replication (Jin 1995, Kim 1995). However, later findings indicate that other non-structural HCV proteins such as the viral polymerase NS5B may interact functionally with the NS3 helicase (Jennings 2008). These interactions need to be investigated further in order to better understand the mechanisms of HCV replication. The N terminus (189 aa) of the NS3 protein has a serine protease activity. However, in order to develop full activity of the protease the NS3 protease domain requires a portion of NS4A (Faila 1994, Bartenschlager 1995, Lin 1995, Tanji 1995, Tomei 1996). NS3 together with the NS4A cofactor are responsible for cleavage of the remaining downstream cleavages of the HCV polyprotein precursor. Since the NS3/NS4A protease function is essential for viral infectivity it is a promising target in the design of antiviral treatments. In 2011 two potent NS3/NS4A inhibitors, boceprevir (Malcolm 2006) and telaprevir (Perni 2006), were approved by FDA and EMA to be used in combination with IFN α and ribavirin. However, several resistance-associated mutations within the HCV NS3/NS4A coding region have been observed. Meanwhile two additional HCV protease inhibitors, paritaprevir and simeprevir have been approved treatment of HCV genotypes 1 and 4, respectively and additional drugs are awaiting approval.

NS4A. The HCV non-structural protein 4A (p4; 54 aa) is a polypeptide that acts as a cofactor of the NS3 serine protease (Faila 1994, Bartenschlager 1995, Lin 1995, Tanji 1995, Tomei 1996). Moreover, this small protein is involved in the targeting of NS3 to the endoplasmic reticulum resulting in a significant increase of NS3 stability (Wölk 2000).

NS4B. The NS4B (p27; 217 aa) is an integral membrane protein that forms oligomers localised in the endoplasmic reticulum (Yu 2006). The N-terminal domain of the NS4B has an amphipathic character that targets the protein to the ER. This domain is crucial in HCV replication (Elazar 2004, Gretton 2005) and therefore an interesting target for the development of HCV therapeutics or vaccines. In addition, a nucleotide-binding motif (129–134 aa) has been identified (Einav 2004). Moreover, NS4B has the capability of RNA binding (Einav 2008). It has already been demonstrated that the protein induces an ER-derived membranous web that may serve as a platform for HCV RNA replication (Egger 2002). In summary, NS4B appears to be the central viral protein responsible for the formation of the HCV RNA replication complex (Blight 2011).

NS5A. The NS5A protein (p56; 458 aa) is a membrane-associated phosphoprotein that has multiple functions in HCV RNA replication, viral assembly, and virion release. It is phosphorylated by different cellular protein kinases indicating an essential role of NS5A in the HCV replication cycle that is still not fully understood. In addition, NS5A has been found to be associated with several other cellular proteins (MacDonald 2004) making it difficult to determine the exact functions of the protein. One important property of NS5A is that it contains a domain of 40 amino acids, the so-called IFN α sensitivity-determining region (ISDR) that plays a significant role in the response to IFN α -based therapy (Enomoto 1995, Enomoto 1996). An increasing number of mutations within the ISDR showed positive correlation with sustained virological response to IFN α -based treatment. A previous study suggests that NS5A interacts with cytosolic cyclophilin A (CypA) and that this interaction is essential for viral replication (Chatterji 2009). Since inhibitors of CypA, e.g., cyclosporins, already exist, these important findings offer new opportunities for the development of potent anti-HCV therapeutic strategies. Furthermore, HCV NS5A seems to play a key role in preventing oxidative stress-mediated apoptosis keeping the host cell alive, thus enabling the virus to further produce progeny virus (Amako 2013). In addition to the viral enzymes, NS5A is also an interesting target for the development of anti-HCV acting therapeutics, due to its multi-functional properties during different stages of HCV replication. Consequently, three drugs targeting NS5A have been developed and approved to date (daclatasvir, ledipasvir, ombitasvir) and further NS5A inhibitors are to come.

NS5B. The non-structural protein 5B (p66; 591 aa) represents the RNA-dependent RNA polymerase of HCV (Behrens 1996). The hydrophobic domain (21 aa) at the C terminus of NS5B inserts into the membrane of the endoplasmic reticulum, while the active sites of the polymerase are located in the cytoplasm (Schmidt-Mende 2001). During HCV RNA replication NS5B is an essential compound of the HCV replication complex within the NS4B-induced membranous web.

The cytosolic domains of the viral enzyme form the typical polymerase right-handed structure with “palm”, “fingers”, and “thumb” subdomains (Ago 1999, Bressanelli 1999, Lesburg 1999). In contrast to mammalian DNA and RNA polymerases the fingers and thumb subdomains are connected resulting in a fully enclosed active site for nucleotide triphosphate binding. This unique structure makes the HCV NS5B polymerase an attractive target for the development of antiviral drugs.

Using the genomic HCV RNA as a template, the NS5B promotes the synthesis of minus-strand RNA that then serves as a template for the synthesis of genomic positive-strand RNA by the polymerase.

Similar to other RNA-dependent polymerases, NS5B is an error-prone

enzyme that incorporates wrong ribonucleotides at a rate of approximately 10⁻³ per nucleotide per generation. Unlike cellular polymerases, the viral NS5B lacks a proofreading mechanism leading to the conservation of misincorporated ribonucleotides. These enzyme properties together with the high rate of viral replication promote a pronounced intra-patient as well as inter-patient HCV evolution.

Currently, one nucleotidic polymerase inhibitor (sofosbuvir) and one non-nucleosidic polymerase inhibitor (dasabuvir) are approved.

F protein, ARFP. In addition to the ten proteins derived from the long HCV ORF, the F (frameshift) or ARF (alternate reading frame) or core+1 protein has been reported (Walewski 2001, Xu 2001, Varaklioti 2002). As the designations indicate, the ARFP is the result of a -2/+1 ribosomal frameshift between codons 8 and 11 of the core protein-encoding region. The ARFP length varies from 126 to 161 amino acids depending on the corresponding genotype. *In vitro* studies have shown that ARFP is a short-lived protein located in the cytoplasm (Roussel 2003) primarily associated with the endoplasmic reticulum (Xu 2003). Detection of anti-F protein antibodies in the serum of HCV positive subjects indicates that the protein is expressed during infection *in vivo* (Walewski 2001, Komurian-Pradel 2004). However, the functions of ARFP in the viral life cycle are still unknown and remain to be elucidated.

Viral life cycle

HCV enters humans via different transmission routes. The most effective mode of transmission is direct blood-to-blood contact, e.g., blood transfusion, needle sharing, organ transplantation, and other invasive procedures. Furthermore, sexual and mother-to-child transmission have also been described as being responsible for HCV infection. After the virus has entered the blood circulation it reaches the basolateral surface of its host cells within the liver, namely the hepatocytes. The not yet fully understood complex mechanisms of virus entry into its target cell and the downstream processes of HCV proliferation are briefly described below.

Adsorption and viral entry

Binding to and entry of HCV into hepatocytes is a very complex multistep process and more and more host factors involved in that process have been identified over the last 18 years. The first candidate as receptor for HCV was the tetraspanin CD81 (Pileri 1998). CD81 is an ubiquitous 25 kd molecule expressed on the surface of a large variety of cells including hepatocytes and

PBMCs that is involved in a post-binding step (Cormier 2004, Koutsoudakis 2006, Bertaud 2006). However, further studies have shown that CD81 alone is not sufficient for HCV viral entry and that cofactors such as scavenger receptor B type I (SR-BI) are needed (Bartosch 2003b, Hsu 2003, Scarselli 2002, Kapadia 2007). These findings together with the identification of other host factors involved in HCV cell entry generate the current model for the early steps of HCV infection (Lupberger 2012, Dubuisson 2014).

Adsorption of HCV to its target cell is the first step of viral entry. This process may be mediated by VLDL or LDL that is reported to be associated with HCV virions in human sera (Bradley 1991, Thomssen 1992, Thomssen 1993). Dependent on the density of viral particles, HCV binding is thought to be initiated by the interaction of virus-associated apolipoprotein E (ApoE) and the heparan sulfate proteoglycans syndecan-1 and syndecan-4 or SR-BI on the surface of host cells (Dao 2012, Shi 2013, Lefevre 2014, Xu 2015). SR-BI is a protein expressed on the surface of the majority of mammalian cells. It acts as a receptor for LDL as well as HDL (Acton 1994, Acton 1996) emphasising the role of these compounds for HCV infectivity. Alternative splicing of the SR-BI transcript leads to the expression of a second isoform of the receptor SR-BII (Webb 1998), which also may be involved in HCV entry into target cells (Grove 2007). SR-BI is capable of binding to HCV-associated lipoproteins as well as the viral glycoprotein E2. For that reason, SR-BI is assumed to represent the bridge step between attachment of HCV and viral entry. This is supported by two studies showing that HCV binding to SR-BI is a prerequisite for subsequent interaction of the virus with CD81 (Kapadia 2007, Zeisel 2007).

The multi-step procedure of HCV cell entry was shown to be even more complex since a cellular factor termed claudin-1 (CLDN1) has been identified as being involved in this process (Evans 2007). CLDN1 is an integral membrane protein that forms a backbone of tight junctions and is highly expressed in the liver (Furuse 1998). Inhibition assays reveal that CLDN1 involvement occurs downstream of the HCV-CD81 interaction (Evans 2007). However, CD81 and CLDN1 seem to form a protein complex prior to viral entry. Recent findings suggest that CLDN1 could also act as a compound enabling cell-to-cell transfer of hepatitis C virus independently of CD81 (Timpe 2007). Furthermore, it was reported that two other members of the claudin family, claudin-6 and claudin-9, may play a role in HCV infection (Zheng 2007, Meertens 2008). The observation that some human cell lines were not susceptible to HCV infection despite expressing SR-BI, CD81, and CLDN1 indicated that other cellular factors must be involved in viral entry (Evans 2007). In fact, a cellular four-transmembrane domain protein named occludin (OCLN) was identified to represent an additional cellular factor essential for the susceptibility of cells to HCV infection (Liu 2009, Ploss 2009). Similar to claudin-1, OCLN is a component of the tight junctions in

hepatocytes. All tested cells expressing SR-BI, CD81, CLDN1, and OCLN were susceptible to HCV. However, recent work identified E-cadherin as an additional factor that is involved in viral entry (Li 2016). This adhesion protein seems to affect HCV uptake indirectly by triggering the required cell surface distribution of CLDN1 and OCLN, respectively. Although the precise mechanism of HCV uptake in hepatocytes is still not understood, these four proteins may represent the complete minimal set of host cell factors necessary for cell-free HCV entry. Nevertheless, recent studies reported two receptor tyrosine kinases EGFR and ephrin receptor A2 (EphA2), the Niemann-Pick C1-like 1 cholesterol uptake receptor (NPC1L1), transferrin receptor 1 (TfR1), and CD63 as cellular cofactors for HCV adsorption and entry into hepatocytes (Lupberger 2011, Sainz 2012, Martin 2013, Park 2013).

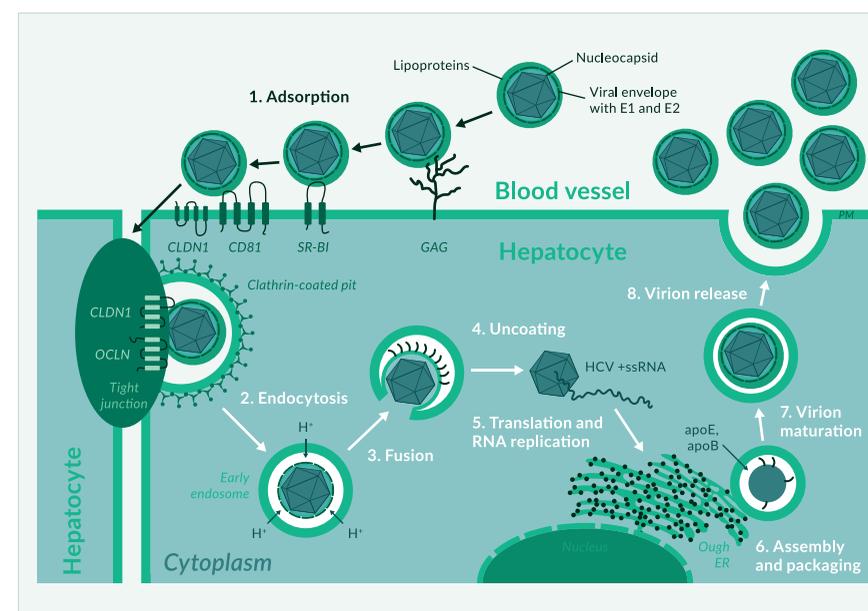


Figure 2. Current model of the HCV lifecycle. Designations of cellular components are in red. For a detailed illustration of viral translation and RNA replication, see Pawlowsky 2007. Abbreviations: HCV +ssRNA, single stranded genomic HCV RNA with positive polarity; rough ER, rough endoplasmic reticulum; PM, plasma membrane. For other abbreviations see text.

After the complex procedure of binding to the different host membrane factors HCV enters the cell in a pH-dependent manner indicating that the virus is internalised via clathrin-mediated endocytosis (Bartosch 2003b, Hsu 2003, Blanchard 2006, Codran 2006). The acidic environment within the endosomes is assumed to trigger HCV E1-E2 glycoprotein-mediated fusion of the viral envelope with the endosome membrane (Blanchard 2006, Meertens 2006, Lavillette 2007).

In summary, HCV adsorption and viral entry into the target cell is a very

complex procedure that is not yet fully understood. Despite having identified several host factors that probably interact with the viral glycoproteins, the precise mechanisms of interaction need to continue to be investigated.

Besides the infection of cells through cell-free HCV it has been documented that HCV can also spread via cell-to-cell transmission *in vitro* (Valli 2006, Valli 2007). This transmission pathway is dependent on several host factors that are also necessary for cell-free HCV infection, including SR-BI, CLDN1, OCLN, EGFR, EphA2, and NPC1L1. However the VLDL pathway, CD81, and TFR1 seem to be dispensable for cell-to-cell transmission in cultivated hepatoma cells (Witteveldt 2009, Barretto 2014). These findings require further investigation in order to analyse the process of cell-to-cell transmission of HCV both *in vitro* and *in vivo*. Antiviral treatment strategies must account for the cellular pathways of both cell-free virus and HCV transmitted via cell-to-cell contact. Cell-to-cell spread of HCV is very important particularly since this transmission route remains inaccessible to humoral immune responses as well as extracellular acting anti-HCV therapeutics.

Translation and post-translational processes

As a result of the fusion of the viral envelope and the endosomal membrane, the genomic HCV RNA is released into the cytoplasm of the cell. As described above, the viral genomic RNA possesses a non-translated region (NTR) at each terminus. The 5'NTR consists of four distinct domains, I-IV. Domains II-IV form an internal ribosome entry site (IRES) involved in ribosome-binding and subsequent cap-independent initiation of translation (Fukushi 1994, Honda 1999, Tsukiyama-Kohara 1992, Wang 1993). The HCV IRES binds to the 40S ribosomal subunit complexed with eukaryotic initiation factors 1A, 2, and 3 (eIF1A, eIF2 and eIF3), GTP and the initiator tRNA, resulting in the 48S preinitiation complex (Jaafar 2016, Spahn 2001, Otto 2002, Sizova 1998, reviewed in Hellen 1999). Subsequently, the 60S ribosomal subunit associates with that complex leading to the formation of the translational active complex for HCV polyprotein synthesis at the endoplasmic reticulum. HCV RNA contains a large ORF encoding a polyprotein precursor. Post-translational cleavages lead to the 10 functional viral proteins Core, E1, E2, p7, NS2-NS5B (see Figure 1B). The viral F protein (or ARF protein) originates from a ribosomal frameshift within the first codons of the core-encoding genome region (Walewski 2001, Xu 2001, Varaklioti 2002). Besides several other cellular factors that have been reported to be involved in HCV RNA translation, various viral proteins and genome regions have been shown to enhance or inhibit viral protein synthesis (Zhang 2002, Kato 2002, Wang 2005, Kou

2006, Bradrick 2006, Song 2006).

The precursor polyprotein is processed by at least four distinct peptidases. The cellular signal peptidase (SP) cleaves the N-terminal viral proteins' immature core protein, E1, E2, and p7 (Hijikata 1991), while the cellular signal peptide peptidase (SPP) is responsible for the cleavage of the E1 signal sequence from the C-terminus of the immature core protein, resulting in the mature form of the core (McLauchlan 2002). The E1 and E2 proteins remain within the lumen of the ER where they are subsequently N-glycosylated, with E1 having 5 N-glycosylation sites and E2 harbouring 11 putative N-glycosylation sites (Duvet 2002).

In addition to the two cellular peptidases HCV encodes two viral enzymes responsible for cleavage of the non-structural proteins NS2 to NS5B within the HCV polyprotein precursor. The zinc-dependent NS2/NS3 cysteine protease consisting of the NS2 protein and the N-terminal portion of NS3 autocatalytically cleaves the junction between NS2 and NS3 (Santolini 1995), whereas the NS3 serine protease cleaves the remaining functional proteins (Bartenschlager 1993, Eckart 1993, Grakoui 1993a, Tomei 1993). However, for its peptidase activity NS3 needs NS4A as a cofactor (Failla 1994, Tanji 1995, Bartenschlager 1995, Lin 1995, Tomei 1996).

HCV RNA replication

The complex process of HCV RNA replication is poorly understood. The key enzyme for viral RNA replication is NS5B, an RNA-dependent RNA polymerase (RdRp) of HCV (Behrens 1996). In addition, several cellular as well as viral factors have been reported to be part of the HCV RNA replication complex. One important viral factor for the formation of the replication complex appears to be NS4B, which is able to induce an ER-derived membranous web (MW) containing most of the non-structural HCV proteins including NS5B (Egger 2002). Further analyses revealed that the MW consists of rough ER, endosomes, mitochondria and cytosolic lipid droplets. The main MW-structures associated with HCV replicase activity are ER-derived protrusions called double membrane vesicles (DMV) which are inducible primarily by HCV NS5A (Romero-Brey 2012). Accordingly, DMV are proposed to be the cytosolic subsites of downstream processes during HCV RNA replication.

HCV NS5B uses the previously released genomic positive-strand HCV RNA as a template for the synthesis of an intermediate minus-strand RNA. After the viral polymerase has bound to its template, the NS3 helicase is assumed to unwind putative secondary structures of the template RNA in order to facilitate the synthesis of minus-strand RNA (Jin 1995, Kim 1995). In turn, again with the assistance of the NS3 helicase, the newly

synthesised antisense RNA molecule serves as the template for the synthesis of numerous plus-strand RNA. The resulting sense RNA may be used subsequently as genomic RNA for HCV progeny as well as for further polyprotein translation.

Using a single molecule HCV RNA detection assay it was shown recently that low level synthesis of single stranded (+) HCV RNA as well as (-) HCV RNA occurs within a few hours of infection and prior to formation of robust replication complexes (Shulla 2015). This indicates that initial HCV RNA replication may ensure sustained infection of the host cell independently of the continuous integrity of the infecting HCV RNA molecule.

Assembly and release

Viral assembly represents the steps of arranging structural viral (glyco) proteins, and the genomic HCV RNA in order to form infectious viral particles (reviewed in Lindenbach 2013).

As is the case for all other steps in the HCV lifecycle, viral assembly is a multi-step procedure involving most viral components along with many cellular factors. Previously it was reported that core protein molecules were able to self-assemble *in vitro*, yielding nucleocapsid-like particles. More recent findings suggest that viral assembly takes place within the ER (Gastaminza 2008) and that cytosolic lipid droplets (cLD) are involved in particle formation (Moradpour 1996, Barba 1997, Miyanari 2007, Shavinskaya 2007, Appel 2008). As one of the first steps of viral assembly it appears that newly synthesised HCV core molecules are relocated from the ER to cLD, where it homodimerises.

HCV NS5A is assumed to play a key role in discharging genomic HCV RNA from replication or translation to core-cLD-complexes (Appel 2008, Masaki 2008, Benga 2010).

Recent studies suggest that HCV NS2 as well as p7 may be coordinators of virion assembly via multiple interactions with several viral as well as host proteins, respectively (Jirasko 2010, Guo 2015). NS2 interacts with the viroporin p7. The resulting NS2-p7 complex is anchored in the ER-membrane with other domains localised in the cytosol. Subsequent cytosolic interaction of NS2-p7 with the NS3-NS4A enzyme complex is proposed to lead to detachment of core molecules from cLD to the site of budding into the ER (Counihan 2011) as well as to the packaging of genomic RNA. Finally, the NS2-p7 complex is presumably responsible for the transport from ER membrane-bound glycoproteins E1-E2 to the site of viral assembly. As a consequence all required components for HCV particle formation are now in close proximity and budding of the assembled structures into the ER occurs.

During the subsequent cellular secretory processes, HCV particles

experience maturation. This includes post-translational glycan modification as well as refolding by the formation of several disulfide bonds (Vieyres 2010). Furthermore, at this stage interaction of HCV particles with lipoproteins is suggested to occur.

Finally, infectious HCV virions are secreted from the plasma membrane.

Model systems for HCV research

For a long time HCV research was limited due to a lack of small animal models and efficient cell culture systems. The development of the first HCV replicon system (HCV RNA molecule, or region of HCV RNA, that replicates autonomously from a single origin of replication) 10 years after the identification of HCV offered the opportunity to investigate the molecular biology of HCV infection in a standardised manner (Lohmann 1999).

HCV replicon systems. Using total RNA derived from the explanted liver of an individual chronically infected with HCV genotype 1b, the entire HCV ORF sequence was amplified and cloned in two overlapping fragments. The flanking NTRs were amplified and cloned separately and all fragments were assembled into a modified full-length sequence. Transfection experiments with *in vitro* transcripts derived from the full-length clones failed to yield viral replication. For this reason, two different subgenomic replicons consisting of the 5' IRES, the neomycin phosphotransferase gene causing resistance to the antibiotic neomycin, the IRES derived from the encephalomyocarditis virus (EMCV) and the NS2/3'NTR or NS3/3'NTR sequence, respectively, were generated.

In vitro transcripts derived from these constructs without the genome region coding for the structural HCV proteins were used to transfect the hepatoma cell line Huh7 (Lohmann 1999). The transcripts are bicistronic, i.e., the first cistron containing the HCV IRES enables the translation of the neomycin phosphotransferase as a tool for efficient selection of successfully transfected cells and the second cistron containing the EMCV IRES directs translation of the HCV-specific proteins. Only some Huh7 clones can replicate replicon-specific RNA in titres of approximately 10^8 positive-strand RNA copies per microgram total RNA. Moreover, all encoded HCV proteins are detected predominantly in the cytoplasm of the transfected Huh7 cells. The development of this replicon was a milestone in HCV research with regard to the investigation of HCV RNA replication and HCV protein analyses.

More recently, the methodology has been improved in order to achieve significantly higher replication efficiency. Enhancement of HCV RNA replication was achieved by the use of replicons harbouring cell culture-adapted point mutations or deletions within the NS genes (Blight

2000, Lohmann 2001, Krieger 2001). Further development has led to the generation of selectable full-length HCV replicons, i.e., genomic replicons that also contain genetic information for the structural proteins Core, E1, and E2 (Pietschmann 2002, Blight 2002). This improvement offered the opportunity to investigate the influence of the structural proteins on HCV replication. Thus it became possible to analyse the intracellular localisation of these proteins although viral assembly and release has not been achieved.

Another important milestone was reached when a subgenomic replicon based on the HCV genotype 2a strain JFH-1 was generated (Kato 2003). This viral strain derived from a Japanese subject with fulminant hepatitis C (Kato 2001). The corresponding replicons showed higher RNA replication efficiency than previous replicons. Moreover, cell lines distinct from Huh7, such as HepG2 or HeLa were transfected efficiently with transcripts derived from the JFH-1 replicon (Date 2004, Kato 2005).

HCV pseudotype virus particles (HCVpp). The generation of retroviral pseudotypes bearing HCV E1 and E2 glycoproteins (HCVpp) offers the opportunity to investigate E1-E2-dependent HCVpp entry into Huh7 cells and primary human hepatocytes (Bartosch 2003a, Hsu 2003, Zhang 2004). In contrast to the HCV replicons where cells were transfected with HCV-specific synthetic RNA molecules, this method allows a detailed analysis of the early steps in the HCV life cycle, e.g., adsorption and viral entry.

Infectious HCV particles in cell culture (HCVcc). Transfection of Huh7 and 'cured' Huh7.5 cells with full-length JFH-1 replicons led for the first time to the production of infectious HCV virions (Zhong 2005, Wakita 2005). The construction of a chimera with the core NS2 region derived from HCV strain J6 (genotype 2a) and the remaining sequence derived from JFH-1 improved infectivity. Importantly, the secreted viral particles are infectious in cell culture (HCVcc) (Wakita 2005, Zhong 2005, Lindenbach 2005) as well as in chimeric mice with human liver grafts as well as in chimpanzees (Lindenbach 2006).

An alternative strategy for the production of infectious HCV particles was developed (Heller 2005): a full-length HCV construct (genotype 1b) was placed between two ribozymes in a plasmid containing a tetracycline-responsive promoter. Huh7 cells were transfected with those plasmids, resulting in efficient viral replication with HCV RNA titres of up to 10^7 copies/mL cell culture supernatant.

The development of cell culture systems that allow the production of infectious HCV represents a breakthrough for HCV research and it is now possible to investigate the whole viral life cycle from viral adsorption to virion release. These studies will help to better understand the mechanisms of HCV pathogenesis and they significantly accelerate the development of HCV-specific antiviral compounds. Nevertheless, hepatoma cell lines do not represent primary human hepatocytes, the host cells of HCV in the

liver. The most relevant biological differences between hepatoma cells and hepatocytes are the ongoing proliferation of hepatoma cells and some differences in cellular morphology. In contrast to hepatoma cells, primary hepatocytes are highly polarised cells that play an important role, e.g., in viral adsorption, entry, and release. Further efforts must be made to develop HCV replication systems reflecting *in vivo* conditions as realistically as possible.

Small animal models. Substantial progress was also achieved in establishing two mouse models for HCV infection via genetically humanised mice (Dorner 2011). In this experiment, immunocompetent mice were transduced using viral vectors containing the genetic information of four human proteins involved in adsorption and entry of HCV into hepatocytes (CD81, SR-BI, CLDN1, OCLN). This humanisation procedure enabled the authors to infect the transduced mice with HCV. Although this mouse model does not enable complete HCV replication in murine hepatocytes it will be useful to investigate the early steps of HCV infection *in vivo*. Moreover, the approach should be suitable for the evaluation of HCV entry inhibitors and vaccine candidates.

A second group of investigators have chosen another promising strategy for HCV-specific humanisation of mice. After depleting murine hepatocytes human CD34⁺ hematopoietic stem cells and hepatocyte progenitors were co-transplanted into transgenic mice leading to efficient engraftment of human leukocytes and hepatocytes, respectively (Washburn 2011). A portion of the humanised mice became infected with primary HCV isolates resulting in low-level HCV RNA in the murine liver. As a consequence HCV infection induced liver inflammation, hepatitis, and fibrosis. Furthermore, due to the co-transplantation of CD34⁺ human hematopoietic stem cells, an HCV-specific T cell immune response could be detected.

Both strategies are promising and have already delivered new insights into viral replication and the pathogenesis of HCV. However, the methods lack some important aspects and need to be improved. As soon as genetically humanised mice that are able to replicate HCV completely are created, they can be used for the investigation of HCV pathogenesis and HCV-specific immune responses. The Washburn method should be improved in order to achieve higher HCV replication rates. A reconstitution of functional human B cells would make this mouse model suitable to study the important HCV-specific antibody response.

Finally, a humanised mouse model that is able to produce infectious HCV accompanied by human-like HCV pathogenesis would be an ideal tool for preclinical monitoring of putative HCV-specific therapeutics and vaccines.

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7. Prophylaxis and vaccination

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Introduction

Understanding of the biology and modes of transmission of hepatitis viruses has significantly improved over the last decades. Even so, prophylactic vaccines are only available for hepatitis A (HAV) and B (HBV). Although an enormous amount of basic and clinical research has been performed in trying to develop a vaccine against hepatitis C (HCV), it is unlikely that either a prophylactic or therapeutic HCV vaccine will be available soon. A phase 3 vaccine trial against hepatitis E (HEV) in China resulted in the vaccine being licensed there; it is currently unknown whether or when this vaccine will become available in other countries. Prophylaxis of HCV, HDV (for patients) and HEV infection therefore involves avoiding the routes of exposure to the respective hepatitis viruses discussed in detail in *Chapters 1–4*.

Prophylaxis of hepatitis viruses

Hepatitis A and E

HAV and HEV are usually transmitted by oral ingestion of contaminated food or water. Thus, particular caution is warranted when individuals from low endemic areas such as Western Europe and the US travel to countries with a high prevalence of HAV and HEV. Several recent outbreaks of HEV infection have occurred in different regions of the world were associated with significant morbidity and mortality, e.g., the recent outbreak of HEV in refugee camps in South Sudan of more than 5000 acute jaundice cases within five months showed a fatality rate of about 10% in pregnant women (CDC 2013). In addition, HEV (but not HAV) can also be a zoonosis. Consumption of offal and wild boar is associated with a risk for HEV. This may have significant implications for immunosuppressed patients as cases of chronic HEV with the development of advanced fibrosis have been described in patients after organ transplantation (Wedemeyer 2012). HEV has frequently been detected in the pork and occupational exposure has frequently been identified as a risk factor for being anti-HEV positive (Pischke 2014). Importantly, zoonotic HEV is usually caused by HEV genotype 3 while HEV genotype 1 can be found in travel-associated HEV

(Wedemeyer 2012; Pischke 2014). It is important to note that HEV is heat sensitive (>70°C; >2 min) (Johns 2016). HAV (Hettman 2016) and HEV can also be transmitted by blood transfusion as recently confirmed in a large study from England screening more than 200,000 blood products (Hewitt 2014). Of note, up to 10% of pooled plasma products can contain HEV RNA in Europe. The overall relevance of HEV transmission by blood products is discussed in more detail in *Chapter 4*. Distinct genetic polymorphisms may be associated with the risk of becoming infected with HAV (Zhang 2012) and HEV (Wedemeyer 2012).

Hepatitis B and D

HBV and HDV were frequently transmitted by blood transfusion before HBsAg testing of blood products was introduced in the 1970s. Since then, vertical transmission and sexual exposure have become the most frequent routes of HBV infection. Medical procedures still represent a potential source for HBV and thus strict and careful application of standard hygienic precautions for all medical interventions are mandatory, and not only in endemic areas. This holds true in particular for immunocompromised individuals who are highly susceptible to HBV as HBV is characterised by a very high infectivity (Wedemeyer 1998). Moreover, immunosuppressed patients are at risk for reactivation of occult HBV after serological recovery from HBV. Treatments with high doses of steroids and rituximab have especially been identified as major risk factors for HBV reactivation (Loomba 2008). The FDA highlighted attention to the potential risk for fatal HBV reactivations in patients receiving B cell depleting therapies (Di Bisceglie 2014). However, also other immunosuppressive drugs may lead to increased HBV replication and thus all patients receiving immune modulating agents should be screened for HBsAg and anti-HBc. The need for pre-emptive antiviral differs according to the HBV serostatus (anti-HBs positive or negative, HBsAg positive or negative) and the level of immunomodulation induced by the respective drug (Perillo 2015).

After a new diagnosis of HBV, family members of the patient need to be tested for their immune status against HBV. Immediate active vaccination is recommended for contacts who are anti-HBc negative. HBsAg positive individuals should use condoms during sexual intercourse if it is not known if the partner has been vaccinated. Non-immune individuals who have experienced an injury and were exposed to HBsAg positive fluids should undergo passive immunisation with anti-HBs as soon as possible, preferentially within 2–12 hours (Cornberg 2011).

Hepatitis C

Less than 1% of individuals who are exposed to HCV by an injury with contaminated needles develop acute HCV infection. At Hannover Medical School, no HCV seroconversions occurred after 166 occupational exposures with anti-HCV positive blood over six years (2000–2005). A systematic literature review identified 22 studies including a total of 6956 injuries with HCV contaminated needles. Only 52 individuals (0.75%) became infected. The risk of acute HCV was lower in Europe at 0.42% compared to eastern Asia at 1.5% (Kubitschke 2007). Thus, the risk of acquiring HCV infection after a needle-stick injury is lower than frequently reported. Global differences in HCV seroconversion rates may suggest that genetic factors provide some level of natural protection. Indeed, distinct polymorphisms have been identified that are associated either with protection from HCV or with a higher likelihood of recovering spontaneously from acute HCV (Schaefer 2011). Factors associated with a higher risk of HCV transmission are likely to be HCV viraemia in the index patient, the amount of transmitted fluid and the duration between contamination of the respective needle and injury. Suggested follow-up procedures after needle stick episode include:

- Testing for HCV RNA immediately and an ALT testing.
- If possible, a HCV RNA quantification in the serum of index patient.
- There is no need for prophylactic treatment with IFN and ribavirin or direct acting antivirals.
- HCV RNA should be performed after 2 and 4 weeks; if the results are negative, HCV RNA testing should be repeated at weeks 6 and 8.
- After 12 and 24 weeks, anti-HCV and ALT levels should be determined; if the results are out of range or positive, HCV RNA testing should be performed.

Sexual transmission has clearly been identified as a risk for HCV, as about 10–20% of patients with acute HCV report this as having been a potential risk factor (Deterding 2009). However, there is also evidence that the risk of acquiring HCV sexually is extremely low in individuals in stable partnerships who avoid injuries: Cohort studies including more than 500 HCV positive patients followed over periods of more than four years could not identify any cases of confirmed HCV transmission. The risk for HCV transmission has recently been estimated to be about 1 per 190,000 sexual contacts (Terrault 2013). There was no association between specific sexual practices and HCV infection in monogamous heterosexual couples. Thus, current guidelines do not recommend the use of condoms in monogamous heterosexual relationships (EASL 2011). However, this does not hold true for HIV positive gay men. Several outbreaks of acute HCV have been described

in this population (Boesecke 2012, Bradshaw 2013). Transmission was associated with more sexual partners, increased levels of high-risk sexual behaviour (in particular fisting) and were more likely to have shared drugs via a nasal or anal route than controls.

Due to the low HCV prevalence in most European countries and a relatively low vertical transmission rate of 1–6%, general screening of pregnant women for anti-HCV is not recommended. Interestingly, transmission may be higher for girls than for boys (European Paediatric Hepatitis C Virus Network 2005). Transmission rates are higher in HIV positive women so pregnant women should be tested for HCV. Other factors possibly associated with high transmission rates are the level of HCV viraemia, maternal intravenous drug use, and the specific HLA types of the children. Immunoregulatory changes during pregnancy reduce the pressure by cytotoxic T cells which may select viruses with optimised replication fitness and thereby facilitate vertical transmission (Honegger 2013). Cesarean sections are not recommended for HCV RNA positive mothers as there is no clear evidence that these reduce transmission rates. It is not clear yet whether direct-acting antivirals (DAAs) against HCV can reduce transmission rates of HCV when given during the last trimester of pregnancy. HCV therapy should be considered in all HCV positive women who want to become pregnant (EASL 2017). Children of HCV positive mothers should be tested for HCV RNA after one month as maternal anti-HCV antibodies can be detected for several months after birth. Mothers with chronic HCV can breastfeed their children as long as they are HIV negative and do not use intravenous drugs (European Paediatric Hepatitis C Virus Network 2001, EASL 2011). This clinical recommendation is supported by experimental data showing inactivation of HCV by human breast milk in a dose dependent manner. Of note this effect is specific to human breast milk and the mechanism is destruction of the lipid envelope but not of viral RNA or capsids (Pfaender 2013).

Medical treatment still represents a risk factor for acquiring HCV. This has been demonstrated for Spain (Martinez-Bauer 2008), Italy (Santantonio 2006), France (Brouard 2008) and the US (Corey 2006). We have reported data from the German Hep-Net Acute HCV Studies and found 38 cases (15% of the entire cohort) of acute HCV patients who reported a medical procedure as the most likely risk factor for having acquired HCV (Deterding 2008, Deterding 2016). Thus, medical treatment *per se* still represents a significant risk factor for HCV – even in high-income countries. Strict adherence to universal precaution guidelines is urgently warranted.

HCV is surprisingly stable and can be infectious for at least six months if stored in liquids at 4° C (Ciesek 2010) and for up to three weeks in bottled water (Doerrbecker 2013). HCV is also associated with filter material used by people who inject drugs (Doerrbecker 2013). Moreover, HCV shows a prolonged survival in lipid-containing fluids such as propofol (Steinmann

2011). These findings demonstrate that it is critical to strictly follow hygienic standards in medical practice to prevent HCV transmission.

Vaccination against HAV

The first active HAV vaccine was licensed in 1995. The currently available inactive vaccines are manufactured from cell culture-adapted HAV, grown either in human fibroblasts or diploid cells (Nothdurft 2008). Two doses of the vaccine are recommended. The second dose should be given between 6 and 18 months after the first dose. All vaccines are highly immunogenic and all vaccinated healthy persons develop protective anti-HAV antibodies. Similar vaccine responses are obtained in both children and adults and no relevant regional differences in response to HAV vaccination have been observed. The weakest vaccine responses have been described for young children receiving a 0, 1 and 2 month schedule (Hammit 2008). Of note, maternal anti-HAV positive children vaccinated at age 6 months have lower vaccine responses and are less likely to maintain HAV antibodies through age 10 years (Spradling 2016). Patients with chronic liver disease do respond to vaccination but may display lower anti-HAV titres (Keeffe 1998). HAV vaccination in HIV positive people is more effective if HIV replication is already suppressed by antiretroviral therapy and patients have higher CD4+ T-cell counts (Tseng 2013). A combined vaccine against HAV and HBV is available that needs to be administered three times, on a 0, 1, and 6 months schedule. More than 80% of healthy individuals have detectable HAV antibodies by day 21 applying an accelerated vaccine schedule of 0, 7 and 21 days using the combined HAV/HBV vaccine, and all study subjects were immune against HAV by 2 months (Kallinowski 2003).

HAV vaccines are very well tolerated and no serious adverse events have been linked with the administration of HAV vaccines (Nothdurft 2008). The vaccine can safely be given together with other vaccines or immunoglobulins without compromising the development of protective antibodies.

Vaccination is recommended for non-immune individuals who plan to travel to endemic countries, medical health professionals, gay men, people in contact with patients with HAV, and individuals with chronic liver diseases. Some studies have suggested that patients with chronic HCV have a higher risk of developing fulminant HAV (Vento 1998), although this finding has not been confirmed by other investigators (Deterding 2006). The recommendation to vaccinate all patients with HCV against HAV has recently been challenged. A meta-analysis including studies on mortality from HAV in people with HCV revealed a number-needed-to-vaccinate to prevent one death of more than 800,000 (Rowe 2012), thus questioning the use of routine HAV vaccination in HCV positive people.

The implementation of childhood vaccination programmes has led to significant and impressive declines of HAV infections in several countries, justifying further efforts aiming at controlling the spread of HAV in endemic countries (Hendrickx 2008). It is important to highlight that most studies have confirmed that HAV vaccination is cost-effective (Rein 2008, Hollinger 2007).

Several long-term follow-up studies after complete HAV vaccinations have been published in recent years (Stuurman 2016). Anti-HAV titres usually decline during the first year after vaccination but remain detectable in almost all individuals for at least 10–15 years after vaccination (Van Herck 2011) which also has been confirmed by systematic reviews (Ott 2012). Based on these studies it was estimated that protective anti-HAV antibodies should persist for ≥ 30 years after successful vaccination (Hammit 2008, Bovier 2010, Spradling 2016).

A single dose administration of an inactivated HAV vaccine can induce protective antibody levels which can persist for more than 10 years. Thus, future research is needed to explore single dose vaccine approaches which would be cost-saving and increase overall vaccine coverage (Ott 2013).

Vaccination against HBV

The HBV vaccine was the first vaccine able to reduce the incidence of cancer. In Taiwan, a significant decline in cases of childhood hepatocellular carcinoma (HCC) has been observed since the implementation of programmes to vaccinate all infants against HBV (Chang 1997). This landmark study impressively highlighted the usefulness of universal vaccination against HBV in endemic countries. The findings were confirmed in various additional studies and a reduced incidence of HCC not only in infants but also in young adults has recently been shown in a 30 year follow-up of a randomised neonatal vaccination study (Qu 2014). Controversial discussions are ongoing regarding to what extent universal vaccination against HBV may be cost-effective in low-endemic places such as the UK, the Netherlands or Scandinavia (Zuckerman 2007). In 1992 the World Health Organization recommended general vaccination against HBV. It should be possible to eradicate HBV by worldwide implementation of this recommendation, because humans are the only epidemiologically relevant host for HBV.

The first plasma-derived HBV vaccine was approved by FDA in 1981. Recombinant vaccines consisting of HBsAg produced in yeast became available in 1986. In the US, two recombinant vaccines are licensed (Recombivax and Engerix-B) while additional vaccines are used in other countries. The vaccines are administered three times, on a 0, 1, and 6 month timetable.

Who should be vaccinated? The German Guidelines (Cornberg 2011)

**This list is based on the German Guidelines for Hepatitis B and can be considered as a recommendation for most countries.*

- HBV high-risk persons working in health care settings including trainees, students, cleaning personnel;
- Personnel in psychiatric facilities or comparable welfare institutions for cerebrally damaged or disturbed patients; other people who are at risk because of blood contact with people who are possibly infected depending on the risk evaluation, e.g., persons giving first aid professionally or voluntarily, employees of ambulance services, police officers, social workers, and prison staff who have contact with drug addicts;
- People with chronic kidney disease, dialysis patients, patients with frequent blood or blood component transfusions (e.g., haemophiliacs), patients prior to extensive surgery (e.g., before operations using heart-lung machine. The urgency of the operation and the patient's wish for vaccination protection are of primary importance);
- People with chronic liver disease including chronic diseases with liver involvement as well as HIV positive people without HBV markers;
- People at risk of contact with HBsAg carriers in the family or shared housing, sexual partners of HBsAg carriers;
- Patients in psychiatric facilities or residents of comparable welfare institutions for cerebrally damaged or disturbed persons as well as persons in sheltered workshops;
- Special high-risk groups, e.g., gay men who are sexually active men, people who inject drugs (PWID), sex workers, prisoners serving extended sentences;
- People at risk of being in contact with HBsAg carriers in facilities (kindergarten, children's homes, nursing homes, school classes, day care groups);
- People travelling to regions with high HBV prevalence for an extended period of time or with expected close contact with the local population;
- People who have been injured by possibly contaminated items, e.g., needle puncture (see post-exposition prophylaxis);
- Infants of HBsAg positive mothers or of mothers with unknown HBsAg status (independent of weight at birth) (see post-exposition prophylaxis);
- Routine testing for previous contact with HBV is not necessary before vaccination unless the person belongs to a risk group and may have acquired immunity against HBV before. Pre-vaccine testing is usually not cost-effective in populations with an anti-HBc prevalence below 20%. Vaccination of HBsAg positive individuals can be performed without any danger – however, it is ineffective.

Efficacy of vaccination against HBV

A response to HBV vaccination is determined by the development of anti-HBs antibodies, detectable in 90–95% of individuals one month after a complete vaccination schedule (Coates 2001). Responses are lower in elderly people and much weaker in immunocompromised persons such as organ transplant recipients, patients receiving haemodialysis and HIV positive individuals who have low CD4 counts. In case of vaccine non-response, another three courses of vaccine should be administered and the dose of the vaccine should be increased. Other possibilities to increase the immunogenicity of HBV vaccines include intradermal application and co-administration of adjuvants and cytokines (Cornberg 2011). The response to vaccination should be monitored in high-risk individuals such as medical health professionals and immunocompromised persons. Some guidelines also recommend testing elderly persons after vaccinations as vaccine response does decline more rapidly in the elderly (Wolters 2003).

Post-exposure prophylaxis

People who are not immune who have been in contact with HBV-contaminated materials (e.g., needles) or who have had recent sex with an HBV positive person should undergo active-passive immunisation (active immunisation plus HBV immunoglobulin) as soon as possible – preferentially within the first 48 hours of exposure to HBV. Individuals previously vaccinated but who have an anti-HBs titre of <10 IU/L should also be vaccinated both actively and passively. No action is required if an anti-HBs titre of >100 IU/L is documented; active vaccination alone is sufficient for persons with intermediate anti-HBs titres between 10 and 100 IU/L (Cornberg 2011).

Safety of HBV vaccines

Several hundred million individuals have been vaccinated against HBV. The vaccine is very well tolerated. Injection site reactions in the first 1 to 3 days and mild general reactions are common, although they are usually not long lasting. Whether there is a causal relationship between the vaccination and the seldom observed neurological disorders occurring around the time of vaccination is not clear. In the majority of these case reports the concomitant events most likely occurred coincidentally and are independent and not causally related. That HBV vaccination causes and induces acute episodes of multiple sclerosis or other demyelinating diseases

have been repeatedly discussed 10 to 15 years ago (Geier 2001, Hernan 2004, Girard 2005). However, there is no scientific proof of such a relationship. Numerous studies have not been able to find a causal relationship between the postulated disease and the vaccination (Sadovnick 2000, Monteyne 2000, Ascherio 2001, Confavreux 2001, Schattner 2005).

Long-term immunogenicity of HBV vaccination

Numerous studies have been published in recent years investigating the long-term efficacy of HBV vaccination. After 10 to 30 years, between one third and two thirds of vaccinated individuals have completely lost anti-HBs antibodies and only a minority maintain titres of >100 IU/L. However, in low/intermediate endemic countries such as Italy, this loss in protective humoral immunity did not lead to many cases of acute or even chronic HBV infection (Zanetti 2005). To what extent memory T cell responses contribute to a relative protection against HBV in the absence of anti-HBs remains to be determined. Nevertheless, in high-endemic countries such as Gambia, a significant proportion of vaccinated infants still seroconvert to anti-HBc indicating active HBV infection (18%) and some children even develop chronic HBV (van der Sande 2007). A very high efficacy of a single booster vaccine after 15 to 30 years has been shown in several studies (e.g. Su 2013, Bruce 2016) suggesting that immune memory is maintained in the majority of initial vaccine responders. However, protective titres are frequently lost again a few years after booster vaccination. Overall, these data indicate that no regular HBV booster doses are recommended in vaccine responders. Still, booster vaccinations should be considered in persons at risk including medical health professionals.

Prevention of vertical HBV transmission

Infants of HBsAg positive mothers should be immunised actively and passively within 12 hours of birth. This is very important as the vertical HBV transmission rate can be reduced from 95% to <5% (Ranger-Rogez 2004). Mothers with high HBV viraemia, of >200,000 million IU/mL, should receive in addition antiviral therapy with a potent HBV polymerase inhibitor (EASL 2012). Randomised trials showed that both tenofovir (Pan 2016) and telbivudine (Han 2011, Wu 2015) can reduce the risk for vertical HBV transmission when antiviral treatment is started during the third trimester of pregnancy. Tenofovir and telbivudine have been classified as Category B drugs by the FDA and can therefore be given during pregnancy as no increased rates of birth defects have been reported (FDA pregnancy

exposure registries 2013). If active/passive immunisation has been performed, there is no need to recommend cesarean section (Wong 2014). Mothers of vaccinated infants can breastfeed unless antiviral medications are being taken by the mother, which can pass through breast milk. If exposure to HBV polymerase inhibitors to infants by breast milk is associated with any specific risk is currently unknown.

Vaccination against HCV

There are no prophylactic or therapeutic vaccines against HCV. As reinfections after spontaneous or treatment-induced recovery from HCV infection have frequently been reported, the aim of a prophylactic vaccine would very likely be not to prevent completely an infection with HCV but rather to modulate immune responses in such a way that the frequency of evolution to a chronic state can be reduced (Torresi 2011).

HCV specific T cell responses play an important role in the natural course of HCV infection. The adaptive T cell response is mediated both by CD4+ helper T cells and CD8+ killer T cells. Several groups have consistently found an association between a strong, multispecific and maintained HCV specific CD4+ and CD8+ T cell response and the resolution of acute HCV infection (Rehermann 2013). While CD4+ T cells seem to be present for several years after recovery, there are conflicting data whether HCV specific CD8+ T cells responses persist or decline over time (Wiegand 2007). However, several studies have observed durable HCV specific T cells in HCV negative individuals who were exposed to HCV by occupational exposure or as household members of HCV positive partners, but who never became HCV RNA positive. A 10-year longitudinal study involving 72 healthcare workers showed that about half of the individuals developed HCV specific T cell responses detectable most frequently four weeks after exposure (Heller 2013). These observations suggest that HCV specific T cells may be induced upon subclinical exposure and may contribute to protection against clinically apparent HCV infection. However, it might also be that repeated subinfectious exposure to HCV may not protect from HCV but rather increase susceptibility by expansion of regulatory T cells which suppress effector T cells (Park 2013). T cell responses are usually much weaker in chronic HCV. The frequency of specific cells is low but also effector function of HCV specific T cells is impaired. Different mechanisms are discussed as being responsible for this impaired T cell function, including higher frequencies of regulatory T cells (Tregs), altered dendritic cell activity, upregulation of inhibitory molecules such as PD-1, CTLA-4 or 2B4 on T cells and escape mutations. HCV proteins can directly or indirectly contribute to altered functions of different immune cells (Rehermann 2013, Owusu Sekyere 2015).

To what extent humoral immune responses against HCV contribute to spontaneous clearance of acute HCV is less clear. Higher levels of neutralising antibodies early during the infection are associated with viral clearance (Pestka 2007). Antibodies with neutralising properties occur at high levels during chronic infection, although HCV constantly escapes these neutralising antibodies (von Hahn 2007). Yet, no completely sterilising humoral anti-HCV immunity exists in the long-term after recovery (Rehermann 2013). Attempts to use neutralising antibodies to prevent HCV reinfection after liver transplant have not been successful even though onset of viraemia may be delayed by administration of HCV antibodies (Gordon 2011, Chung 2013). Still, novel neutralising antibodies have been developed which also prevented HEV infection in a humanised mouse model of HCV infection (Desombere 2016). Furthermore, induction of neutralising antibodies by vaccination was possible with protected infection in mice (Li 2016).

Few phase I vaccine studies based either on vaccination with HCV peptides, HCV proteins alone or in combination with distinct adjuvants or recombinant viral vectors expressing HCV proteins have been completed (Torresi 2011). HCV specific T cells or antibodies against HCV were induced by these vaccines in healthy individuals. Particular broad, rather strong and sustained CD4 and CD8+ T cell responses could be induced by a vaccine based on human and chimpanzee adenoviruses expressing non-structural HCV proteins (Barnes 2012, Swadling 2014). Studies in chimpanzees have shown that it is likely that a vaccine will not be completely protective against heterologous HCV infections. However, a reasonable approach might be the development of a vaccine that does not confer 100% protection against acute infection but prevents progression of acute HCV to chronic infection. In any case, there are no vaccine programmes that have reached phase 3 yet (Halliday 2011). Therapeutic vaccination against HCV has also been explored (Klade 2008, Wedemeyer 2009, Torresi 2011). These studies show that induction of HCV specific humoral or cellular immune responses is possible even in chronically infected individuals. The first studies showed a modest antiviral efficacy of HCV vaccination in some patients (Sallberg 2009, Habersetzer 2011). Therapeutic vaccination was also able to enhance responses to interferon α and ribavirin treatment (Pockros 2010, Di Bisceglie 2014). However, even with potent viral-vector based vaccines, most patients do not restore HCV specific T cell immunity upon vaccination (Swadling 2016) – unless there is a mismatch between the endogenous virus and the vaccine (Kelly 2016). Considering the approval of extremely potent and safe direct acting antivirals against HCV, therapeutic vaccination is no longer explored as a treatment concept for chronic HCV.

Vaccination against HEV

A phase 2 vaccine trial performed in Nepal with 2000 soldiers showed a 95% efficacy for an HEV recombinant protein (Shrestha 2007). However, the development of this vaccine was stopped. In September 2010, data from a very large phase 3 trial were reported involving about 110,000 individuals in China (Zhu 2010). The vaccine efficacy of HEV-239 was 100% after three doses to prevent cases of symptomatic acute HEV. Further observation confirmed the ability of the vaccine to prevent clinical hepatitis. However, the induction of HEV antibodies does not induce sterilising immunity and thus does not completely protect from HEV infection. Still, vaccination largely reduces infection rates with a RR of 0.15 during further follow-up of the Chinese vaccine trial (Huang 2014). Similarly, naturally acquired immunity against HEV does not provide complete protection (Huang 2014). It remains to be formally determined if the HEV genotype 1-derived vaccine also prevents against zoonotic HEV genotype 3, while the vaccine was effective in China against HEV genotype 4. HEV-specific T cell immunity has been shown to be cross-HEV genotype-specific in patients with acute HEV (Gisa 2016). One can therefore assume that the vaccine should induce pan-genotypic immunity. Moreover, vaccine efficacy in special risk groups such patients with end-stage liver disease, immunocompromised individuals or elderly persons are unknown. Finally, the duration of protection needs to be determined as antibody titres have been shown to decline after vaccination (Shrestha 2007, Zhu 2010, Wedemeyer 2011). To what extent cellular immunity against HEV is important in the context of HEV vaccination is also unknown but HEV specific T cell response has been associated with the control of chronic (Suneetha 2012) and acute (Gisa 2016, Brown 2016) HEV infection. It is currently unknown if and when the vaccine HEV-239 will become available in other countries. Until then, preventive hygienic measures remain the only option to avoid HEV infection.

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8. Hepatitis B: diagnostic tests

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Introduction

The diagnosis of hepatitis B virus (HBV) infection was initiated by the discovery of the Australia antigen (Hepatitis B surface antigen, HBsAg). During the ensuing decades, serologic assays were established for HBsAg and other HBV antigens and antibodies. Advances in molecular biology techniques led to the development of polymerase chain reaction (PCR) assays for direct determination of hepatitis B virus DNA (HBV DNA).

Diagnosis of HBV infection tests for a series of serological markers of HBV and excludes alternative etiological agents such as hepatitis A, C, D, and E viruses. Serological tests are used to distinguish acute, self-limited infections from chronic HBV infections and to monitor vaccine-induced immunity. These tests are also performed to determine if the patient should be considered for antiviral therapy. Nucleic acid testing for HBV DNA is used as the standard to quantify HBV viral load and measures, together with HBV antigens and HBV antibodies, the effectiveness of therapeutic agents.

Other causes of chronic liver disease should be systematically looked for including coinfection with HCV, HDV, HEV or HIV. Cytomegalovirus, Epstein-Barr virus, enteroviruses, other hepatotoxic drugs, and even herbal medicines should be considered when appropriate. Moreover, comorbidities, including alcoholic, autoimmune and metabolic liver disease with steatosis or steatohepatitis should be assessed. Finally, vaccination status and previous test results should be used to guide appropriate testing.

Serological tests for HBV

Collection and transport

Serological tests for viral antigens can be performed on either serum or plasma (Yang 2002). The World Health Organization (WHO) has defined an international standard for normalisation of expression of HBV DNA concentrations already long time ago (Quint 1990). Serum HBV DNA levels should be expressed in IU/mL to ensure comparability; the same assay should be used in the same patient to evaluate antiviral efficacy. Both HBV antigens and antibodies are stable at room temperature for days, at 4°C for months, and frozen at -20°C to -70°C for many years. Care should be taken

to avoid hemolysis of the sample because it may interfere with the ability of the assay to accurately detect these markers. Vigilance must be taken to avoid the degradation of the viral nucleic acid in the specimen, which can result in falsely low or no measurable viral load. Serum should therefore be removed from clotted blood within 4 hours of collection and stored at -20°C to -70°C (Krayden 1998). Alternatively, the presence of EDTA in plasma is known to stabilise viral nucleic acids. EDTA blood can be stored for up to five days at 4°C without affecting the viral load. Polymerase chain reaction-based tests that are routinely used as standard can use either serum or plasma. In principle, the diagnosis of HBV infection can also be made by the detection of HBsAg or hepatitis B core antigen (HBcAg) in liver tissues by immunohistochemical staining.

Hepatitis B surface antigen and antibody

Hepatitis B surface antigen (HBsAg) is the serologic hallmark of acute and chronic HBV infection. The HBsAg level is a reflection of the transcriptional activity of the matrix of HBV infection, the covalently closed circular HBV DNA (cccDNA). It is an important marker that not only indicates active hepatitis B infection but can also predict clinical and treatment outcomes. It can usually be detected by chemiluminescent microparticle immunoassay (CMIA) technology. Immunoassays are standardised against the WHO international standard, relatively inexpensive, fully automated and express HBsAg titres in IU/mL.

HBsAg appears in serum 1 to 10 weeks after acute exposure to HBV, prior to the onset of hepatitis and elevation of serum alanine aminotransferase. HBsAg usually becomes undetectable after four to six months in patients who recover from hepatitis B. Persistence of HBsAg for more than six months implies chronic infection. It is estimated that about 5 percent of immunocompetent adult patients with genuine acute hepatitis B progress to chronic infection (Chu 1989). Among patients with chronic HBV infection, the rate of clearance of HBsAg is approximately 0.5 to 1 percent per year (Liaw 1991). The disappearance of HBsAg is frequent, but not always followed by the appearance of hepatitis B surface antibody (anti-HBs). In most patients, anti-HBs persists for decades, thereby conferring long-term immunity. The coexistence of HBsAg and anti-HBs has been reported in HBsAg positive individuals (Tsang 1986, Dufour 2000). In most instances, the antibodies are unable to neutralise the circulating virions. These individuals should therefore be regarded as carriers of the hepatitis B virus.

In recent years the quantification of HBsAg levels (qHBsAg) has become more important. Assays for qHBsAg are fully automated and have high output. The two most reported assays for the quantification of serum HBsAg

are the ARCHITECT HBsAg QT (Abbott Laboratories) and the Elecsys HBsAg II Quant (Roche Diagnostics). These assays detect all forms of circulating HBsAg: virion-associated as well as subviral filamentous and spherical particles (Bayliss 2013), possibly as well as HBsAg produced from integrated viral envelope DNA, which needs to be put into consideration in different clinical settings. qHBsAg titres are higher in HBeAg(+) than in HBeAg(-) patients and are negatively correlated with liver fibrosis in HBeAg(+) patients. In HBeAg(-) chronic hepatitis B, an HBsAg level <1000 IU/mL and an HBV DNA titre <2000 IU/mL accurately identifies inactive carriers (Brunetto 2010). During PEG-IFN treatment, HBsAg quantification is used as an on-treatment stopping rule to identify patients who will not benefit from therapy, and treatment may be stopped or switched at week 12 (EASL 2012). In contrast, in patients with nucleos(t)ide therapy the measurement of qHBsAg levels over time during antiviral therapy have not yielded definite answers yet in helping to distinguish patients that will clinically resolve chronic hepatitis B infection with HBsAg loss or seroconversion. Interestingly, stopping antiviral treatment in association with low HBsAg titres seems to be a new area of HBV related clinical research (Papatheodoridis 2016). In clinical practice, HBsAg quantification is a simple and reproducible tool that can be used in association with HBV DNA to classify patients during the natural history of HBV and to monitor therapy and the use of both parameters has been linked to the assessment of a ship's "longitude and latitude" position in the ocean (Martinot-Peignoux 2013).

Since most HBsAg assays relay on elisa technique sophisticated laboratory equipment is needed to perform the assay. To allow screening in resource limited settings, rapid diagnostic test (RDT) have been developed for the detection of HBsAg. The latest assays e.g. VIKIA HBsAg, Alere Determine HBsAg or DRW-HBsAg v2 assay showed in recent studies high sensitivity and were able to detect HBsAg mutation variants and might present in the future powerful tools for screening campaigns (Servant-Delmas 2015).

Hepatitis B core antibody

Hepatitis B core antibody (Anti-HBc) can be detected throughout the course of HBV infection in the serum and it appears after HBsAg.

During acute infection, anti-HBc is predominantly class IgM, which is an important marker of HBV infection during the window period between the disappearance of HBsAg and the appearance of anti-HBs. IgM anti-HBc may remain detectable for up to two years after acute infection. Furthermore, the titre of IgM anti-HBc may increase to detectable levels during exacerbations of chronic hepatitis B (Maruyama 1994). This can present a diagnostic

problem, incorrectly suggesting acute hepatitis B. Other common causes of acute exacerbation of chronic hepatitis B are superinfection with hepatitis D virus (delta virus) or hepatitis C virus. IgG anti-HBc persists along with anti-HBs in patients who recover from acute hepatitis B. It also persists in association with HBsAg in those who progress to chronic HBV infection.

Isolated detection of anti-HBc can occur in three settings: during the window period of acute hepatitis B after disappearance of HBsAg when the anti-HBc is predominantly IgM; many years after recovery from acute hepatitis B when anti-HBs has fallen to undetectable levels; and after many years of chronic HBV infection when the HBsAg titre has decreased to below the level of detection.

HBV DNA can be detected in the liver of most persons with isolated anti-HBc. Transmission of HBV infection has been reported from blood and organ donors with isolated anti-HBc. There are, in a small percentage of cases, false positive isolated anti-HBc test results.

The evaluation of individuals with isolated anti-HBc should include repeated testing for anti-HBc, HBsAg, anti-HBe, and anti-HBs. Those who remain isolated anti-HBc positive should be tested for the presence of IgM anti-HBc to rule out recent HBV infection. Individuals with evidence of chronic liver disease should be tested for HBV DNA to exclude low-level chronic HBV infection.

Hepatitis B e antigen and antibody

Hepatitis B e antigen (HBeAg) is a secretory protein processed from the precore protein. It is generally considered to be a marker of HBV replication and infectivity. HBeAg to anti-HBe seroconversion occurs early in patients with acute infection, prior to HBsAg to anti-HBs seroconversion. However, HBeAg seroconversion may be delayed for years to decades in patients with chronic HBV infection. In such patients, the presence of HBeAg is usually associated with the detection of high levels of HBV DNA in serum and active liver disease and is associated with higher rates of transmission of HBV infection. However, HBeAg positive patients with perinatally acquired HBV infection may have normal serum ALT concentrations and minimal inflammation in the liver (Chang 1988).

Seroconversion from HBeAg to anti-HBe is usually associated with a decrease in serum HBV DNA and remission of liver disease. However, some patients continue to have active liver disease after HBeAg seroconversion. Such individuals may have low levels of wild type HBV or HBV variants with a stop codon in the precore or dual nucleotide substitutions in the core promoter region that prevent or decrease the production of HBeAg (Carman 1989).

Serum HBV DNA assays

Qualitative and quantitative tests for HBV DNA in serum have been developed to assess HBV replication. Currently, most HBV DNA assays use real-time PCR techniques, report results in IU/mL, have a lower limit of detection of up to 9 IU/mL and a range of linearity of up to 9 log₁₀ IU/mL.

Recovery from acute hepatitis B is usually accompanied by the disappearance of HBV DNA in serum. However, HBV DNA may remain detectable in serum for many years if tested by PCR assays (Cornberg 2011) suggesting that the replication machinery of the virus persists but is controlled by the immune system (occult infection with low amounts of HBV DNA in the absence of HBsAg).

In patients with spontaneous or treatment-induced HBeAg seroconversion in chronic hepatitis B, PCR assays may remain positive except in patients with HBsAg loss or seroconversion. By contrast, most patients who develop HBeAg seroconversion during nucleos(t)ide analogue therapy have undetectable serum HBV DNA. In fact, many patients receiving nucleos(t)ide analogue therapy remain HBeAg positive despite having undetectable serum HBV DNA for months or years. The explanation for this phenomenon is likely related to the lack of direct effect of nucleos(t)ide analogues on cccDNA and viral RNA transcription and viral protein expression.

HBV DNA levels are also detectable in patients with HBeAg negative chronic hepatitis, although levels are generally lower than in patients with HBeAg positive chronic hepatitis. Because of the fluctuations in HBV DNA levels there is no absolute single cutoff level that is reliable for differentiating patients in the inactive carrier state from those with HBeAg negative chronic hepatitis B (Chu 2002).

HBV genotypes

HBV can be classified actually into ten genotypes (A to J) and four major serotypes with approximately 4 and 8% intergroup nucleotide divergence across the complete genome.

Genotypes A-D, F, H, and I are classified further into subgenotypes (Kramvis 2014). There have been reports about differing therapeutic responses with nucleos(t)ide analogues and interferon α with respect to different genotypes giving a greater chance of therapeutic response with IFN in genotype A. Furthermore, some genotypes, such as B and C, may have a greater risk for the development of hepatocellular carcinomas. HBV genotyping can be determined using several methods; most diagnostic laboratories use commercial available line probe assays (e.g. Inno-Lipa[®]), or Sanger sequencing but other assays such as reverse hybridisation,

restriction fragment length polymorphism (RFLP), -specific PCR assays, sequence analysis, microarray (DNACHIP), real time PCR and fluorescence polarisation assay (Villar 2015) can be used. Nevertheless, in contrast to hepatitis C, the diagnosis of HBV genotypes in the clinical setting is not routine (Thursz 2011).

Antiviral resistance testing

Hepatitis B virus (HBV) mutations associated with resistance to HBV drugs arise frequently, and these can sometimes lead to treatment failure and progression to advanced liver disease. Considerable research is focused into the mechanisms of resistance to nucleos(t)ides and the selection of mutants. The genes that encode the polymerase and envelope proteins of HBV overlap, so resistance mutations in the polymerase usually affect the hepatitis B surface antigen; these alterations affect infectivity, vaccine efficacy, pathogenesis of liver disease, and transmission throughout the population (*see Chapter 2*). Associations between HBV genotype and resistance phenotype have allowed cross-resistance profiles to be determined for many commonly detected mutants, so genotyping assays can be used to adapt therapy. *In vitro* phenotyping procedures are established in a rather small number of HBV laboratories and are not commercially available. Known mutations can be detected by commercially available tests (line probe assay e.g. Inno-Lipa®) with a threshold of about 5% or by Sanger sequencing of the viral polymerase gene with a threshold of about 20%. Determination of novel mutations remains for research-oriented labs with full-length sequencing methods or novel ultra-deep next generation sequencing techniques (NGS) that allow detection of mutants with threshold below 1%.

Future markers that are not yet in clinical practice

HBV core-related antigen (HBcrAg)

The so-called hepatitis B core-related antigen (HBcrAg) assay utilizes a mixture of monoclonal antibodies isolated from HBV core antigen – immunized mice to detect and quantify HBV core antigen (HBcAg), free HBeAg, HBeAg-antibody complex, and the 22kDa precore protein (p22cr). HBcrAg can be measured using a commercially available chemiluminescent enzyme immunoassay and the levels are quantified in U/ml (Lumipulse, Belgium). The assay's measurement linear range spans from 3 to 7logU/ml

but shows some sensitivity issues below 3log U/ml; samples above 7log U/ml need to be diluted. Several reports suggest that HBcrAg levels correlate with serum HBV-DNA in untreated patients with CHB and might be useful to differentiate HBeAg negative patients with active and inactive disease. A correlation between HBcrAg levels and the size of the intrahepatic cccDNA pool has been suggested in cohorts of patients with genotype B/C, either untreated or undergoing NA treatment. HBcrAg has been also shown to correlate with intrahepatic viral RNA levels in Asian patients treated with NAs (reviewed in Charre 2019). A recent study found that HBcrAg is strongly correlated with total HBV DNA, cccDNA and pgRNA levels both in HBeAg positive and HBeAg negative patients. CccDNA transcriptional activity, calculated by pgRNA/cccDNA ratio, was only correlated to HBcrAg, but not to qHBsAg in HBeAg negative patients, suggesting that HBcrAg might be a better surrogate marker of cccDNA transcriptional activity than qHBsAg. Patients who were negative for HBcrAg (<3log U/ml) showed less liver cccDNA and lower cccDNA activity than HBcrAg positive patients. Furthermore, several recent studies (mainly in Asian patients) could link high or increasing HBcrAg levels with an elevated risk for HCC development (Tada 2016, Honda 2016). With more studies needed ahead, HBcrAg could become useful in the evaluation of new antiviral therapies aiming at a functional cure of HBV infection either by directly or indirectly targeting the intrahepatic cccDNA pool (Testoni 2019).

Quantitative HBV-RNA in serum

Another interesting marker is the quantitative detection of HBV-RNAs in serum. HBV RNAs can be detected in patients' sera at levels ranging between 0.1 and 1% of HBV-DNA levels in the absence of antiviral treatment. Several observations suggest a wide heterogeneity of circulating HBV RNA species that may vary depending on different stages of chronic HBV infection. Methods of quantification of serum HBV RNAs differ according to studies. There are two main strategies to detect and quantify HBV transcripts. One is based on 3'end amplification that amplifies the majority of HBV transcripts (except truncated RNAs) as they share the same 3'end. Quantification of circulating truncated RNAs by assays using a primer in the cryptic polyA signal site could reflect the transcriptional activity of viral integration. The second strategy is based on 5' amplification specifically targeting 3.5kb transcripts, pgRNA and and preC mRNA. For testing, low input volumes are needed; however, their sensitivity remains to be improved: currently, the LOD ranges from 1.85log copies/ml to 3.4 log copies/ml, and there is currently no direct standardization of HBV RNA quantification. (reviewd in Charre 2019). Several studies could show that encapsidated and enveloped HBV-RNA can be detected in serum of chronic HBV infected patients in high

quantity (Wong 2016, van Bömmel 2015). Furthermore, HBV-RNA in serum is highly correlated with intrahepatic pregenomic RNA as a surrogate for active cccDNA in NA or pegylated IFN treated and untreated conditions (Giersch 2017). In line with this data, patients with favourable response during pegylated IFN or nucleos(t)ide analogue treatments (van Bömmel 2015, Jansen 2016) could be predicted by quantitative monitoring HBV-RNA in serum. Only few studies so far have shown a moderate correlation of RNAs with qHBsAg in pretreated patients, in HBeAg neg patients there was only a weak correlation (Liu 2019). The loss of serum HBV RNAs may reflect the transcription silencing of cccDNA and may be an indicator for safe withdrawal from antiviral treatment. Theoretically, the most promising area for clinical application of serum HBV RNAs might be the prediction of relapse and sustained response, especially HBsAg loss after treatment discontinuation, but further studies are needed: for example, to better understand how serum HBV RNAs, specifically pgRNA, is released from hepatocytes, in the virion or naked capsid? Is the egress mechanism different between HBV DNA containing particles and RNA containing particles? Taken together, these data indicate that HBV-RNA might become an important marker for the monitoring of intrahepatic replication during antiviral treatments in clinical practice in the near future.

Quantitative anti-HBcAg

Finally, higher anti-HBc levels detected by quantitative measurements of anti-HBcAg was hypothesised to reflect a stronger host-adaptive anti-HBV immunity (Yan 2013). Interestingly, recent studies (in Asian patients) could show that quantitative level of anti-HBc is a new additional predictor of pegylated IFN and nucleos(t)ide analogue treatments efficacy in HBeAg positive patients and might be used for pretreatment stratification (Fan 2016).

Assessment of liver disease

As a first step, the causal relationship between HBV infection and liver disease has to be established and an assessment of the severity of liver disease needs to be performed. Not all patients with chronic HBV infection have persistently elevated aminotransferases. Patients in the immune-tolerant phase have persistently normal ALT levels and a proportion of patients with HBeAg negative chronic HBV may have intermittently normal ALT levels. Therefore appropriate, longitudinal long-term follow-up is crucial.

The assessment of the severity of liver disease should include: biochemical markers, including aspartate aminotransferase (AST) and

alanine aminotransferase (ALT), gammaglutamyl transpeptidase (GGT), alkaline phosphatase, prothrombin time and serum albumin, blood counts, and hepatic ultrasound. Usually, ALT levels are higher than AST. However, when the disease progresses to cirrhosis, the ratio may be reversed. A progressive decline in serum albumin concentrations and prolongation of the prothrombin time, often accompanied by a drop in platelet counts, are characteristically observed once cirrhosis has developed (EASL 2012).

Acute HBV infection

The diagnosis of acute HBV is based upon the detection of HBsAg and IgM anti-HBc. During the initial phase of infection, markers of HBV replication, HBeAg and HBV DNA, are also present. Recovery is accompanied by the disappearance of HBV DNA, HBeAg to anti-HBe seroconversion, and subsequently HBsAg loss or seroconversion to anti-HBs.

The differential diagnosis of HBsAg positive acute hepatitis includes acute HBV, exacerbations of chronic HBV, reactivation of chronic HBV, superinfection of a hepatitis B carrier with hepatitis C or D virus (Tassopoulos 1987), and acute hepatitis due to drugs or other toxins in an HBV carrier.

Past HBV infection

Previous HBV infection is characterised by the presence of anti-HBs and/or IgG anti-HBc. Immunity to HBV infection after vaccination is indicated by the presence of anti-HBs only.

HBsAg

- If negative, acute HBV infection is ruled out (Dufour 2000).
- If positive, the patient is infected with HBV. A repeat test six months later will determine if the infection has resolved or is chronic.

Anti-HBs

- If negative, the patient has no apparent immunity to HBV.
- If positive, the patient is considered immune to HBV (either because of resolved infection (anti-HBc positive) or vaccination (anti-HBc negative)).

Anti-HBc IgM

In rare cases, anti-HBc immunoglobulin M (IgM) may be the only HBV marker detected during the early reconvalescence or 'window period' when the HBsAg and anti-HBs tests are negative. Because current tests for HBsAg are very sensitive, an anti-HBc IgM that is typically positive with acute HBV infection is not generally required to diagnose active infection. Because some chronic HBV carriers remain anti-HBc IgM positive for years, epidemiological information is necessary to confirm that the infection is indeed acute. A negative anti-HBc IgM in the presence of a positive HBsAg suggests that the infection is likely chronic. For these reasons, routine testing for anti-HBc IgM is not generally recommended to screen for acutely infected patients.

Chronic HBV infection

Chronic HBV infection is defined by the continued presence of HBsAg in the blood for longer than six months. Additional tests for HBV replication, HBeAg and serum HBV DNA, should be performed to determine if the patient should be considered for antiviral therapy. In addition HDV coinfection needs to be ruled out by testing for anti-HDV. All patients with chronic HBV infection should be regularly monitored for progression of liver disease because HBV DNA and ALT levels vary during the course of infection. In addition, patients who are not candidates for treatment at the time of presentation may become candidates for treatment during follow-up.

HBeAg negative patients who have normal serum ALT and low (<2000 IU/mL) or undetectable HBV DNA are considered to be in an inactive carrier state. These patients generally have a good prognosis and antiviral treatment is not indicated. However, serial tests are necessary to accurately differentiate them from patients with HBeAg negative chronic hepatitis who have fluctuating ALT and/or HBV DNA levels (Lok 2007). Patients who are truly inactive carriers should continue to be monitored but at less frequent intervals. HBeAg negative patients with elevated serum ALT concentrations should be tested for serum HBV DNA to determine if the liver disease is related to persistent HBV replication.

HBsAg

- If negative, chronic HBV infection is typically ruled out.
- If positive, the patient is considered HBV infected. Chronic infection is diagnosed when the HBsAg remains detectable for more than six months.

Antibody to hepatitis B core protein

- If negative, past infection with HBV is typically ruled out.
- If positive, the patient has been infected with HBV. Infection may be resolved (HBsAg negative) or ongoing (HBsAg positive). If the infection is resolved, the person is considered naturally immune to HBV infection.

Antibody to hepatitis B surface protein

- If negative, the patient has no apparent immunity to HBV.
- If positive, the patient is considered immune to HBV (either because of resolved infection or as the result of prior vaccination). Very rarely (less than 1%) chronic carriers can be positive for HBsAg and antibody to hepatitis B surface protein (anti-HBs) at the same time (Tsang 1986, Dufour 2000). In such cases, the patient is considered infectious.

Serum transaminases

Once an individual has been diagnosed with chronic HBV infection, follow-up testing must be performed for alanine aminotransferase (ALT), a marker of liver cell inflammation. Repeat periodic testing is indicated because the ALT levels can fluctuate (e.g., from less than the upper limit of normal to intermittently or consistently elevated). Sustained and intermittent elevations in ALT beyond the upper limit of normal are indicative of hepatic inflammation and correlate with an increased risk of progressive liver disease. It must be noted that the normal ALT ranges are both age and sex dependent and, occasionally, individuals with severe liver disease may not manifest elevated ALT (Cornberg 2011, EASL 2012).

Occult HBV infection

This is defined as the presence of detectable HBV DNA by PCR in patients who are negative for HBsAg. Most of these patients have very low or undetectable serum HBV DNA levels accounting for the failure to detect HBsAg. Infections with HBV variants that decrease HBsAg production or have mutations in the S gene with altered S epitopes evading detection in serology assays for HBsAg are uncommon. HBV DNA is often detected in the liver and transplantation of livers from these persons can result in *de novo* HBV infection (Margeridon-Thermet 2009).

Assessment of HBV immunity

Immunity to HBV is acquired from a resolved infection or from vaccination. The HBV vaccine has been shown to induce protective immunity in 90% to 95% of vaccinees. Most vaccinees will have protective levels of anti-HBs for 5 to 10 years after vaccination, although the exact duration of immunity remains undefined.

Anti-HBs

- If the anti-HBs level is less than 10 mIU/mL, this implies that the person is not immune to HBV. In individuals who have received a complete course of HBV vaccine, the level of anti-HBs may drop to less than 10 mIU/mL after five to 10 years, but these individuals might still be considered to be immune, based on their vaccination history (Maruyama 1994). In clinical practice, these individuals should receive a booster vaccination.
- If the anti-HBs result is greater than 10 mIU/mL, the person is considered to be immune. Immunity may be due to immunisation or resolved natural infection. These two states can be distinguished by testing for antibody to hepatitis B core protein (anti-HBc), which is present in subjects that have had HBV infection but absent in vaccinees (see below)

Anti-HBc

- If the anti-HBc total test is positive, this is compatible with current or resolved HBV infection. A negative HBsAg confirms a resolved infection. HBV vaccination does not induce anti-HBc.

Liver biopsy and noninvasive liver transient elastography

Liver biopsy is still the standard procedure for determining the degree of necroinflammation and fibrosis since hepatic morphology can assist the decision to start treatment. Biopsy is also useful for evaluating other possible causes of liver disease such as fatty liver disease. Although liver biopsy is an invasive procedure, the risk of severe complications is low. It is important that the size of the needle biopsy specimen be large enough to accurately assess the degree of liver injury and fibrosis. A liver biopsy is usually not required in patients with clinical evidence of cirrhosis or in those in whom treatment is indicated irrespective of the grade of activity or the stage of fibrosis.

There is growing interest in the use of noninvasive methods, including serum markers and transient elastography, to assess hepatic fibrosis to

complement or avoid a liver biopsy. Transient elastography offers high diagnostic accuracy for the detection of cirrhosis, although the results may be confounded by severe inflammation associated with high ALT levels and the optimal cut-off of liver stiffness measurements for HBV varies among studies (Cornberg 2011, EASL 2012, Terrault 2016, *see also Chapter 17*).

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9. Treatment of hepatitis B infections

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Introduction

Individuals with hepatitis B virus (HBV) infection carry a significantly increased risk of life-threatening complications such as hepatic decompensation, liver cirrhosis and hepatocellular carcinoma (HCC) (Beasley 1988). The goal of treatment of chronic HBV infection is to improve survival of the infected person by preventing progression to liver cirrhosis or end-stage liver disease, HCC and death; and prevention of transmission of HBV to others (EASL 2017, Terrault 2016, Sarin 2016). Long term observational studies of the natural course of HBV infections have shown that the level of serum HBV DNA correlates higher with the risk of developing late sequel as cirrhosis and HCC as compared to other patient or virus related factors (Chen 2006, Iloeje 2006) (Figure 1). Moreover, it has now become apparent that deep and continuous suppression of HBV replication can revert liver fibrosis or even cirrhosis in most patients (Chan 2010b, Schiff 2011, Marcellin 2013).

Three categories of cure have been defined for HBV infections: virological, functional (also referred to as immunologic cure), and partial cure. Virological cure means the suppression of the replication of HBV to undetectable levels, and it has become the major goal in treatment of chronic HBV infections. HBeAg seroconversion is another treatment endpoint, provided that HBV replication remains durably suppressed to low levels. The loss of HBsAg or HBeAg seroconversion to anti-HBs can be considered as stable remission of HBV infections and it is often referred to as functional cure. However, this goal is difficult to achieve by available treatment options. Complete clearance of HBV from an infected liver is impossible or achieve by current treatment options because even after HBeAg seroconversion the HBV infections will persist on cellular levels.

Two drug classes are available for the treatment of chronic HBV infections: the immune modulator interferon α (standard or pegylated (PEG)-INF α) as well as nucleoside or nucleotide analogues (NA), which act as reverse transcriptase inhibitors of the HBV polymerase. Currently, the nucleoside analogues lamivudine (LAM), telbivudine (LdT), entecavir (ETV) and the acyclic nucleotide analogues adefovir dipivoxil (ADV) and tenofovir in the two formulations tenofovir disoproxil fumarate (TDF) and tenofovir

alafenamide (TAF) are available. Due to this broad spectrum of therapeutic options and by employing laboratory tests, ultrasound and risk calculators, disease progression and complications can be prevented in many patients if the infection is diagnosed early and treated effectively. A number of regional and international treatment guidelines for HBV infections have been published over the last decade, each one reflecting regional differences in access to drugs, laboratory tests and health care. New therapies aiming at increasing the rate of HBsAg seroconversions or even eradicating chronic HBV infections continue to be developed, but yet in early stages of clinical studies.

Goals of treatment

Due to persistence of episomal covalently closed circular DNA (cccDNA), a template of the HBV genome located in the nucleus of infected hepatocytes, a complete eradication of HBV infection is currently impossible (Rehermann 1996). Reactivation of a HBV infection can occur in certain circumstances from these nuclear reservoirs even decades after HBsAg loss, for instance during immunosuppressive therapy. Accordingly, the aim of treatment of chronic hepatitis B is currently not to eradicate the infection but to reduce complications such as liver failure and HCC and to increase survival (EASL 2017, Cornberg 2011, KASL 2013, Terrault 2016, WHO 2015, Sarin 2016). In patients with acute hepatitis B, preventing the risk of acute liver failure is the main treatment goal. These aims can be approached by successful long term suppression of the HBV replication. Ideally, an immunologic control over the infection can be achieved. Immunologic control includes serologic response, which is characterised by seroconversion of HBeAg to anti-HBe or by loss of HBsAg, as well as a stage of low level replication during which, even in absence of serologic response, an indication for re-treatment is not given. This goal can be referred to as sustained immune control.

To determine the success of antiviral therapy surrogate markers are used during and after treatment. These parameters include virologic parameters (serological status of HBeAg and HBsAg, HBsAg levels, HBV DNA levels) and patient-related parameters (aminotransferases, liver histology).

Table 1. Scoring systems for the assessment of individual risk of HCC development during long term treatment with NAs

Score	Patients evaluated	Included parameters	Cut off (points)	Performance
CU-HCC	Asian patients: 1005 in training and 424 in validation cohort	Age, albumin, bilirubin, HBV DNA, cirrhosis	5	97% NPV over 10 years
GAG-HCC	Asian patients (n=820)	Age, sex, HBV DNA, cirrhosis	101	99% NPV over 10 years
REACH-B	Asian non-cirrhotic patients: 3584 in trainings and 1505 validation cohort	Age, sex, ALT, HBV DNA, HBeAg status	8	98% NPV over 10 years
PAGE-B	European patients: 1325 in training and 490 in validation cohort	Age, sex, platelets	< 6	100 % NPV over up to 5 years

Endpoints of treatment

Suppression of HBV replication. In two large long-term studies a close correlation of baseline HBV DNA levels and subsequent disease progression was demonstrated. In the REVEAL study, 3774 untreated HBV-infected individuals were followed over a mean time period of 11.4 years in Taiwan (Chen 2006, Iloeje 2006). HBV DNA levels at baseline were the strongest predictors of cirrhosis and HCC development (Figure 1). In multivariate models, the relative risk of cirrhosis increased when HBV DNA reached levels greater than 300 copies/mL, independent of whether patients were negative or positive for HBeAg. In addition, individuals with HBV DNA levels $\geq 10^4$ copies/mL (or $\geq 2,000$ IU/mL) were found to have a 3-15 fold greater incidence of HCC as compared to those with a viral load $< 10^4$ copies/mL. On the other hand, suppression of HBV DNA over some years shows a time dependent reversion of liver fibrosis as well as a decrease of the HCC risk.

The regression of liver fibrosis during antiviral treatment was impressively demonstrated in a subanalysis of two trials evaluating 348 patients who underwent biopsies before and after five years of TDF monotherapy (Figure 2) (Marcellin 2013). Of those patients, 88% experienced an improvement in overall liver histology as measured by an improvement of at least two points in the Knodell score of HAI (histologic activity index) (Figure 5). Of the 94 patients who had cirrhosis at the start of therapy, 73% experienced regression of cirrhosis, and 72% had at least a two-point reduction in fibrosis scoring. The positive effect of and effective antiviral treatment on liver histology was also shown in a subgroup of 59 patients from a rollover study including two phase III trials on the efficacy of ETV in treatment-naïve patients. Liver biopsies taken at baseline and

after a median treatment duration of 6 years (range, 3-7 years) showed a histologic improvement, defined as a decrease of 2 points or greater in the Knodell necroinflammatory score in absence of worsening of the Knodell fibrosis score in 96% of patients. In addition, an improvement of more than 1 point in the Ishak fibrosis score was seen in 88% of patients, including all 10 patients who had advanced fibrosis or cirrhosis when they entered the phase III studies (Chang 2010a).

The decrease of HCC incidence in HBV infected individuals during antiviral treatment was illustrated by results of a retrospective analysis comparing HBV infected individuals receiving with those not receiving antiviral treatment between 1997 and 2010 in Taiwan (Wu 2014). Among the patients receiving treatment with NAs, the incidence rate of HCCs over 7 years of follow up was 7.3 % as compared to 22.9% among patients without antiviral treatment. However, the HCC risk is not affected immediately after the initiation of antiviral treatment. Thus, the incidence of HCCs was shown to start decreasing after 5 years of effective HBV DNA suppression by either entecavir or tenofovir, and after eight years of treatment it was similar to individuals without HBV infection in a multicentric European cohort (Papatheodoridis 2017, Papatheodoridis 2018). The presence of liver cirrhosis strongly determines the remaining HCC risk. However, also patients with liver cirrhosis show a decreasing incidence in HCC development during treatment (Su 2016). Overall, these data indicate that with potent NAs HCC risk can be reduced but not eliminated.

Estimating the individual risk for HCC development during effective long term treatment with NAs is currently an important challenge for the treating physician. Several scoring systems have been proposed including the CU-HCC-, GAG-HCC- und REACH-B-Score. A comparison of the performance of these three scores in Asian individuals receiving ETV for the treatment of chronic HBV infections found them to be equally precise in predicting HCC development (Wong 2013). For European individuals, the PAGE-B score, which is based on different parameters, seems to allow a more precise prediction as compared to the other scores (Papatheodorides 2014). An overview of the different scoring systems is given in table 1. Although the interpretation of the cut off results of those scores and the corresponding management strategies have not definitely been defined yet, those risk calculators can be used for evidence-based personalised tailoring of monitor algorithms of chronically HBV infected individuals.

Based on these observations, a durable suppression of HBV replication monitored by HBV DNA levels in serum has become the most urgent aim in the treatment of HBV infections (Cornberg 2011, EASL 2017, Terrault 2016, Sarin 2016, WHO 2015). Treatment is recommended to be continued until one of the following end-point has been achieved:

HBeAg seroconversion. In HBeAg positive patients, seroconversion

from HBeAg to anti-HBe was found to be a reliable surrogate marker for prognosis of chronic HBV infection leading in many cases to an inactive HBsAg carrier state (Figure 3). In these patients, HBsAg remains detectable but HBV replication continues at low or even undetectable levels and transaminases are generally within normal ranges.

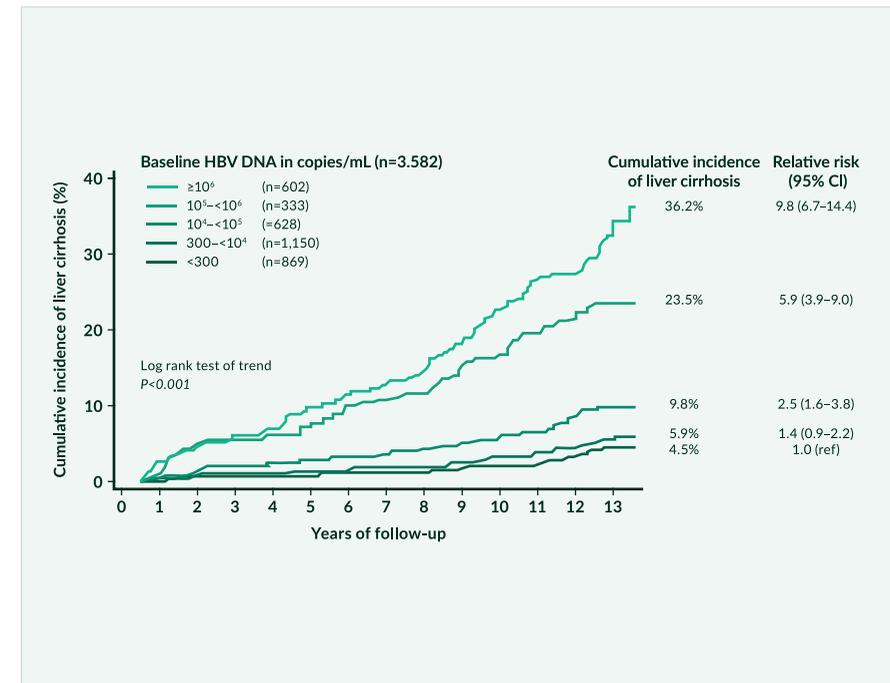


Figure 1. Cumulative incidence of liver cirrhosis in untreated HBV-infected individuals within a mean observation period of 11.4 years (REVEAL Study). The incidence of liver cirrhosis increases over time depending on baseline HBV DNA levels (Iloeje 2006). The relative risk for developing HCC was 1.4 in patients with HBV DNA levels of 300 to 1,000 and increased to 2.4 in patients with 1,000-10,000 to 5.4 in patients with 10,000 to 100,000 and to 6.7 in patients with HBV DNA levels >1 million copies/mL. A similar association between HBV DNA levels and the risk of HCC development was shown (Chen 2006).

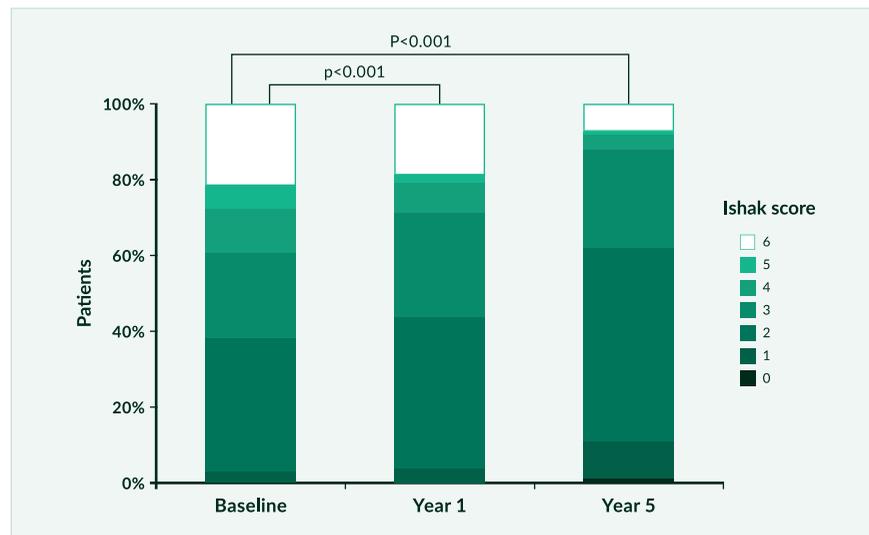


Figure 2. Changes in liver histology after five years of treatment with TDF. In a study looking at 348 patients with paired liver biopsies, regression of liver fibrosis and even liver cirrhosis (Ishak score 5 and 6) was found in the majority of patients (adapted from Marcellin 2013). A similar extent of the regression of liver fibrosis was observed during up to seven years of treatment with ETV (Chang 2010a).

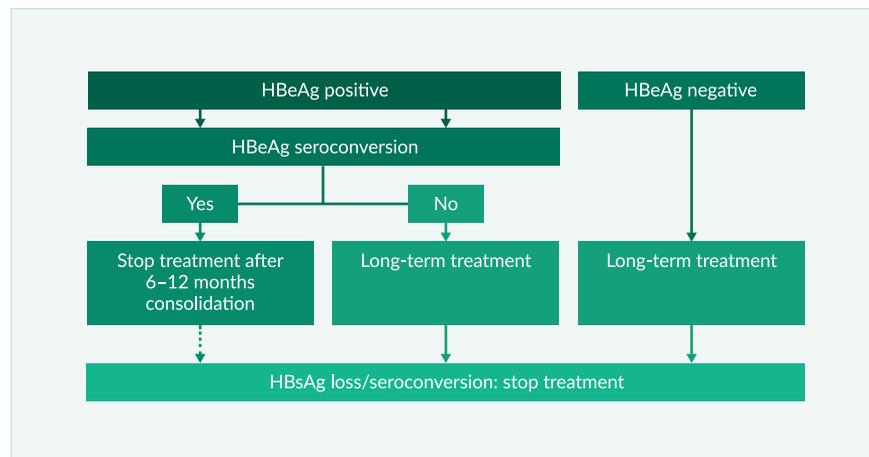


Figure 3. Possible endpoints of treatment of chronic Hepatitis B. After achieving HBeAg seroconversion or HBsAg loss or seroconversion, antiviral treatment can be stopped. However, it is recommended to maintain treatment for a period of 6-12 months after HBeAg or HBsAg seroconversion (modified from [EASL 2017]).

HBeAg seroconversions that appear during antiviral treatment can be considered as a lasting immune response in the majority of patients. In a meta-analysis in 76% of patients achieving HBeAg seroconversion this was stable after treatment discontinuation (Papatheodoridis 2016). On the other hand, long-term observations reveal that HBeAg seroconversion cannot always be taken as a guarantee of long-term remission. A reactivation of the

disease with “sero-reversion” (HBeAg becoming detectable again) as well as a transition to HBeAg negative chronic hepatitis B with increased, often fluctuating, HBV DNA levels, can occur in 30-50% of patients (Hadziyannis 1995, Hadziyannis 2001, Hadziyannis 2006, van Hees 2018). Therefore, HBeAg seroconversion should only be regarded as a treatment endpoint in conjunction with durable and complete suppression of HBV replication.

The age of an individual in which an HBeAg seroconversion occurs seems to have an important influence on the development of complications of HBV infections. In a recent long-term observational study in 483 HBeAg positive patients achieving spontaneous HBeAg seroconversion, it was shown that over 15 years after HBeAg seroconversion the incidence of cirrhosis and HCC was lower for patients who had achieved HBeAg seroconversion at an age <30 years old compared to patients achieving seroconversion at an age >40 years old (Chen 2010). This observation raises the question whether HBeAg seroconversions appearing during antiviral treatment in patients older than 40 years might also be associated with a higher remaining risk of complications compared to patients achieving HBeAg seroconversion at a younger age.

There is ongoing discussion whether and how long a consolidation treatment (6-12 months) should be maintained following HBeAg seroconversion. As a result, Asian guidelines recommend stopping treatment after HBeAg seroconversion, whereas American and European guidelines favour treatment continuation, but allow discontinuation in selected patients with close subsequent monitoring.

HBsAg loss. Because HBsAg loss or seroconversion is associated with a complete and definitive remission of the activity of chronic hepatitis B and an improved long-term outcome, it is currently regarded as stable remission of HBV infections or a “functional cure”, although HBV cccDNA still persists in infected hepatocytes and reactivations may occur. Unfortunately, HBsAg loss can be induced in only a limited number of patients by treatment (in up to 10% of HBeAg positives and in <1% of HBeAg negatives). This is not improved even by long term treatment with NAs up to eight years (Marcellin 2014). The probability of HBsAg seroclearance during therapy with NAs is linked to a decrease in HBsAg levels during the early treatment period. As HBsAg levels remain unchanged in most patients during the first years of treatment it seems therefore unlikely that a longer duration of NA treatment will further increase rates of HBsAg losses (Figure 4) (Marcellin 2011).

Sustained immune control. The term “sustained immune control” can be used to describe a stage that follows the discontinuation of treatment for hepatitis B and applies to both, NA or PegIFN based treatments. Sustained immune control and it should be equated with “absence of virological treatment indication” and refers to a stage with low HBV replication (ideally < 2,000 IU/mL) and normal ALT levels in spite of detectable HBsAg (and possibly

HBeAg). However, durability of this immune control is not guaranteed due to the fluctuating course of HBeAg negative chronic hepatitis B.

For treatment with PEG-IFN α in both, HBeAg positive and -negative patients, inducing an immune control status, characterised by persistent suppression of viral replication with HBV DNA levels $<2,000$ IU/mL and normalisation of ALT levels was defined as another, combined treatment endpoint (Marcellin 2009). If this condition is maintained over time, it increases the probability of HBsAg loss and reduces the development of liver fibrosis and HCC. Late relapse beyond 6 months post-treatment has been described, but a sustained response at 1 year post-treatment appears to be durable through long-term follow-up (Marcellin 2009). However, the immune control status needs to be regularly monitored, and treatment needs to be re-introduced in case of increase of HBV replication. Immune control defined as “absence of treatment indication” was recently shown to be an important end point after discontinuation of long term antiviral treatment in HBeAg negative patients (Berg 2017). For patients presenting any signs of liver fibrosis or family history of HCC, immune control should not be regarded as the treatment endpoint but rather the complete suppression of HBV replication.

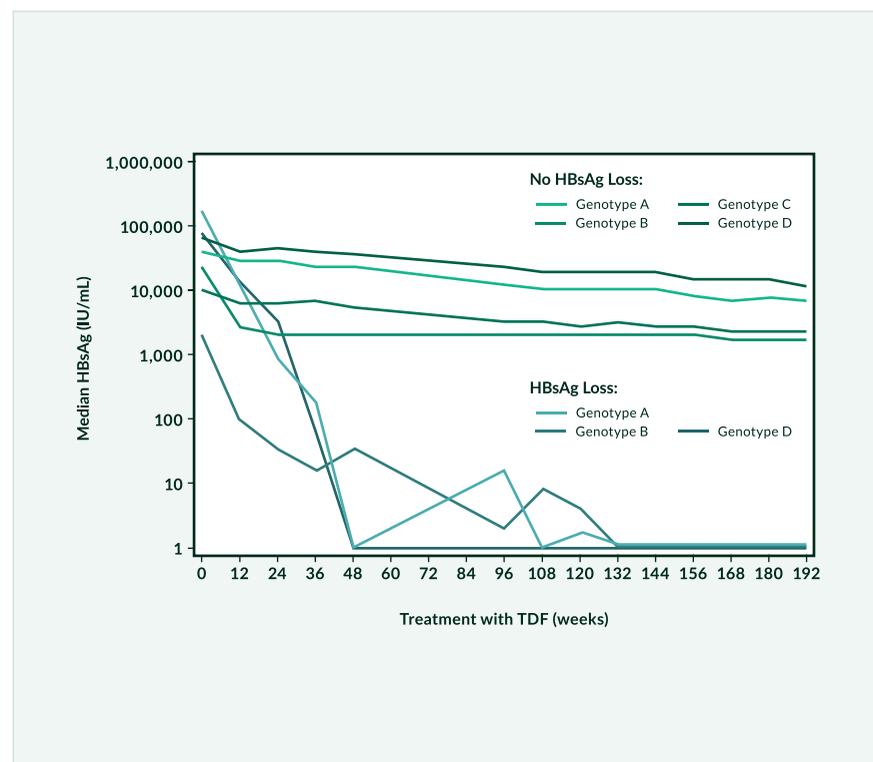


Figure 4. Patients in the TDF studies 102 and 103 who lost HBsAg showed a significant decline in HBsAg levels already in the early treatment phase (Marcellin 2011).

Criteria for treatment response

Virologic response

- Sustained decrease of HBV DNA, to at least $<2,000$ IU/mL (corresponding to $<10,000$ copies/mL), ideally to <60 IU/mL (<300 copies/mL).
- Sustained HBeAg seroconversion in HBeAg positive patients.
- Ideally, loss of HBsAg with or without appearance of anti-HBs.

Biochemical response

- Sustained ALT normalisation.

Histologic response

- Reduction of fibrosis (histological staging).
- Reduction of inflammatory activity (histological grading).

Potential long-term effects

- Avoidance of cirrhosis, hepatocellular carcinoma (HCC), transplantation, and death.

Indication for antiviral therapy

Treatment of acute hepatitis B

Acute hepatitis B resolves spontaneously in 95-99% of cases (McMahon 1985, Tassopoulos 1987, Liaw 2009). Therefore, treatment of acute HBV infections with the currently available drugs is generally not indicated. In patients with a potentially life-threatening disease course as severe or fulminant acute hepatitis B antiviral treatment should, however, be considered as there are observations suggesting that antiviral treatment might reduce mortality in patients experiencing fulminant hepatitis during acute HBV infection. Thus, in a trial comparing treatment with LAM 100 mg/day versus no treatment in 80 Chinese patients with fulminant hepatitis B, a mortality of 7.5% was found in patients receiving LAM treatment compared to 25% in the control group ($p=0.03$) (Yu 2010). The result of this and another study also demonstrated that the earlier the treatment was initiated, the better were the results obtained (Kumar 2007). A rapid decline of HBV DNA load was a good predictor for treatment outcome (Kumar 2007). In contrast, a lower mortality was not seen when antiviral therapy was initiated late in the course of severe acute hepatitis B in patients with already manifested acute liver failure and advanced hepatic encephalopathy (Wang 2014). Several case reports from Europe also indicate that patients with severe and fulminant hepatitis B may benefit from early antiviral therapy with LAM or other NAs by reducing the need for high-urgency liver transplantation (Tillmann 2006). As a result, antiviral treatment of fulminant or severe

acute hepatitis B with NA is recommended by current treatment guidelines (Terrault 2015, EASL 2017, WHO 2015, Sarin 2016). Interferon therapy is contraindicated in patients with acute HBV infection because of the risk of liver failure by increasing the inflammatory activity of the HBV infection (Tassopoulos 1997). The endpoint of treatment of acute HBV infections is HBsAg clearance (EASL 2017, WHO 2015, Sarin 2016).

Table 2. Key guideline recommendations for indication for antiviral treatment of HBV infection

AASLD (Terrault 2015)	<p>Consider treatment:</p> <ul style="list-style-type: none"> Normal ALT and HBV DNA >1,000,000 IU/mL, >40 years of age with liver biopsy showing significant necroinflammation or fibrosis HBeAg(+): HBV DNA >20,000 IU/mL + ALT ≤2x ULN + biopsy shows moderate/severe inflammation or significant fibrosis HBeAg(+): HBV DNA >20,000 IU/mL + ALT >2x ULN HBeAg(-): HBV DNA >2,000 IU/mL + ALT >2x ULN Liver cirrhosis and HBV DNA > 2,000 IU/mL <p>Consider biopsy:</p> <ul style="list-style-type: none"> HBeAg(+): HBV DNA >20,000 IU/mL + ALT >2x ULN + compensated HBeAg(+): HBV DNA >20,000 IU/mL + ALT 1-2x ULN + age >40 years or family history of HCC HBeAg(-): HBV DNA >2000 to 20,000 IU/mL + ALT 1-2x ULN
APASL (Sarin 2016)	<p>Consider treatment:</p> <ul style="list-style-type: none"> All patients: HBV DNA detectable + moderate to severe inflammation or significant fibrosis HBeAg(+): HBV DNA >20,000 IU/mL + ALT >2x ULN HBeAg(-): HBV DNA >2,000 IU/mL + ALT >2x ULN Decompensated liver cirrhosis Compensated liver cirrhosis + HBV DNA >2,000 IU/mL (regardless of ALT levels)
EASL (EASL 2012)	<p>Consider treatment:</p> <ul style="list-style-type: none"> HBV DNA >2000 IU/mL + moderate to severe necroinflammation and/or ALT > ULN HBV DNA >20,000 IU/mL, ALT >2x ULN without liver histology
Belgian (Colle 2007)	<p>Consider treatment:</p> <ul style="list-style-type: none"> HBeAg(+): HBV DNA >20,000 IU/mL + ALT >2x ULN (or moderate/severe hepatitis on biopsy) HBeAg(-): HBV DNA ≥2,000 IU/mL and elevated ALT <p>Consider biopsy:</p> <ul style="list-style-type: none"> Fluctuating or minimally elevated ALT (especially in those older than 35-40 years)
Dutch (Buster 2008)	<p>Consider treatment:</p> <ul style="list-style-type: none"> HBeAg(+) and HBeAg(-): HBV DNA ≥20,000 IU/mL and ALT ≥2x ULN or active necrotic inflammation HBeAg(-): HBV DNA ≥2000 to 20,000 IU/mL and ALT ≥2x ULN (and absence of any other cause of hepatitis)
German (Cornberg 2011)	<p>Consider treatment:</p> <ul style="list-style-type: none"> BV DNA >2000 IU/mL + minimal inflammation/low fibrosis or ALT elevation

Italian (Carosi 2011)	<p>Consider treatment:</p> <ul style="list-style-type: none"> HBeAg(+): HBV DNA >20,000 IU/mL + ALT > ULN and/or METAVIR ≥ F2 or Ishak ≥ S3 HBeAg(-): HBV DNA >2,000 IU/mL + ALT > ULN and/or METAVIR ≥ F2 or Ishak ≥ S3 <p>Consider biopsy:</p> <ul style="list-style-type: none"> When fibrosis is suspected by non-invasive evaluation
Turkish TASL (Akarca 2008)	<p>Consider treatment:</p> <ul style="list-style-type: none"> HBV DNA >2,000 IU/mL + histological fibrosis >2 HBV DNA >20,000 IU/mL + any histological finding + ALT >2x ULN
Korean (KASL 2012)	<p>Consider treatment:</p> <ul style="list-style-type: none"> HBeAg(+): HBV DNA >20,000 IU/mL + ALT >2x ULN or ALT 1-2x ULN and moderate-to-severe degree of inflammation or periportal fibrosis HBeAg(-): HBV DNA >2000 IU/mL + ALT >2x ULN or ALT 1-2x ULN and moderate-to-severe degree of inflammation or periportal fibrosis
WHO (2015)	<p>Consider treatment:</p> <ul style="list-style-type: none"> HBeAg(+) + HBeAg (-): HBV DNA >20,000 IU/mL + persistently abnormal ALT if HBV DNA measurement is unavailable: persistently abnormal ALT levels (regardless of HBeAg status) after exclusion of glucose tolerance, dyslipidaemia and fatty liver

For detailed recommendations please refer to the original publications.

Treatment of chronic hepatitis B

All individuals with detectable HBV DNA should be considered as potential candidates for antiviral therapy (EASL 2017, Terrault 2016, Chen 2006, Iloeje 2006). There is widespread agreement that the decision whether to initiate treatment should be made on the criteria: 1) serum HBV DNA levels, 2) ALT elevation and 3) histologic changes of liver tissue (Akarca 2008, Carosi 2011, Cornberg 2011, KASL 2012, EASL 2017, Terrault 2016, Sarin 2016). Indication for treatment should also take into account age, health status, family history of HCC or cirrhosis and extrahepatic manifestations. Differentiation between HBeAg positive and HBeAg negative chronic hepatitis B is not necessary anymore for treatment indication, although with respect to the choice of the appropriate antiviral drug (NAs vs. interferon α) these criteria may be still useful. Current recommendations for treatment indications may vary across different regions, and the criteria of different national and international societies are displayed in Table 1 (Akarca 2008, Carosi 2011, Colle 2007, Cornberg 2011, EASL 2017, Buster 2008, KASL 2012, Terrault 2016, WHO 2015, Sarin 2016). In comparison with previous recommendations, in most of these guidelines the most relevant factor for a decision to initiate treatment has shifted from histological proven

disease activity to the serum level of HBV DNA. Thus, most guidelines now recommend antiviral treatment for patients with HBV DNA levels >2,000 IU/mL (corresponding to >10,000 copies/mL) in association with a sign of ongoing hepatitis (elevated ALT levels or liver fibrosis demonstrated by liver histology greater than A1/F1 or, alternatively, non-invasive tools such as liver elastography or serologic algorithms such as fibrotest).

The question which indications are needed to start antiviral treatment in patients with liver cirrhosis is answered controversially across different treatment guidelines. Most guidelines agree upon recommending treatment for all patients with liver cirrhosis or high-grade liver fibrosis and any measurable HBV DNA (Cornberg 2011, KASL 2012, EASL 2017, Sarin 2016). The WHO guidelines recommend treatment initiation in all patients with cirrhosis, even if the HBV DNA level is low or undetectable (WHO 2015), which is a more practical approach for settings in which quantification of HBV DNA is unavailable. In other treatment guidelines antiviral treatment is recommended for patients with chronic HBV infection and compensated cirrhosis, if HBV DNA levels are greater than 2,000 IU/mL, regardless of ALT levels (Terrault 2016, Sarin 2016). The decision path for antiviral treatment proposed by the German guidelines is depicted in Figure 2 (Cornberg 2011). In patients with decompensated cirrhosis with Child-Pugh score B or C, standard or pegylated interferon- α is contraindicated. For patients without liver cirrhosis, the treatment indication is linked to disease stages:

Inactive chronic HBsAg carriers (or patients with HBeAg negative HBV infection), characterised by negative HBeAg and positive anti-HBe, HBV DNA levels <2,000 IU/mL and serum aminotransferases within normal ranges do not have an indication for antiviral therapy (Brunetto 2011, EASL 2017, WHO 2015, Terrault 2016, Sarin 2016). However, differentiation between true inactive HBsAg carriers and patients with chronic HBeAg negative hepatitis may be difficult in some cases and HBV DNA needs to be monitored on a regular basis. Elevated transaminases are no reliable parameter for assessing the stage of liver fibrosis and long-term prognosis of HBV-infected patients. On the other hand, even in patients with normal or slightly elevated aminotransferases there can be a significant risk for the development of HBV associated complications (Chen 2006, Iloeje 2006, Kumar 2008). HBsAg levels may be helpful to predict reactivation of HBV replication and inflammatory activity (Martinot-Peignoux 2013). It is reasonable to assess fibrosis progression by non-invasive methods or to perform a liver biopsy in these individuals and to control the levels of HBV DNA and ALT at three-month intervals and start treatment if moderate inflammation or advanced fibrosis becomes evident (Table 1).

HBV immune tolerant patients (or patients with HBeAg positive HBV infection) are mostly under 30 years old and can be recognised by their high HBV DNA levels, detectable HBeAg, normal ALT levels and minimal or absence of significant

histological changes. The risk of disease progression in these individuals is very low (Tseng 2015). According to most practice guidelines, immediate therapy is not required as long as severe fibrosis development can be ruled out (Akarca 2007, Balik 2008, Carosi 2008, Buster 2008, Cornberg 2011, EASL 2017, KASL 2012, Terrault 2016, Sarin 2016). The WHO guidelines represent an exemption in this point as they do not advice antiviral treatment in immunotolerant individuals only up to an age of 30 years. Indeed, regarding the long lead in time for HCC development, it can be assumed that immune tolerant HBV with elevated risk for HCC development such as positive family history and patients from high endemic areas like East Asia or Africa may benefit from early antiviral therapy despite normal ALT levels. Treatment with either TDF or a combination of TDF plus emtricitabine was recently shown to be equally effective in suppressing HBV replication in in Asian immune tolerant patients with high-level viraemia (Chan 2014). However, the HBeAg loss rates were only 2-6% after 195 weeks of treatment and thus lower as in immune active patients. Studies are under way to further clarify this issue, especially to answer the question whether early intervention with antiviral therapy will positively influence the long-term risk for HCC.

Table 3. Indication for antiviral treatment according to the EASL guidelines for the treatment of chronic HBV infection (EASL 2017).

HBeAg positive or negative chronic hepatitis B	HBV DNA >2,000 IU/mL, ALT >ULN and/or at least moderate liver necroinflammation or fibrosis	→	Treatment
	HBV DNA >20,000 IU/mL and ALT >2 x ULN regardless of fibrosis	→	
HBeAg positive chronic infection	>30 years	→	
HBV infection and cirrhosis	Any detectable HBV DNA level	→	
Family history of HCC or cirrhosis; extrahepatic manifestations	Any detectable HBV DNA level	→	Treatment possible, even if typical indications not fulfilled
<i>Special populations:</i>			
HBV infected individuals undergoing immunosuppression	HBsAg positive	→	ETV or TDF or TAF as prophylaxis*
	HBsAg negative, anti-HBc positive	→	NA prophylaxis if high risk of HBV reactivation*
HBV infection in pregnancy		→	NA treatment if benefit outweighs risk**

ULN = upper limit of normal; *=for details see [Mallet 2016]; **for details see [EASL 2017]

How to treat

Therapy of chronic Hepatitis B is possible with either PEG-INF α or with NA. (Figure 6).

The option of PEG-INF α treatment should be considered for all patients. However, if a patient does not fulfil the criteria for a higher likelihood of response to treatment with PEG-INF α , has contraindications, or is intolerant to PEG-INF α , long-term therapy with an NA is recommended (Figure 6). If a NA is chosen, several parameters have to be considered prior to therapy: the antiviral efficacy of the drug, the resistance barrier, potential side effects and the stage of liver disease. The preferred regimens are ETV, TDF or TAF as monotherapies as first line treatment as recommended in guidelines due to their strong antiviral efficacy and low rate (ETV) or to date even absence (TDF, TAF) of reported resistance (EASL 2017, WHO 2015, Terrault 2016, Sarin 2016). LAM, ADV and LdT are still licensed, but due to their weaker antiviral performance and substantial risk of resistance development no longer recommended for treatment of hepatitis B (EASL 2017).

However, if the initial viral load is low and liver cirrhosis has been excluded, any approved NA may be used for treatment. (Table 3).

Treatment options

Currently available drugs for the treatment of HBV infections are listed in table 4. Because of a limited tolerability due to adverse events, duration of treatment with PEG-INF α via subcutaneous injection is limited to a period of up to 48 weeks. NAs are orally administered and can achieve suppression of HBV DNA in almost all patients, but they have to be used for an undefined period unless one of the endpoints is achieved (see above). Planned discontinuation of long term NA treatment represents a novel approach to induce immune control in HBeAg negative patients. The efficacy of NAs can be hampered by emergence of HBV resistance. Response rates during treatment with different drugs are shown in Figure 7.

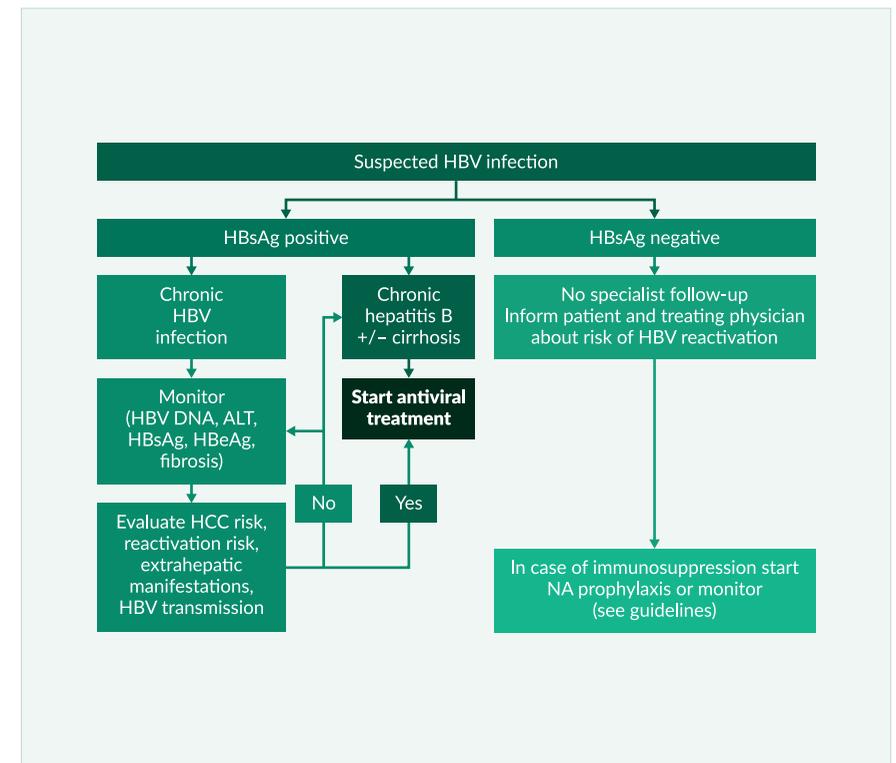


Figure 5. Treatment algorithm for chronic HBV infection according to the EASL Guidelines 2017. For treatment with nucleoside or nucleotide analogues, agents with high genetic barrier against resistance such as entecavir or tenofovir should preferably be chosen (modified from EASL 2017).

Table 4. Recommendations for the use of nucleos(t)ide analogues in clinical practice

Drug	Advantage	Disadvantage	Recommendation
Lamivudine (LAM)	Low treatment costs Oral solution available for children or individual dosage in case of renal impairment	High risk of resistance in long-term monotherapy Cross-resistance to ETV and LdT	Use as first-line therapy only in selected patients with low viral load Use in pregnancy possible
Adefovir dipivoxil (ADV)	Experience in combination with LAM No cross-resistance to LAM	Moderate antiviral activity Primary non-response in 10–20% of cases Slow viral kinetics during therapy Risk of viral resistance in long-term monotherapy Nephrotoxicity	Not to be used as first-line or mono therapy
Telbivudine (LdT)	High antiviral efficacy No cross-resistance to entecavir	Moderate risk for viral resistance in long-term monotherapy Neuropathy and myopathy	First-line therapy Can be combined with TDF
Entecavir (ETV)	High antiviral efficacy Low risk for viral resistance in long-term monotherapy in lamivudine-naïve patients Combination therapy with TDF as rescue therapy Oral solution available for individual dosage in case of renal impairment	In LAM-experienced patients high risk for the development of viral resistance and virologic failure in long-term monotherapy	First-line therapy Can be combined with TDF Recommended for pre-emptive treatment in patients with immunosuppression
Tenofovir disoproxil fumarate (TDF)	High antiviral efficacy Low risk for viral resistance in long-term monotherapy Oral solution available for individual dosage in case of renal impairment	Rare Nephrotoxicity* Decrease in bone mineral density	First- and any second-line therapy Can be combined with ETV, LdT or LAM if needed Recommended for pre-emptive treatment in patients with immunosuppression
Tenofovir alafenamide (TAF)	Comparable antiviral efficacy as TDF in HBeAg positive and negative patients Smaller risk of bone density loss or kidney damage than TDF		First- and second line treatment for patients with compensated liver disease

* in HBV mono-infected patients no renal toxicity was observed in 8 years of TDF treatment

Table 5. Overview of currently approved drugs for the treatment of HBV infections*

Drug	Name	Dose	Duration
Interferon α			
Standard Interferon α -2a	Roferon®	2.5–5 mio. U/m ² body surface 3x/week	4–6 months
Standard Interferon α -2b	Intron A®	5–10 mio. IU 3x/week	4–6 months
Pegylated Interferon α -2a	Pegasys®	180 µg/week	48 weeks
Nucleoside analogues			
Lamivudine (LAM)	Epivir®, Zeffix®	100 mg/day	long-term
Telbivudine (LdT)	Tyzeka®, Sebivo®	600 mg/day	long-term
Entecavir (ETV)	Baraclude®	0.5 mg/day	long-term
		1 mg/day for patients with lamivudine resistance	long-term
Nucleotide analogues			
Adefovir dipivoxil (ADV)	Hepsera®	10 mg/day	long-term
Tenofovir disoproxil fumarate (TDF)	Viread®	300 mg/day	long-term
Tenofovir alafenamide (TAF)	Vemlidy®	25 mg/day	long-term

* generic drugs are not listed

Interferons

INF α is a natural occurring cytokine with immune modulatory, anti-proliferative and antiviral activity. During treatment, the therapeutic efficacy of INF α can often be clinically recognised by a self-limited increase of ALT levels to at least twice the baseline levels. These ALT flares are often associated with virologic response.

The main aim of INF α treatment is to induce a long-term remission after a finite treatment duration. Response to IFN α can be either HBeAg seroconversion or durable suppression of HBV DNA to low or undetectable levels. In these responders the chance for HBsAg loss in the long-term is relatively high.

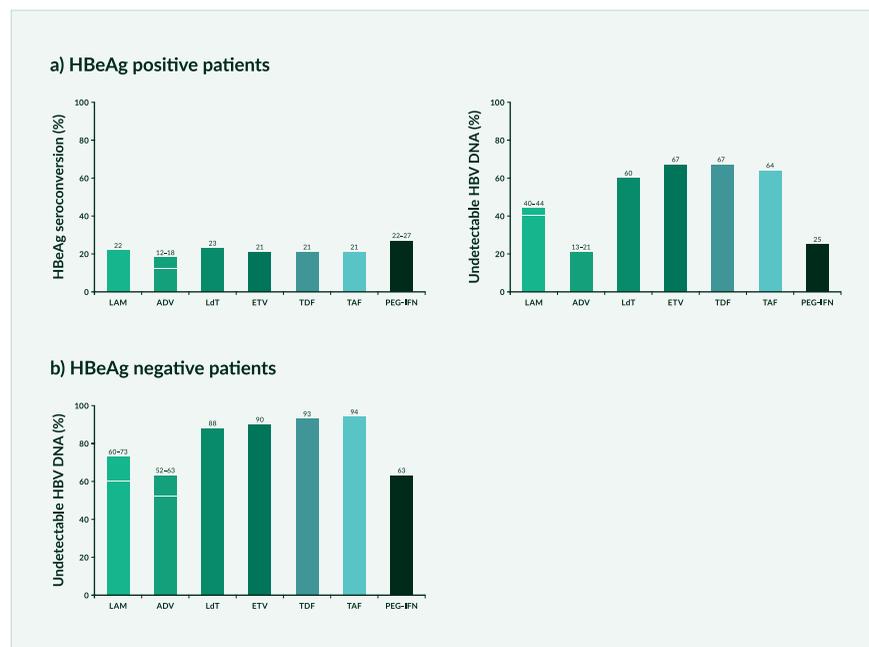


Figure 6. One-year efficacy of medications currently approved for the treatment of chronic HBV infection (Lok 2009; Buti 2016, Chan 2016). Treatment efficacy is expressed as suppression of HBV DNA below the limit of detection and rates of HBeAg seroconversion. As no head-to-head trials comparing the substances have been undertaken, differences in antiviral efficacy have to be interpreted with caution.

Standard IFN α . Standard IFN α was approved for treatment of chronic hepatitis B in 1992. IFN α is applied in dosages ranging from 5 million units (MU) to 10 MU every other day or thrice weekly. In a meta-analysis, a significant improvement in endpoints was shown in patients with HBeAg positive chronic hepatitis B being treated with standard IFN compared to untreated patients (Craxí 2003). Complete remission of fibrotic changes was observed in some patients and the loss of HBsAg occurred comparatively often. Furthermore, there was a trend towards reduction of hepatic decompensation (treated 8.9% vs. untreated 13.3%), hepatocellular carcinoma (1.9 vs. 3.2%), and liver associated deaths (4.9 vs. 8.7%) (Craxí 2003).

A significant decrease in ALT and in HBV DNA serum levels was also shown for standard IFN α in the treatment of HBeAg negative chronic hepatitis B (Brunetto 2003). However, a high percentage (25-89%) of these patients relapses after the end of treatment showing elevation of ALT levels and a return of HBV DNA levels. The relapse rate seems to be higher when treatment duration is short (16 to 24 weeks) compared to longer treatment (12 to 24 months). A retrospective comparison of IFN therapies lasting from 5 to 12 months showed that with longer treatment the chance of a long-term response was 1.6 times higher (normalisation of ALT, HBV DNA $<1 \times 10^6$ copies/mL 1-7 years after end of therapy). The overall response rates were 54% at the end of therapy, 24% at 1 year after therapy, and 18% 7 years after

therapy (Manesis 2001).

Patients with long-term response to treatment have a more favourable course than patients who were untreated, unresponsive, or who had a relapse interferon α therapy with respect to progression to liver cirrhosis, liver associated deaths, and development of hepatocellular carcinoma (Brunetto 2003, Lampertico 2003). However, due to higher antiviral efficacy PEG-IFN α should be preferred to standard IFN α .

PEG-IFN α . The addition of a polyethylene glycol molecule to the IFN resulted in a significant increase in half-life, thereby allowing administration once weekly. Two types of subcutaneously administered PEG-IFN α were developed: PEG-IFN α -2a and PEG-IFN α -2b, of which PEG-IFN α -2a was licensed for the treatment of chronic HBV infections in a weekly dose of 180 μ g for 48 weeks in both HBeAg positive and HBeAg negative patients. However, PEG-IFN α -2b shows similar efficacy. After one year on treatment with PEG-IFN α -2a and α -2b, 22% to 27% of patients were reported to achieve HBeAg seroconversion (Janssen 2005, Lau 2005).

The safety profiles of standard IFN α and PEG-IFN α are similar. Following therapy termination a relatively high relapse rate is to be expected (>50%). The dose of 180 μ g per week applied for 48 weeks was recently shown to exert a stronger antiviral efficacy compared to administration for 24 weeks or to administration of 90 μ g per week (Liaw 2011). Treatment durations longer than 48 weeks are not recommended in current guidelines.

PEG-IFN α in HBeAg positive patients. Four randomised, controlled studies investigating the efficacy of PEG-IFN α in HBeAg positive patients have been conducted (Crespo 1994, Chan 2005, Janssen 2005, Lau 2005). These studies compared 180 μ g PEG-IFN α per week to standard IFN, LAM, and/or a combination treatment with PEG-IFN α + LAM for 48 weeks. Sustained HBeAg seroconversion at the end of follow-up (week 72) was significantly higher in patients treated with PEG-IFN α -2a alone or in combination with LAM than in patients treated with LAM alone (32% and 27% versus 19%) (Marcellin 2004).

PEG-IFN α in HBeAg negative patients. The efficacy and safety of 48 weeks treatment with 180 μ g PEG-IFN α -2a once weekly plus placebo, plus 100 mg LAM daily, or LAM alone was compared in 177, 179, and 181 HBeAg negative patients, respectively. After 24 weeks of follow-up, the percentage of patients with normalisation of ALT levels or HBV DNA levels below 20,000 copies/mL was significantly higher with PEG-IFN α -2a monotherapy (59% and 43%, respectively) and PEG-IFN α -2a plus LAM (60% and 44%) than with LAM monotherapy (44% and 29%); the rates of sustained suppression of HBV DNA below 400 copies/mL were 19% with PEG-IFN α -2a monotherapy, 20% with combination therapy, and 7% with LAM alone (Lau 2005).

Prolongation of PEG-IFN α treatment beyond 48 weeks may increase

sustained response rates in HBeAg negative patients. This was found in an Italian study in 128 mainly genotype D-infected HBeAg negative patients who were randomised to either treatment with 180 µg PEG-IFN α-2a per week for 48 weeks or an additional treatment with PEG-IFN α-2a at the dose of 135µg per week for another 48 weeks. Additionally, in a third arm patients received combination treatment with PEG-IFN α-2a 180µg/week and LAM 100 mg/day, followed by 48 weeks of PEG-IFN α-2a in the dosage of 135 µg/week. As a result, 48 weeks after the end of treatment 26% of patients who had received 96 weeks of PEG-IFN α-2a containing treatment showed HBV DNA levels <2,000 IU/mL as compared to only 12% of the patients who had received PEG-IFN α-2a for 48 weeks. Combination with LAM showed no additional effect (Lampertico 2013). However, prediction of response and management of side effects during prolonged treatment with PEG-IFN α has not yet been established and it is not recommended for clinical practice.

Importantly, it was shown that PEG-IFN α obviously induces immune modulatory effects which lead to considerable HBsAg clearance rates during the long-term follow-up period after treatment termination. In a study, 97 HBeAg positive patients with chronic HBV infection who had received treatment with standard IFN α were retrospectively analysed for a median period of 14 (range, 5-20) years. During the observation period, 28 patients (29%) of this cohort lost HBsAg (Moucari 2009). In another study in 315 HBeAg negative patients who were treated with either PEG-IFN α-2a, LAM 100 mg or a combination of both drugs for 48 weeks, three years after the end of treatment, the rate of HBsAg loss was 8.7% in those who had been treated with PEG-IFN α-2a alone or in combination with LAM while no patient treated with LAM as monotherapy cleared HBsAg (Marcellin 2009a). Of the patients who had received a PEG-IFN α-2a and who still had undetectable HBV DNA three years after treatment, 44% had lost HBsAg.

Nucleoside and nucleotide analogues

NAs inhibit HBV replication by competing with the natural substrate deoxyadenosine triphosphate (dATP) and causing termination of the HBV DNA chain prolongation. They represent two different subclasses of reverse transcriptase inhibitors: while both are based on purines or pyrimidines, acyclic nucleotide analogues have an open (acyclic) ribose ring that confers greater binding capacity to resistant HBV polymerase strains.

The treatment duration for NAs is not defined but a short-term application of these agents for 48 weeks is associated with prompt relapse in viraemia and they should be administered for longer periods. Treatment efficacy of NAs is defined by complete suppression of HBV DNA levels in serum. This should be achieved within 6-12 months if agents with high risk

for resistance development as LAM, ADV, and LdT are used.

Effective long-term control of HBV replication with NAs is associated with a reduction of long-term complications such as liver cirrhosis and the development of HCC, especially in patients with liver cirrhosis (Toy 2009, Hosaka 2012) (Figure 8). Studies with different NAs have demonstrated that suppression of HBV replication is associated with a significant decrease in histologic inflammatory activity and fibrosis, including partial reversion of liver cirrhosis (Chen 2006, Iloeje 2006, Mommeja-Marin 2003, Chen 2010, Marcellin 2011, Schiff 2011). With increasing treatment duration HBeAg seroconversion rates increase, but even after 8 years of treatment they do not exceed 40-50% of treated patients (Liaw 2000, Lok 2000). There is also evidence that effective inhibition of HBV replication can reduce HBV cccDNA, possibly running parallel to the decline in serum HBsAg levels (Werle-Lapostolle 2004, Wursthorn 2006).

As treatment of HBeAg negative patients does not lead to an endpoint in most patients even after after more than a decade of therapy with NAs new concepts are assessed. Discontinuation of long term NA treatment may represent a novel approach to induce sustained immune control as well as serologic response in a significant proportion of HBeAg negative patients (van Bömmel 2018).

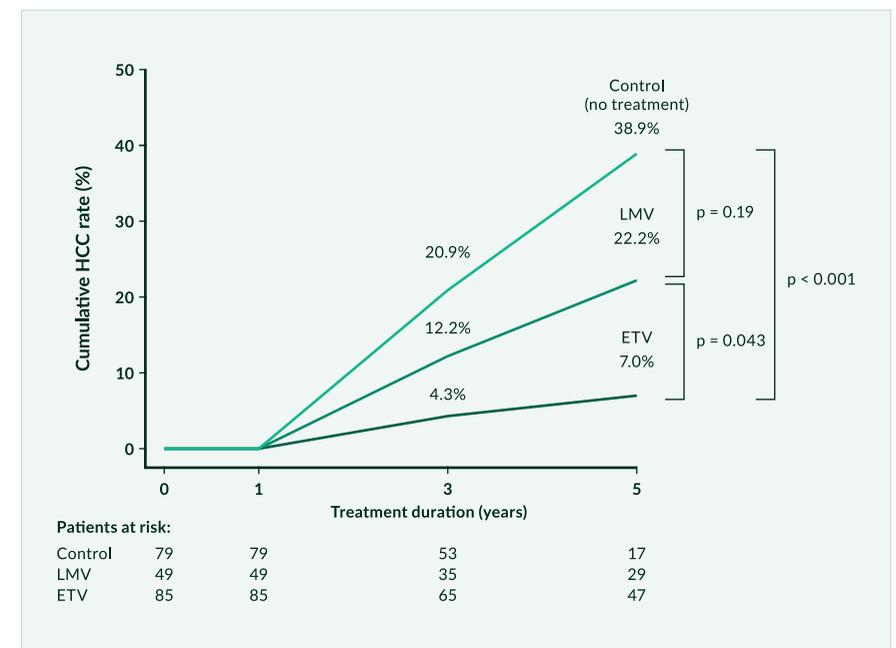


Figure 7. Comparison of HCC rates between patients with chronic hepatitis B and liver cirrhosis receiving either ETV or LMV or no treatment. The figure shows HCC cumulative incidences in the entecavir (ETV)-treated group, in the lamivudine (LAM)-treated, and the control group. The HCC rates after 5 years were the lowest in the ETV treated patients. This effect was not observed in patients without cirrhosis (Hosaka 2013).

Lamivudine (LAM). LAM, a (-) enantiomer of 2' -3' dideoxy-3'-thiacytidine, is a nucleoside analogue that was approved for the treatment of chronic HBV infection in 1988 with a daily dose of 100 mg. This dose was chosen based on a preliminary trial that randomly assigned 32 patients to receive 25, 100, or 300 mg of LAM daily for a total of 12 weeks (Dienstag 1995). In this study the dose of 100 mg was more effective than 25 mg and was similar to 300 mg in reducing HBV DNA levels. LAM exerts its therapeutic action in its phosphorylated form. By inhibiting both the RNA- and DNA-dependent DNA polymerase activities, the synthesis of both the first strand and the second strand of HBV DNA are interrupted.

Long-term LAM treatment is associated with an increasing rate of antiviral drug resistance reaching approximately 70% after 5 years in patients with HBeAg positive HBV infections. Therefore, in many guidelines LAM is not considered a first-line agent in the treatment of chronic HBV infection any more. However, LAM still may play a role in combination regimens or in patients with mild chronic hepatitis B expressing low levels of HBV DNA (<10⁵ copies/mL). An early and complete virologic response to LAM within 6 months of therapy (<400 copies/mL) constitutes a prerequisite for long-term control of HBV infection without the risk of developing resistance.

Adefovir dipivoxil (ADV). Adefovir dipivoxil was approved for treatment of chronic hepatitis B in the US in 2002 and in Europe in 2003. It is an oral diester prodrug of adefovir, an acyclic nucleotide adenosine analogue that is active in its diphosphate form. Because the acyclic nucleotide already contains a phosphate-mimetic group, it needs only two, instead of three, phosphorylation steps to reach the active metabolite stage. ADV was the first substance with simultaneous activity against wild type, pre-core, and LAM-resistant HBV variants. It is active *in vitro* against a number of DNA viruses other than HBV and retroviruses (i.e., HIV). The dose of 10 mg per day was derived from a study comparing 10 mg versus 30 mg/d. The higher dosage leads to stronger suppression of HBV DNA levels but also to renal toxicity with an increase of creatinine levels (Hadziyannis 2003).

ADV was the first acyclic nucleotide that was widely used in the treatment of LAM resistant HBV infections. However, the antiviral effect of ADV in the licensed dosage of 10 mg/day is rather weak as compared to other available antivirals (Figure 7); this disadvantage makes ADV vulnerable to HBV resistance (Hadziyannis 2006a). For this, ADV should not be used as first line monotherapy.

Telbivudine (LdT). Telbivudine is a thymidine analogue which is active against HBV but at least *in vitro* not active against other viruses, including HIV and hepatitis C virus (HCV). LdT at 600 mg/day expresses higher antiviral activity compared to either LAM at 100 mg/day or ADV at 10 mg/day (Figure 7). More patients achieved HBeAg loss within 48 weeks compared to other NAs.

LdT was reported to be non-mutagenic, non-carcinogenic, non-teratogenic, and to cause no mitochondrial toxicity. A favourable safety profile at a daily dose of 600 mg was demonstrated (Hou 2008, Lai 2007). However, CK elevations were observed more often as compared to the group treated with LAM and neurotoxicity may be an issue when LdT is administered in combination with PEG-INF α (Fleischer 2009). Thus, in the GLOBE trial, during a period of 104 weeks grades 3/4 elevations in CK levels were observed in 88 of 680 (12.9%) patients who received LdT and in 28 of 687 (4.1%) patients who received LAM ($p < 0.001$) (Liaw 2009). However, rhabdomyolysis was not observed. Peripheral neuropathy was described in 9 of 48 (18.75%) patients who received combination therapy of PEG-INF α and LdT and only in 10 of 3500 (0.28%) patients who received LdT monotherapy (Goncalves 2009).

In comparison to other NAs, some patients receiving treatment with LdT were shown to experience an increase in GFR rates. This effect was most pronounced in patients with mild renal insufficiency (Sun 2013). However, it is not clear if this translates into a clinical benefit of LdT.

Resistance to LdT has been found to occur in up to 21% of patients after 2 years of treatment (Tenney 2009), predominantly in those who did not achieve undetectable HBV DNA level by 24 weeks of treatment (Zeuzem 2009). LdT shows cross-resistance to LAM and ETV. As a consequence LdT should not be used in LAM or ETV refractory patients.

Entecavir (ETV). Entecavir, a cyclopentyl guanosine nucleoside analogue, is a selective inhibitor of HBV replication and was approved in 2006. Entecavir blocks all three polymerase steps involved in the replication process of the hepatitis B virus: first, base priming; second, reverse transcription of the negative strand from the pregenomic messenger RNA; third, synthesis of the positive strand of HBV DNA. In comparison to all other nucleoside and nucleotide analogues, ETV is more efficiently phosphorylated to its active triphosphate compound by cellular kinases. It is a potent inhibitor of wild-type HBV but is less effective against LAM-resistant HBV mutants. Therefore, ETV was approved at a dose of 0.5 mg per day for treating naïve HBeAg positive and HBeAg negative patients, but at the dose of 1 mg per day for patients with prior treatment with LAM (Lai 2005, Sherman 2008).

Treatment-naïve HBeAg positive patients achieved undetectable HBV DNA levels in 67%, 74% and 94% after one, two and five years of therapy, respectively (Figure 5, Figure 9) (Chang 2010). Long-term studies in ETV responding patients demonstrated that response was maintained in nearly all these patients over an observational period of up to six years. So far, the rate of resistance at six years of treatment is estimated to be approximately 1.2% for treatment-naïve patients (Tenney 2009). Loss of HBsAg occurs in 5% of treatment-naïve individuals after two years of ETV therapy (Gish 2010).

A non-randomised Italian study in a mixed population of predominantly HBeAg negative patients could demonstrate undetectable HBV DNA levels in 91% and 97% of patients at 1 and 2 years of ETV treatment, respectively (Lampertico 2010).

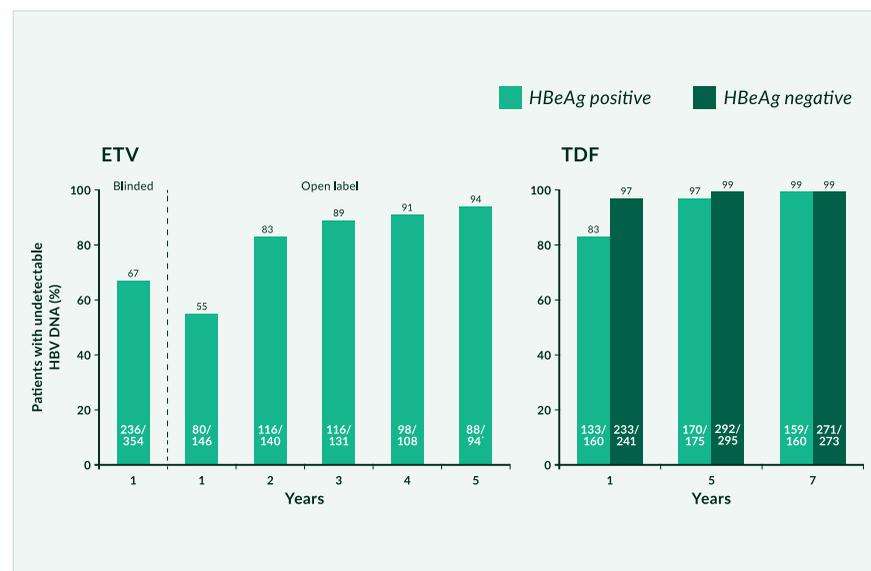


Figure 8. Long-term treatment with potent nucleos(t)ide analogues leads to suppression of HBV DNA to undetectable levels in most patients. (Marcellin 2008, Chang 2010, Marcellin 2013, Buti 2015). The long-term cohort ETV-901 consists of HBeAg positive patients initially treated in the study ETV-022 (ETV 0.5 mg/day), which was designed for a duration of one year and then continued open label.

In LAM-resistant patients ETV is less potent. Only 19% and 40% of these patients achieved undetectable HBV DNA after one and two years, respectively, despite an increased dose of 1 mg/day (Gish 2007, Sherman 2008). Due to cross-resistance up to 45% of patients with LAM resistance develop resistance against ETV after 5 years of treatment (Tenney 2009).

ETV has a favourable tolerability profile and can be easily adjusted to renal function. However, ETV may cause severe lactic acidosis in patients with impaired liver function and a MELD score of >20 points (Lange 2009).

Tenofovir (TFV). Tenofovir is available in two different formulas. It is an acyclic nucleoside phosphonate, or nucleotide analogue, and structurally closely related to ADV. TFV has selective activity against retroviruses and hepadnaviruses and is approved for the treatment of HIV infection and chronic hepatitis B. Tenofovir disoproxil fumarate (TDF), an ester prodrug form of tenofovir (PMPA; (R)-9-(2-phosphonylmethoxypropyl) showed marked antiviral efficacy over eight years (HBV DNA <400 copies/mL) in almost all treatment-naïve -negative and -positive patients (Table 5). HBeAg loss and HBeAg seroconversion were found in 47% and 31% of patients,

respectively. Of the HBeAg positive patients remaining under observation for 8 years, 8.5% experienced HBsAg loss (Marcellin 2014). Other clinical studies showing a high efficacy of TDF in LAM-resistant HBV (van Bömmel 2010, Levrero 2010). Due to possibly existing cross-resistance to ADV, the efficacy of TDF might be hampered by the presence of ADV resistance in patients with high HBV viraemia; however, a breakthrough of HBV DNA during TDF treatment in patients with previous ADV failure or in treatment-naïve patients was not been observed (van Bömmel 2010, Levrero 2010, Berg 2014).

Table 6. Treatment end points in HBeAg positive and HBeAg negative patients after 8 years of treatment with TDF (Marcellin 2014).*

	% HBeAg negative		% HBeAg positive	
	ITT	observed	ITT	observed
HBV DNA				
< 69 IU/mL	75	99.6	58	98
< 29 IU/mL	74	99	58	97
HBeAg				
loss/seroconversion	NA	NA	32/21	47/31
HBsAg				
loss/seroconversion	1.1/0.7	1.1/0.7	12.9/10.3	1.5/8.5

* Patients were originally randomised to treatment with either TDF 300 mg or ADV 10 mg per day. After one year, patients receiving ADV were switched to TDF. The two studies shown here (102 and 103) included more than 600 HBeAg positive and HBeAg negative treatment naïve patients.

TDF is generally well tolerated and not associated with severe side effects. For HBV mono-infected, treatment-naïve patients, renal safety during TDF monotherapy was investigated in three studies. In a randomised study comprising HBeAg negative patients, none of 212 patients treated with TDF for six years and none of 112 patients who were treated with ADV for one year and then switched to TDF for five years had a decrease in GFR to levels of <50 mL/min or an increase of serum creatinine levels to >0.5 mg/dL (Buti 2015). In a similar study in HBeAg positive patients, of 130 patients treated with TDF for 3 years and of 76 patients treated with ADV for one year and consecutively with TDF for 2 years, only one patient showed an increase in serum creatinine levels >0.5 mg/dL starting at year two (Heathcote 2011). In a sub-analysis of both studies in 152 HBeAg positive and HBeAg negative Asian patients, no increase of serum creatinine >0.5 mg/dL or of eGFR <50 mL/min was found in up to 3 years of TDF treatment (Liaw 2009a). In addition, a benefit in renal function was found in treated patients when compared to untreated patients with HBV infection, which might reflect a

lower incidence of glomerulonephritis caused by HBsAg induced immune complexes in treated patients (Mauss 2011).

Tenofovir alafenamide fumarate (TAF, or (9-[[®]-2-[[[(S)-[[[(S)-1-(isopropoxycarbonyl)ethyl]amino] phenoxyphosphinyl]methoxy]propyl] adenin), was approved for the treatment of HBV infections in 2016. TAF is following a novel pro-drug mechanism of action and has a higher bioavailability and increased plasma stability compared to TDF. The resulting lower daily dose of 25 mg (vs. 245 mg for TDF) has been shown to be as effective as the TDF formulation in both HBeAg positive patients, but with fewer negative effects on bone and kidney biomarkers (Buti 2016, Chan 2016). Thus, in both studies, patients receiving TAF experienced a significantly smaller mean decrease in spine and hip bone mineral density at week 48 compared to patients receiving TDF. The median decrease in estimated glomerular filtration rate (eGFR) from baseline to week 48 was smaller in the TAF treated patients, although the clinical relevance of this observation remains under debate.

The use of tenofovir in HIV-coinfected patients is discussed in detail in Chapter 15.

Combination therapy as first-line treatment. Combination treatments with different NAs or NAs with PEG-IFN α were studied in different patient cohorts. However, in most trials combinations were not superior to mono therapies, and due to insufficient knowledge how to choose patients that will benefit from first line combination treatments they are currently not recommended.

There is only one study comparing a combination therapy with LAM and ADV to LAM monotherapy in untreated patients (Sung 2008). In this study, there was no difference in the virologic and biochemical response between both groups. However, the rate of LAM resistance was much lower in the combination group.

A study assessing the combination of LAM with LdT showed no benefit for combination therapy (Lai 2005).

In another trial, 379 treatment-naïve patients were randomised to receive either ETV 0.5 mg/day as monotherapy (n = 186) or in combination with TDF (n = 198) (Lok 2012). By week 96, 76 % of patients in the monotherapy and 83 % in the combination arm showed suppression of HBV DNA below 50 IU/mL (p = 0.088). In a post hoc subgroup analysis, combination therapy was superior to ETV as monotherapy in HBeAg positive patients with baseline HBV DNA > 8 log IU/mL. In a double-blind study in 126 HBeAg positive immune tolerant individuals with high levels of HBV DNA (mean 8.41 log₁₀ IU/mL) were randomly assigned to two arms given either oral TDF 300 mg per day and placebo (n = 64) or a combination of TDF and emtricitabine (200 mg, n = 62) for 192 weeks. At week 192, 55% of patients in the TDF

monotherapy group and 76% of patients in the combination group had levels of HBV DNA <69 IU/mL (p=0.016). However, HBeAg seroconversion or HBsAg loss was reported in only few patients and this was not different across both groups (Chan 2014).

Especially in patients with liver cirrhosis, a fast and complete suppression of HBV replication is desirable. A monotherapy with ETV was found to be as safe and effective as monotherapy with TDF, and an addition of emtricitabine to TDF showed no improvement in response (Liaw 2011). Therefore, in these patients as well, combination treatment is currently not recommended.

Although a combination of NAs and PEG IFN α theoretically represents a more promising approach as two different mechanisms of action could potentially be synergistic, the results from clinical studies do not support this strategy. A more pronounced on-treatment virologic response at week 48 of treatment was observed with combination therapy as compared to LAM or PEG-IFN α alone in one study (Chan 2005). But a combination of LAM plus PEG-IFN α failed to demonstrate serologic or clinical benefit when evaluated at the end of follow-up in most studies (Janssen 2005).

Combination therapies of PEG-IFN α with more potent NAs such as ETV or TDF may be more attractive. A combination treatment of ETV and PEG-IFN 2 α after 4 years of complete response to ETV was found to be superior to continuation of ETV treatment by HBeAg and HBsAg loss and seroconversion rates (Ning 2014). A recent randomised study investigating the efficacy of a combination treatment of PEG-IFN α and TDF alone or in combination in 740 patients with chronic hepatitis B found that patients treated with TDF plus PEG-IFN 2 α for 48 weeks achieved significantly higher rates of HBsAg loss at week 72 (9.1%) than patients treated with either TDF (0%) or PEG-IFN 2 α (2.8%) at week 72 (Marcellin 2016). However, due to the short follow up of these patients and the low rate of HBsAg losses overall, a combination treatment of NAs plus PEG-IFN α can still not be recommended.

Combination treatment with LdT and PEG-IFN α is contraindicated. Peripheral neuropathy was described in 9 of 48 (18.8%) patients who received combination therapy of PEG-IFN α and LdT, as compared to only in 10 of 3,500 (0.28%) patients who received LdT monotherapy (Goncalves 2009).

Choosing the right treatment option

At first, the feasibility of PEG-IFN α therapy should be evaluated (Figure 6). However, if a patient does not fulfil the criteria for PEG-IFN α , has contraindications or low likelihood for response, or is intolerant to interferon, long-term therapy with NAs is recommended. If an NA is chosen several parameters have to be considered prior to therapy: the antiviral

efficacy of the drug, the durability of response, the resistance barrier, expected side effects and the stage of liver disease.

If the initial viral load is low and liver cirrhosis has been excluded, any approved NA may be used, however, more recent guidelines recommend the use of either ETV or TDF for first-line treatment (EASL 2017, WHO 2015, Terrault 2016, Sarin 2016). The use of LAM should be restricted to patients with mild fibrosis and HBV DNA levels $<2,000$ IU/mL (or $<10^4$ copies/mL). For patients with high-level HBV replication ($>2 \times 10^8$ IU/mL or $>10^9$ copies/mL) only drugs with a high genetic barrier should be used (i.e., ETV or TDF) (Table 4).

Prognostic factors for treatment response

Several factors are associated with long-term remission and may help to guide treatment decisions. Pre-treatment factors predictive of HBeAg seroconversion are low viral load, high ALT levels (above 2-5 x ULN) and high histological grading (Flink 2006, Hadziyannis 2006a, Lai 2007, Perrillo 1990, Perrillo 2002, Wong 1993, Yuen 2007, Zoulim 2008, Buster 2009). These general baseline predictors are relevant especially for treatment regimens with PEG-IFN α but may in part be relevant also for NAs (Table 6).

A pooled analysis from the two largest trials using PEG-IFN α -2a or -2b in chronic hepatitis B tried to calculate a score predicting successful interferon therapy based on an individual patient's characteristics (viral load, ALT level, HBV genotype, age, gender). However, this approach may only be feasible in HBeAg positive patients (Buster 2009).

HBV genotypes and treatment response. HBV genotypes have been shown to be associated with IFN α treatment success. Patients with HBV genotype A, prevalent in northern Europe and the US, show a much higher rate of HBeAg and HBsAg seroconversion than patients with HBV genotype D, prevalent in the south of Europe, or the HBV genotypes B or C originating from Asia (Keeffe 2007, Wiegand 2008). During treatment with nucleos(t)ide analogues, suppression of HBV replication and induction of HBeAg loss can be achieved regardless of the present genotype. However, HBsAg loss was almost exclusively observed in patients with genotypes A or D.

Table 7. Predictors of response to antiviral therapy

	Nucleos(t)ide analogues	PEG-IFN α
Before treatment		Low viral load (HBV DNA $\leq 10^7$ IU/mL), high serum ALT levels (above 3 times ULN), high activity scores on liver biopsy (at least A2)
During treatment	Undetectable HBV DNA in a real-time PCR assay at 24 or 48 weeks is associated with HBeAg seroconversion in HBeAg positive patients and lower incidence of resistance	HBV DNA decrease $<20,000$ IU/mL at 12 weeks is associated with 50% chance of HBeAg seroconversion in HBeAg positive patients and with a 50% chance of sustained response in HBeAg negative patients
HBsAg decrease	HBsAg decrease at weeks 12 and 24 may predict HBsAg seroconversion	
HBV genotype	HBV genotype shows no influence on suppression of HBV DNA levels. HBsAg seroconversions mostly observed for genotypes A and D	Association with HBV genotype A and B and response to IFN α is higher than with genotypes C and D, however the association is weak and HBV genotype should not be the only argument for treatment decision

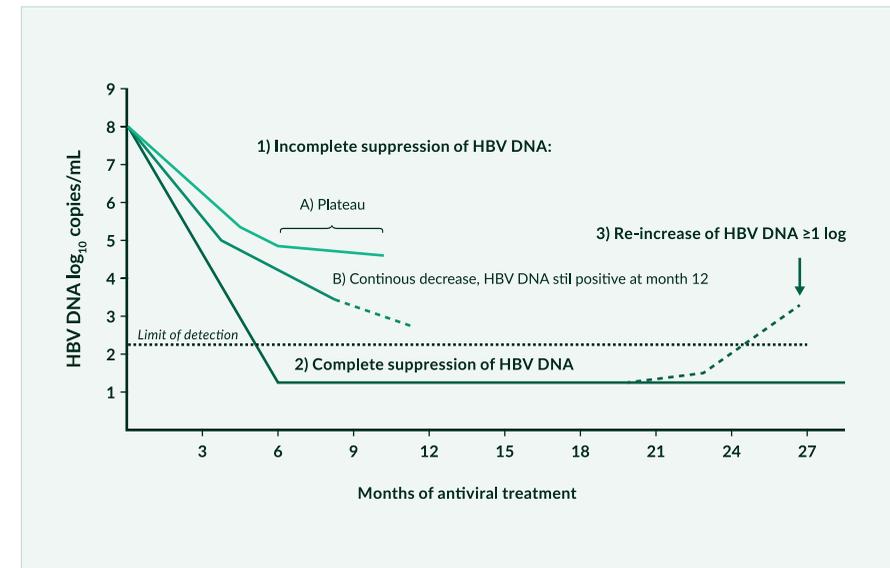


Figure 9. Possible courses of HBV DNA levels during treatment with nucleoside or nucleotide analogues. Incomplete suppression of HBV DNA results in either a "plateau phase" or in a continuous slow decline. A plateau phase represents a high risk for selection of resistant HBV variants, therefore treatment should be changed to a more effective agent or combination therapy. A continuous slow decline should induce a treatment change after 6 months if drugs with a low genetic barrier like LAM or LdT are used. If drugs with a high genetic barrier like ETV or TDF are taken, a continuous slow decline can be monitored for at least 12 months without increased risk of HBV resistance.

HBV DNA levels and treatment response. During antiviral therapy, the decrease of HBV DNA levels from baseline is the most important tool in monitoring treatment efficacy. Complete response to antiviral therapy is defined as suppression of HBV DNA below the limit of detection as measured by a sensitive real time PCR assay (Figure 10). Incomplete suppression is characterised by persistent HBV replication despite antiviral therapy. Ongoing HBV replication should be avoided to prevent the selection of resistant HBV strains by replication of the virus in the presence of drug in the so-called “plateau phases”. A breakthrough of HBV DNA despite continuous NA treatment may be caused by viral resistance; however, if NAs with high genetic barrier against resistance as ETV or TDF are used, non-adherence to the antiviral treatment is more likely. Measuring of HBV DNA kinetics early during therapy will help to guide antiviral treatment and to establish early stopping rules or add-on strategies to avoid antiviral failure (Figure 10).

Incomplete or partial virologic response to NAs is defined as a decrease of HBV DNA $>1 \log_{10}$ IU/mL but remaining measurable (Lavanchy 2004) (Figure 10). The definition of partial response depends on the type of treatment; thus, for agents with a high genetic barrier against resistance like ETV or TDF partial response is defined after 12 months and for substances with a low genetic barrier like LAM or LdT, after 6 months of monotherapy. In case of partial response to a drug with a low genetic barrier, an appropriate rescue therapy should be initiated. By current guidelines, a combination treatment with an NA is recommended for these patients. However, it was recently shown that patients with partial response to LAM or to ADV have a high probability of responding to TDF monotherapy, without risking the development of resistance (Heathcote 2011, Marcellin 2011b, van Bömmel 2010, Berg 2014). Patients with a partial response to ADV were also shown to have a high probability of responding to a subsequent monotherapy with ETV, irrespective of the presence of mutations associated with HBV resistance to ADV (Leung 2009, Leung 2009a).

For patients with partial response to a drug with a high genetic barrier as ETV or TDF, current guidelines also recommend the initiation of a combination treatment. However, this might be necessary only in a minority of patients, as recently published long-term studies have shown that the continuation of a first-line monotherapy with ETV or TDF increases the percentage of patients with undetectable HBV DNA over time without leading to resistance development (Chang 2010, Marcellin 2011b, Snow-Lampert 2011, Marcellin 2014) (Figure 9). Thus, during monotherapy with TDF in HBeAg positive and HBeAg negative patients, an increase of patients with complete suppression of HBV DNA between the end of the first and the end of the fifth year of treatment from 81% and 90% to 100% was shown. For monotherapy with ETV at 1 mg/day, an increase from 55% to 91% and

94% after the fourth and fifth years was demonstrated (Chang 2010). In case of incomplete viral suppression at week 48, a continuation of monotherapy with TDF or ETV 1 mg is advisable as long as HBV DNA levels decrease continuously. However, the debate on whether switching or adding a second drug as optimal management is not yet resolved.

Since only 30-35% of all patients treated with PEG-IFN α reach HBeAg seroconversion after 48 weeks, it has been assessed how to predict the probability of response to PEG-IFN α by kinetics of HBV DNA during treatment. In one retrospective analysis early prediction of stable seroconversion was possible by week 12 of therapy if HBV DNA had reached levels below $5 \log_{10}$ IU/mL within this short treatment period (Fried 2005). In 53% of these patients, HBeAg seroconversion was observed while patients with HBV DNA levels of 5 to $9 \log_{10}$ copies/mL or levels above $9 \log_{10}$ IU/mL achieved HBeAg seroconversion in only 17% and 14%, respectively.

Time point of HBeAg loss. In one study with 172 patients who were treated with PEG-IFN α -2b as monotherapy or in combination with LAM, the loss of HBeAg within the first 32 weeks of treatment was shown to be an on-treatment predictor for HBsAg loss during a mean period of 3.5 years after the end of treatment. HBsAg loss was found in 36% of the patients with early HBeAg loss and only in 4% of the patients with HBeAg loss after 32 weeks of treatment (Buster 2009).

HBsAg levels and treatment response. Response of HBeAg positive and HBeAg negative patients to PEG-IFN treatment can be predicted by measuring HBsAg levels before and changes of HBsAg levels during treatment (Figure 11).

During PEG-IFN treatment for HBeAg positive chronic HBV infection, an absence of a decline in HBsAg levels at week 12 of treatment reduces the probability of response to $<5\%$ in one study (Sonnefeld 2010). In the NEPTUNE trial investigating the predictive value of HBsAg levels in 114 HBeAg positive patients receiving PEG-IFN α -2a over 48 weeks, it was shown that in patients achieving suppression of HBsAg to levels $<1,500$ IU/mL after 12 weeks of treatment, the chance of reaching HBeAg seroconversion, suppression of HBV DNA to levels $<2,000$ IU/mL and HBsAg loss 6 months after treatment was 58%, 52% and 10%, compared to 42%, 31% and 0% in patients with HBsAg levels between 1,500-20,000 IU/mL. In this study, patients still showing HBsAg levels $>20,000$ IU/mL after 12 weeks of treatment achieved none of the endpoints (Liaw 2011). Beyond that, the probability of HBeAg loss rose to 68% in patients with elevation of ALT levels $>2 \times$ the upper limit of normal at treatment initiation (Figure 12).

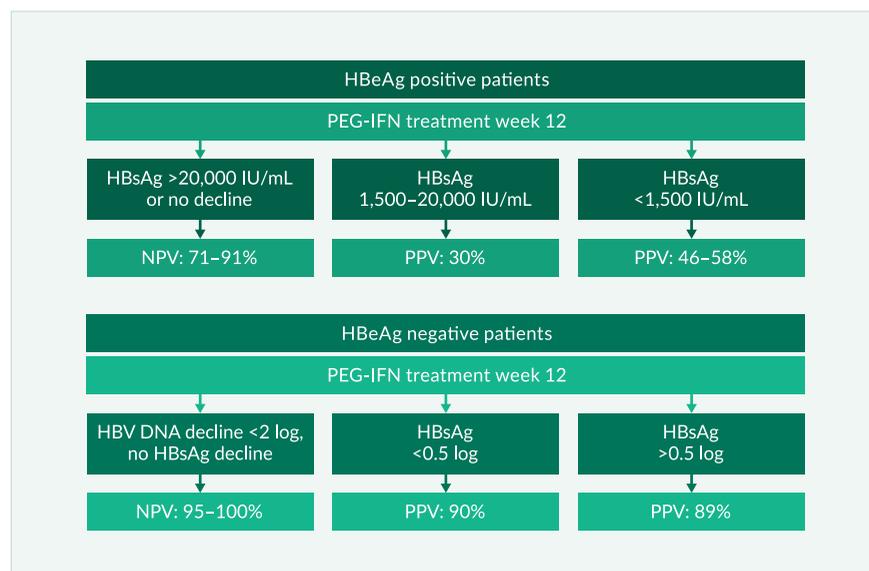


Figure 10. On-treatment prediction of treatment response by HBsAg levels. In different trials, an association of the decline in HBsAg levels within the first 12 weeks of PEG-IFN α treatment and treatment response defined as HBV DNA levels $<2,000$ copies/mL six months after treatment was found (Zonneveld 2010, Piratvisuth 2011, Lau 2009, Liaw 2011, Rijckborst 2010, Moucari 2009). Patients showing no decline in HBsAg levels at week 12 had only a very small chance of long-term response

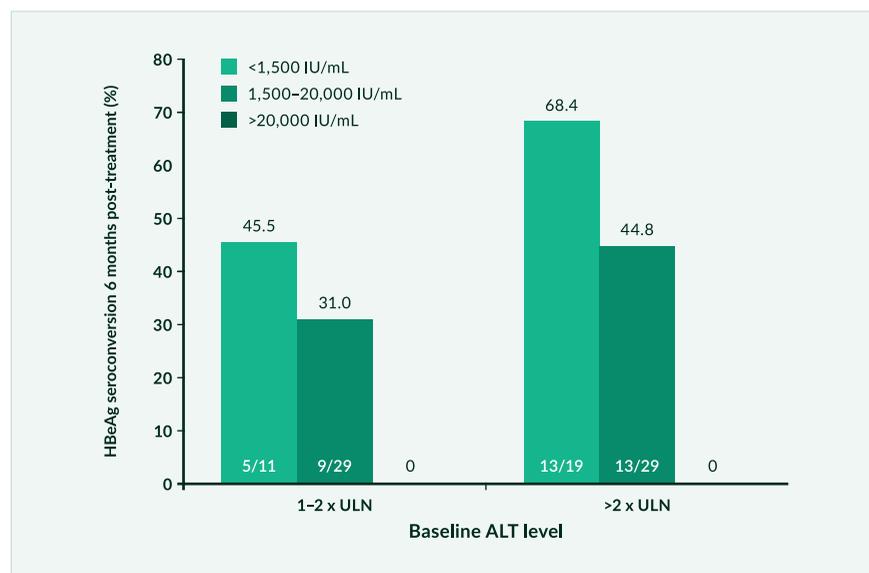


Figure 11. The level of HBsAg levels after 12 weeks of treatment with PEG-IFN α -2a is predictive for HBeAg seroconversion six months after treatment. A combination of ALT levels and HBsAg decline improves positive predictive value in these patients (Liaw 2011).

Also, in HBeAg negative patients the decrease of HBsAg after 12 weeks of PEG-IFN α treatment can predict long-term response. This prediction can be made even more precise regarding the kinetics of both HBsAg and

HBV DNA. In another study comprising 48 patients who were treated with PEG-IFN α -2a, a decrease in serum HBsAg levels of 0.5 and 1 \log_{10} IU/mL at weeks 12 and 24 of therapy was associated with a positive predictive value for HBsAg loss of 90% and 97% at week 96 after treatment, respectively (Moucari 2009).

Monitoring before and during antiviral therapy

Before therapy, HBV DNA levels should be measured with a highly sensitive assay. These results should be reassessed 1-2 months after initiation of therapy. In addition, ALT levels reflecting the inflammatory activity as well as creatinine levels to monitor eventual renal toxicity of NAs should be measured. HBV genotyping is only recommended in patients who are considered candidates for treatment with IFN. HBV resistance testing can be useful in patients with prior failure to more than one NA, but this is not a standard diagnostic approach.

Table 8. Recommendation for laboratory tests for monitoring antiviral therapy

Tests before antiviral treatment	
HBV DNA quantitative	All patients
HBeAg, anti-HBe	All patients
HBsAg quantitative	If IFN-based treatment is planned
HBV genotype	If IFN-based treatment is planned
ALT level	All patients
Creatinine level	All patients
Tests during antiviral treatment	
	Interval
HBV DNA quantitative	After 4-6 weeks, after 12 weeks, then every 3-6 months
HBeAg, anti-HBe	3-6 months, if HBV DNA is undetectable
HBsAg, anti-HBs	3-6 months, in HBeAg positive patients after HBeAg seroconversion and in HBeAg negative patients if HBV DNA is undetectable
HBV resistance test	If HBV DNA increases >1 log during antiviral treatment and pretreatment history is not known, but first check on treatment adherence!
ALT level	Initially every month, then every 3-6 months
Creatinine level*	Every 3-6 months
Other chemistry tests	Every 3-6 months

* According to the manufacturer creatinine levels should initially be monitored every 4 weeks during treatment with TDF or ADV; however, recent treatment guidelines recommend monitoring every 3 months (Terrault 2016, Sarin 2016).

During therapy, HBV DNA, ALT and creatinine levels should be measured initially, after 4 to 6 weeks and then every 3 months. The early identification of viral resistance and an early adjustment of therapy are crucial. Patients with suppression of HBV replication to levels <300 copies/mL (60 IU/mL) for at least 2 years may be scheduled at 6 months intervals (Table 7). However, no studies have been performed that support this procedure.

HBsAg and, in HBeAg positive patients, HBeAg and anti-HBe should also be measured once HBV DNA levels have become undetectable to detect serologic response).

Because the risk for HCC development remains increased even in patients with complete viral suppression during long-term treatment with NA, these patients should regularly receive ultrasound examinations (Papatheodoridis 2014). For the estimation of the individual risk of HCC development, newly introduced score systems can be helpful (see below).

Treatment duration and stopping rules

After loss of HBsAg or seroconversion to anti-HBs antiviral treatment patients can be safely withdrawn from treatment with NAs. This was demonstrated in a recent study assessing the long-term outcome of patients withdrawing from NA treatment after HBsAg clearance. In this study, 27 (5%) out of 520 CHB patients who received NA for prolonged periods ultimately lost serum HBsAg and were subsequently followed for a mean of 44 (12–117) months (Chen 2014).

In HBeAg positive patients continuous treatment with nucleos(t)ide analogues is necessary as long as HBeAg seroconversion is not achieved. Even after seroconversion antiviral therapy should be continued for at least another 12 months to reduce the risk of “sero-reversion” upon stopping the nucleos(t)ide analogue therapy (EASL 2017, WHO 2015, Terrault 2016).

Criteria for optimal treatment duration with NAs are still lacking for patients with HBeAg negative chronic hepatitis B. Therefore, currently unlimited treatment with NAs is recommended. Discontinuation of NA treatment may be considered in selected patients (see below). In patients with liver cirrhosis oral antiviral treatment should not be discontinued at any time point because of the risk of liver decompensation during a virologic and inflammatory rebound.

PEG-IFN α should be administered for 48 weeks in HBeAg positive and HBeAg negative patients. If no decrease in HBV DNA or/and in HBsAg levels can be noted after 12 weeks of treatment, response becomes unlikely and treatment may be stopped early in agreement with the patient.

Controlled cessation of long-term treatment with NAs in HBeAg negative patients

In HBeAg negative patients receiving antiviral treatment, HBsAg loss occurs only occasionally ($<1\%$). Safety and costs of long term treatment with NAs are a concern for these patients. Current recommendations regarding treatment termination follow different strategies. Thus, AASLD guidelines state that NA treatment should be continued in HBeAg negative patients until HBsAg loss, while APASL guidelines recommend that treatment may be withdrawn after at least 2 years of treatment with undetectable HBV DNA (EASL 2017, Terrault 2016, Sarin 2016). EASL guidelines are the first to mention discontinuation of NA treatment as approach to increase immunologic response. All three guidelines recommend excluding patients with cirrhosis from treatment termination unless they have cleared HBsAg. After stopping long-term NA-treatment, a virologic and a biochemical relapse of the HBV infection is common, but some patients have been shown to clear HBsAg following this relapse, and others to develop a stable, low-level replicating HBV infection with no indication for further antiviral treatment (Figure 13). In the FINITE study, the first randomised study to assess the efficacy of NA discontinuation in HBeAg negative patients, the primary endpoint was not relapse in HBV DNA levels, but the rates of HBsAg loss and long-term immunological control defined as HBV DNA levels $<2,000$ IU/mL (Berg 2017). For this, patients were assessed week 144 after withdrawal of monotherapy with tenofovir (TDF). Patients included in the study had HBV DNA at undetectable levels for ≥ 3.5 years and were randomly assigned to either stop ($n = 21$) or continue ($n = 21$) TDF. After discontinuation of TDF, HBV DNA became detectable in all patients. However, a total of 62% ($n = 13$) remained off-therapy until week 144 (Figure 14). Four patients (19%) experienced HBsAg loss. Eight patients had to restart therapy with TDF due to ALT flares.

Prediction of response to NA discontinuation has not yet been established yet. HBsAg levels may be a marker to guide treatment cessation in HBeAg negative patients. The effect of stopping therapy after a long-term ADV treatment of 4 to 5 years with complete viral suppression was evaluated in a small cohort of Greek patients (Hadziyannis 2008). Despite the fact that all patients suffered a slight virologic relapse within 3 months of stopping therapy, most patients went below detection over the following 4 years without any therapy. Moreover, 28% of the patients lost HBsAg. Loss of HBsAg after stopping treatment was associated with low HBsAg titres at the time point of treatment withdrawal; however, due to the few currently available experiences stopping rules have not been established so far. Also other studies have found the association between low HBsAg levels at the time point of treatment withdrawal and HBsAg loss or consolidation of

the HBV infection thereafter. HBsAg levels of $<2 \log_{10}$ IU/mL at treatment withdrawal were associated with a lower relapse rate after 1–2 years (15% vs. 85%) (Liang 2011).

The predictors of off-treatment response were recently assessed in a meta-analysis including 25 studies with more than 1700 patients in whom NAs were discontinued (Papatheodoridis 2016). The duration of suppression of HBV DNA was shown to be the most important predictor of a durable off-therapy, and the probability of a viral relapse was lower in patients with suppression of HBV DNA for 24 months compared to 12 months (36% vs. 75%). Low HBsAg levels at the time point of treatment cessation were shown to be another positive predictor of treatment response (Wang 2016). However, more prospective studies are certainly needed for validation of these observations and for the refined definition of termination of NA treatment. Severe liver damage due to ALT flares was only observed in patients with cirrhosis so far (Papatheodoridis 2016).

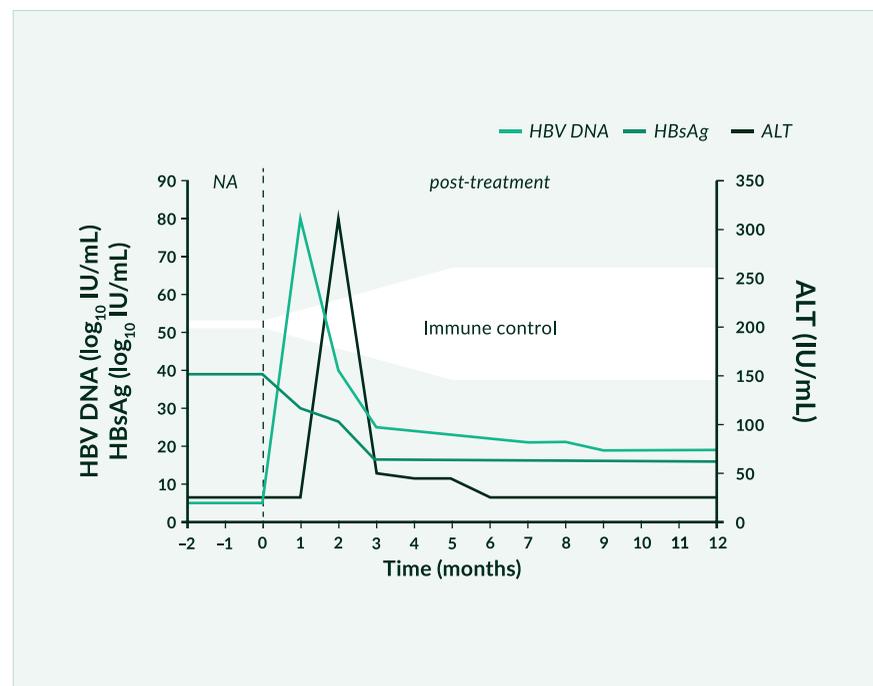


Figure 12. Suggested association of NA treatment discontinuation and the arising of sustained immune control. After a temporary increase, HBV DNA levels durably drop below the limit of treatment indication ($<2,000$ IU/mL) and alanine aminotransferase (ALT) levels normalise. HBsAg levels show some decline under increased immune control, in some patients even to undetectable levels (van Bömmel 2018).

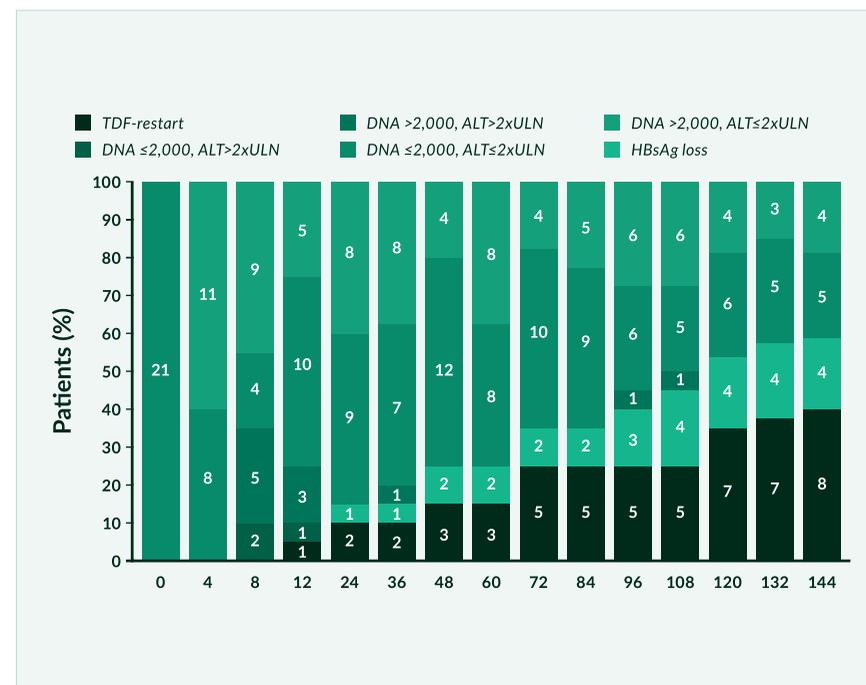


Figure 13. HBV DNA and alanine aminotransferase (ALT)* levels, HBsAg loss and retreatment with TDF in 21 HBeAg- negative patients during 144 weeks after discontinuation of TDF treatment (figure taken from Berg 2017). The number of patients with different post- NA treatment are given in the bars. *ULN defined as 45 U/L. ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; ULN, upper limit of normal

Management of HBV resistance

Resistance development. The mechanism of action of NAs is a competitive inhibition of the HBV polymerase. During treatment with these substances, HBV variants bearing mutations within the HBV polymerase gene may become selected from the HBV quasispecies, a phenomenon which is defined as genotypic resistance.

Phenotypic resistance is defined as decreased susceptibility (*in vitro* testing) to inhibition by antiviral drugs associated with genotypic resistance (Figure 15).

Cross-resistance of HBV to antiviral treatment has been described within the groups of nucleoside and nucleotide analogues, respectively (Figure 16). If a resistant population becomes the majority in an individual, treatment might fail and a viral breakthrough during treatment may appear which may be associated with severe and sometimes fatal reactivation (Zoulim 2012).

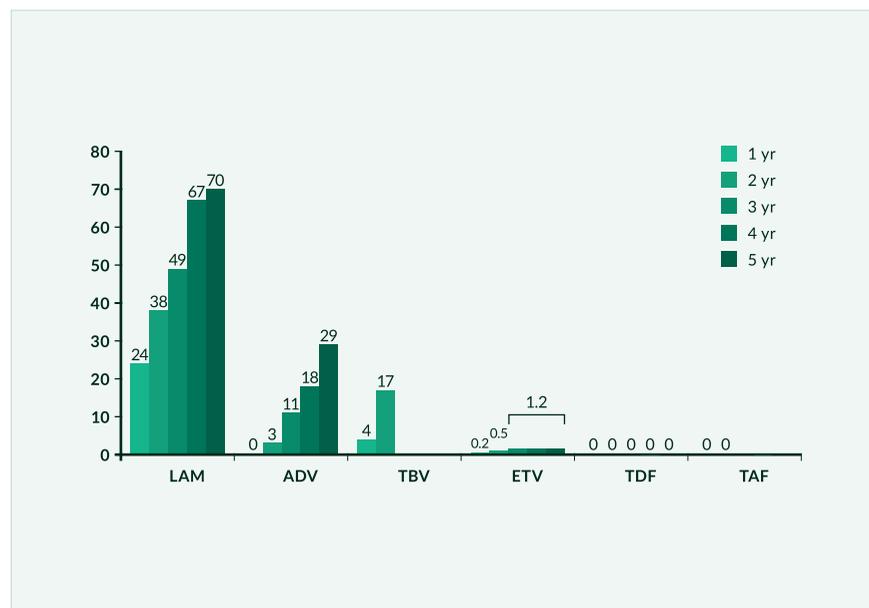


Figure 14. Cumulative incidence of HBV resistance. These numbers are average estimates based on different studies. Overall, resistance rates have been higher in HBeAg positive patients than in HBe antigen negative patients. Long-term data for ADV has only been reported for HBeAg negative patients and thus resistance rates may be even higher for HBe antigen positive individuals. Data for ETV is biased since both patients with best responses (e.g., HBeAg seroconversion) and patients with suboptimal virologic responses (>700,000 copies/mL after one year of treatment) were withdrawn from the study. For TDF and TAF, no viral breakthrough associated with HBV resistance has been described yet (modified from [EASL 2017]).

Theoretically, all available NAs may select resistant HBV strains. However, resistance is very rare in treatment-naïve patients who receive substances with strong antiviral activity, i.e., TDF or ETV, but resistance rates against LdT, ADV and especially LAM are significantly higher (Figure 14).

Interestingly, for patients treated with TDF no resistance has ever been reported, not even in patients who were pretreated with ADV, although ADV resistance-associated mutations might slightly decrease response to TDF (van Bömmel 2012, Kitrinis 2014, Berg 2014).

Detection of HBV resistance. Generally, a confirmed re-increase of HBV DNA >1 log from nadir during treatment with nucleoside/nucleotide analogues is considered being a potential viral breakthrough caused by HBV resistance (Figure 10). Genotypic resistance testing is not available to most treating physicians and is generally not recommended (Cornberg 2011, EASL 2017, Terrault 2016). However, genotypic resistance testing might be helpful in individual cases. It has to be considered that most viral breakthroughs in treatment-naïve patients receiving ETV or TDF are the result of adherence issues. Therefore, patient adherence should be assessed before genotypic resistance testing is done.

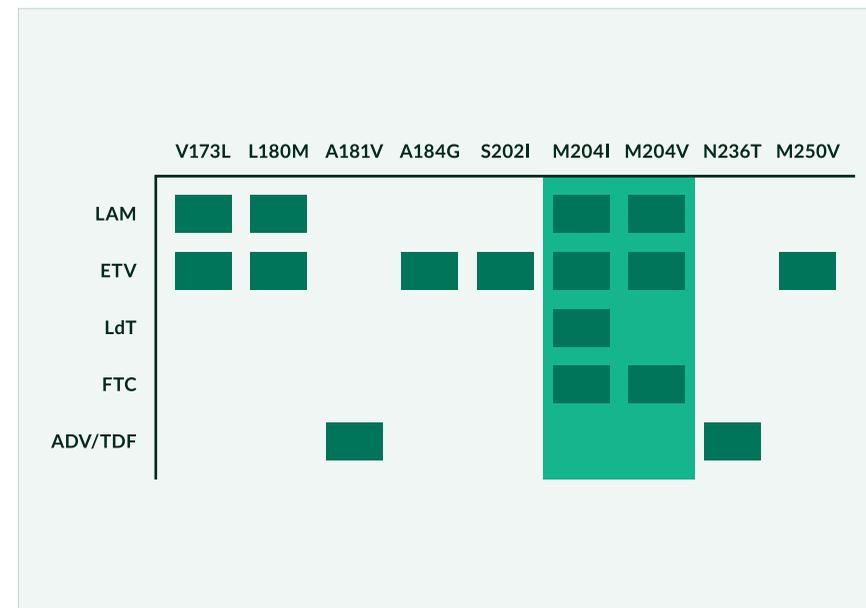


Figure 15. Resistance patterns of different antiviral drugs used for the treatment of chronic hepatitis B. The numbers indicate the respective amino acid position in the HBV polymerase gene. For ETV, resistance at positions rt204 plus an additional mutation at position rt184, rt202 or rt250 is required to lead to clinically significant drug resistance. The mutations rtA181V and rtN236T cause resistance against ADV and weaker response to TDF in some patients; however, to date, viral breakthrough while on TDF treatment has not yet been shown to be associated with HBV variants.

Avoidance of HBV resistance. HBV resistance occurs most frequently in patients treated with LAM, LdT or ADV, therefore many guidelines discourage physicians to use these NAs in first line treatment. The selection of resistant HBV strains becomes more likely if HBV DNA levels do not become suppressed to undetectable levels within 6 months of treatment with these NAs. Therefore, in patients undergoing treatment with these substances, who show detectable HBV DNA after 6 to 12 months of treatment, the treatment should be adjusted (Cornberg 2011, EASL 2017, Terrault 2016). Also, patients with high viral load (>10⁹ copies/mL) are at increased risk of resistance and should not be treated with these substances. First-line treatment with ETV or TDF is recommended by many guidelines to avoid HBV resistance (Cornberg 2011, EASL 2017, Terrault 2016, WHO 2015).

Treatment of HBV resistance. Generally, resistance against a nucleoside analogue should be treated with a nucleotide analogue and vice versa (Figure 13). In real life, treatment with TDF has been shown to suppress most kinds of HBV variants associated with resistance against either nucleoside or nucleotide analogues. Thus, a switch to a monotherapy with TDF was shown to be very effective in patients with resistance to LAM and also in patients with resistance to ADV in European and in Asian patients (van Bömmel 2010, Huang 2017). In a randomised study, it was shown that patients with

resistance to LAM did not show better response to a combination treatment of TDF plus emtricitabine as compared to TDF monotherapy (Fung 2014). In another study, it was observed that monotherapy with TDF was superior to entecavir-adefovir combination treatment in NA resistant patients with suboptimal response to lamivudine-adefovir (Lee 2017). However, some of these patients with genotypic ADV resistance, especially those with HBV DNA levels $>10^7$ copies/mL showed delayed or incomplete response to TDF (van Bömmel 2010). ETV was shown to be effective as monotherapy in patients with resistance to ADV. General recommendations for the management of HBV resistance are given in Table 8.

Table 9. Recommendations for the treatment of HBV resistance

Resistance to nucleoside analogues	Recommended therapeutic option
lamivudine	tenofovir (TDF, TAF), ADV*
telbivudine	tenofovir (TDF, TAF), ADV*
entecavir	tenofovir (TDF, TAF), adefovir*
Resistance to nucleotide analogues	Recommended therapeutic option
adefovir (LAM-naïve)	entecavir, tenofovir (TDF, TAF), (telbivudine), (lamivudine)
adefovir (LAM-resistant)	tenofovir (TDF, TAF)
tenofovir (no <i>in vivo</i> data available)	entecavir, (telbivudine), (lamivudine)

* in case tenofovir is not available

The combination of ADV and LAM in the presence of LAM resistance delays the development of ADV resistance considerably compared to switching to ADV monotherapy (Lampertico 2007). However, combination treatment consisting of one nucleotide and one nucleoside analogue is not necessary for the majority of patients if TDF is available. However combination of TDF with a nucleoside analogue might be useful in patients with multiple pre-treatments who have accumulated different resistance mutations (Petersen 2012, van Bömmel 2012). In a therapeutic setting where TDF is unavailable a combination treatment with ADV should be initiated if resistance to LAM, LdT or ETV occurs.

Treatment of HBV infections in special populations

Pregnancy. Globally, the vertical transmission from the mother to the newborn is the most frequent cause of HBV infection, and the highest risk is during delivery. A combination of hepatitis B immunoglobulin and vaccination given within 12 hours after birth can reduce the risk of perinatal

transmission from >90 to $<10\%$ (WHO 2015). Still for a neonate born to a mother with high levels of HBV DNA ($>200,000$ IU/mL) the risk of perinatal transmission is considerable. Therefore, antiviral treatment is generally recommended in these women (Cornberg 2011, EASL 2017, Terrault 2016, WHO 2015). PEG-IFN α is contraindicated in pregnant women. Antivirals studied in pregnant women are LAM, LdT and TDF. In pregnant women with high levels of HBV DNA, LAM treatment during the last trimester of pregnancy was reported to reduce the risk of intrauterine and perinatal transmission of HBV if given in addition to passive and active vaccination by HBIG and HBV (van Zonneveld 2003). LdT administered for an average of 15 weeks at the end of pregnancy plus active-passive immunisation to neonates reduced vertical transmission rates from 23% to 4% compared to immunisation alone (Han 2011). Because of its high antiviral potency, TDF is often considered the treatment of choice.

The risk of teratogenicity of NAs is assessed by a classification based on data gathered in clinical trials as well as through the FDA Pregnancy Registry. TDF and LdT are listed as pregnancy category B drugs and LAM, whereas ADV and ETV as category C drugs. However, other side effects for the new born cannot completely be ruled out. A recent study reported that bone mineral content of infants of HIV infected mothers exposed to TDF (N=74) was 12% lower than that of infants not exposed to TDF (n=69) (Siberry 2015). Although the significance of this observation is yet unclear, antiviral treatment during pregnancy should be carefully monitored and limited to the second and third trimester. However, the optimal treatment duration has not been studied. As exacerbations of the HBV infection may occur, women with HBV should be monitored closely after delivery (ter Borg 2008).

Immunosuppression. During immunosuppressive treatment, a reactivation of an asymptomatic or inactive HBV infection can occur in 20% to 50% of patients (Lok 2009). Reactivations can occur in HBsAg carriers, but also in HBsAg negative but anti-hepatitis B core antibody (HBc)-positive patients. These reactivations are characterised by an increase in HBV replication followed by an increase in liver inflammation during immune reconstitution resulting in liver damage or even liver failure in some patients (Feld 2010, Roche 2011).

HBV reactivation was especially frequently observed during treatment with corticosteroids and antitumour necrosis factor therapies (i.e., infliximab, etanercept, adalimumab), anti-CD20 therapies (i.e., rituximab-containing chemotherapy) and trans-arterial chemoembolisation for HCC (Vassilopoulos 2007, Moses 2006, Park 2005, Rutgeerts 2009, Mallet 2015).

Prior to initiating immunosuppressive therapies, screening for HBV infection is recommended (Lok 2009, EASL 2017). Pre-emptive treatment with nucleoside/nucleotide analogues should be initiated in all patients with

active HBV infection before any immunosuppressive treatment. HBsAg positive inactive HBV carriers have a diminished risk of HBV reactivation and mortality when pre-emptive treatment is conducted. Inactive carriers receiving immunosuppressive treatment with methotrexate or azathioprine in monotherapy represent an exemption as these patients have a low risk of HBV reactivation (Mallet 2016).

HBsAg negative/anti-HBc positive patients should only receive pre-emptive treatment if treatment with rituximab or human stem cell therapy is planned (Lok 1991, Zurawska 2012).

If available, a highly potent antiviral as ETV or TDF should be used for pre-emptive treatment. This recommendation is based on some recent reports revealing lower rates of HBV reactivation in patients treated with ETV as compared to patients treated with LAM. However, reactivations may still occur albeit in a low frequency. This was recently demonstrated in a randomised controlled trial of HBsAg negative/anti-HBc positive patients receiving chemotherapy including an anti-CD20 agent. In these patients, HBV reactivation occurred in 18% of the untreated compared to 2% of those patients receiving prophylaxis with ETV ($p < 0.05$) (Lau 2003).

Novel treatments for HBV infections

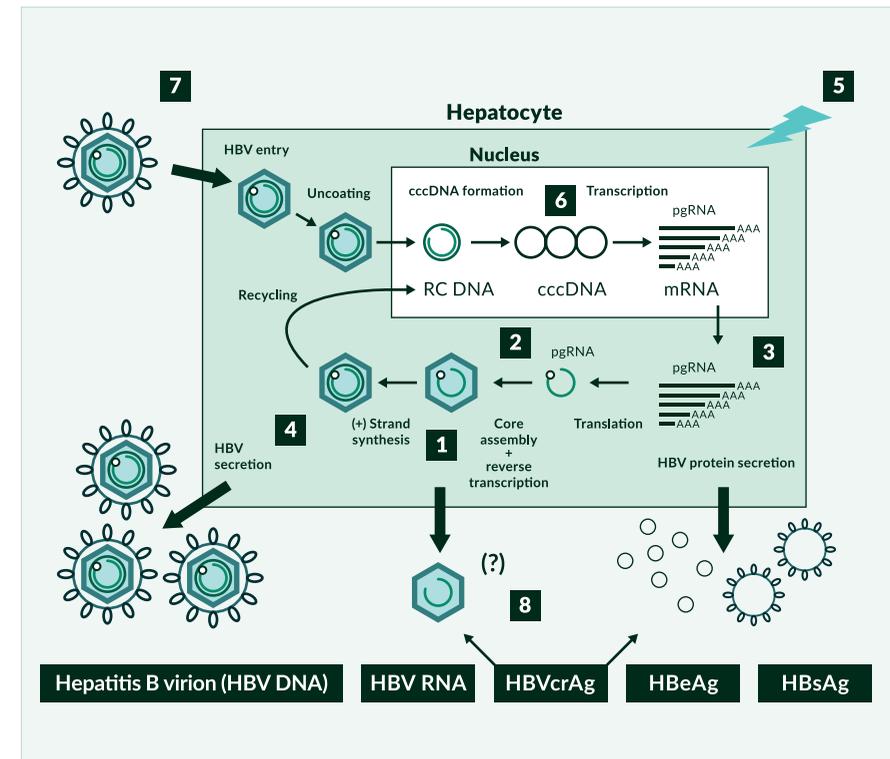


Figure 16. Novel approaches to treat HBV infections (selection). They include direct inhibition of the viral life cycle by HBV polymerase inhibitors (1), inhibition of pgRNA encapsidation with HBc allosteric modulators (CpAMs) (2), HBV RNA interference with siRNA molecules inhibition of HBsAg secretion by nucleic acid polymers (3), inhibition of HBsAg secretion by nucleic acid polymers (4), stimulation of the innate immune system as toll like or receptor stimulation or the adaptive immune system as check point (e.g., programmed cell death (PD-1)) inhibitors (5), cotransporting polypeptide degradation of cccDNA by APOBEC3A/B deminases or CRISPR-associated system 9 (Cas9) proteins (6) HBV entry through sodium taurocholate blocade (7). Novel HBV serum markers are currently investigated for their value in treatment response monitoring, including HBV RNA, HBV core related antigen (HBcrAg), quantitative HBeAg and HBsAg components (8).

The complete eradication of HBV from infected individuals cannot be achieved by any of the currently available treatment strategies, and this is due to persistence of HBV cccDNA. Trials investigating the possibility of improving outcomes in the treatment of HBV infections by combination treatment of PEG-IFN α with NAs in different doses and durations and by using novel NAs are ongoing. Besifovir (LB80380) is an acyclic nucleotide phosphonate with a molecular structure similar to that of ADV and TDF. In a phase IIb, open-label, multicentre study in 114 treatment naïve patients randomised to besifovir 90 mg or 150 mg daily or to ETV 0.5 mg daily for 48 weeks, an equally strong antiviral activity as compared to ETV was shown

for besifovir. Thus, suppression of HBV DNA to undetectable levels was found in 64, 63 and 58 %, and HBeAg seroconversion in 11, 15 and 9.5 %, respectively (Lai 2014). Of note, 94% of patients receiving besifovir had reduced serum L-carnitine, but the L-carnitine levels returned to normal with supplement.

Numerous novel substances are under investigation which might offer more potent suppression of HBV replication and, ideally, even eradication of the infection. Multiple strategies are being followed, some of which are targeting the innate or the adaptive immune system and some targeting the HBV replication cycle at different steps and to date it is not clear which approach is more promising. (Figure 17). Some of these novel drugs are investigated in pre-clinical or in early clinical studies and preliminary results have already been published for some approaches.

Thus, restoring the production of antiviral cytokines, which is often impaired in HBV infected individuals is followed by stimulation of toll like receptors (TLRs) which are located on plasmacytoid dendritic cells and myeloid cells. In HBV infected woodchucks it has been shown that treatment with the oral TLR7 agonist GS-9620 was followed by a marked decrease in serum HBsAg levels. HBsAg seroconversion occurred in several of these animals (Menne 2015). In contrast, in a trial in 26 patients with chronic HBV infection there was no effect of different doses of GS-9620 on HBsAg levels when given over 24 weeks (Boni 2016). However, stimulating effects of the HBV specific immune response were demonstrated including the acquisition of an activated natural killer cell type.

Interruption of HBV replication by interference with different key mechanisms of the HBV replication cycle is also currently investigated. The use of RNAi to inhibit the replication of HBV has been evaluated in animal models.

The siRNA molecules ARC-520 (phase II: NCT02604212 and NCT02604199; Arrowhead Research Corporation, Pasadena, CA, USA), ARB-1467 (phase II: NCT02631096; Arbutus Biopharma, Burnaby, British Columbia, Canada) and ALN-HBV (phase I/II: NCT02826018; Alnylam, Cambridge, MA, USA) are currently investigated in clinical trials. The compound ARC-520 was shown to induce a durable and deep suppression of HBV proteins and HBV DNA in a phase II study (Yuen 2015). The nucleic acid polymer (NAP) Rep2139 (Replicor, Montreal, Quebec, Canada), has been shown to inhibit the secretion of HBsAg by an unidentified mechanism. Its combination with pegIFN- α in clinical trials (NCT02233075) has been shown to result in a significant suppression of HBsAg and HBV DNA levels and a high rate of HBsAg seroconversion.

It is likely that these substances will be used in combination with either NAs or PEG-IFN α and there is a need for new bio markers which reflect the level of HBV replication and the efficacy of these new compounds when HBV DNA is suppressed to undetectable levels. For this purpose, new markers

such as quantitative HBeAg, HBV core-related antigen (HBVcrAg) and HBV RNA are currently under investigation and these molecules might be useful to tailor individual treatments and to increase response rates in the future.

An important goal for new drugs for treatment of HBV infections will be to move patients closer towards complete eradication of HBV. To date it seems, however, too early to predict the role of those novel compounds in future HBV treatments, but this fast developing field of research deserves continuous attention.

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10. Hepatitis D – diagnosis and treatment

Heiner Wedemeyer

Introduction

Hepatitis delta is the most severe form of viral hepatitis in humans. The hepatitis delta virus (HDV) is a defective RNA virus which requires the hepatitis B virus (HBV) surface antigen (HBsAg) for complete replication and transmission, while the full extent of the HBV helper function is unexplored (Rizzetto 1983, Taylor 2012). Hence, HDV occurs only in HBsAg positive individuals either as acute coinfection or as superinfection in patients with chronic HBV (Wedemeyer 2010) (Figure 1). Several studies have shown that chronic HDV infection leads to more severe liver disease than chronic HBV mono-infection, with an accelerated course of fibrosis progression, possibly a slightly increased risk of hepatocellular carcinoma and early decompensation in the setting of established cirrhosis (Hughes 2011, Manesis 2013, Beguelin 2017). Simultaneous HBV and HDV infection has also been shown to be more severe than infection with HBV alone in chimpanzees (Dienes 1990). An easy to apply clinical score has been suggested to predict the likelihood of experiencing a clinical event for patients with HDV, the baseline-event-anticipation (BEA) score (Calle-Serrano 2014). So far, only interferon α treatment is recommended against HDV (Deterding 2019) and has been linked to improve the clinical long-term outcome (Farci 2004, Wranke 2017, Yurdaydin 2018). Data on the use of pegylated interferon (PEG-IFN) confirm earlier findings, leading to prolonged virological off-treatment responses in about one quarter of patients but long-term HDV RNA relapses may occur (Heidrich 2014). Thus, HBsAg clearance should be the preferred endpoint of interferon-based therapies of HDV. Still, suppression of HDV RNA in the presence of HBsAg has been associated with improved clinical outcomes. Alternative treatment options including HBV entry inhibitors and prenylation inhibitors (www.clinicaltrials.gov) are currently in phase 3 clinical development.

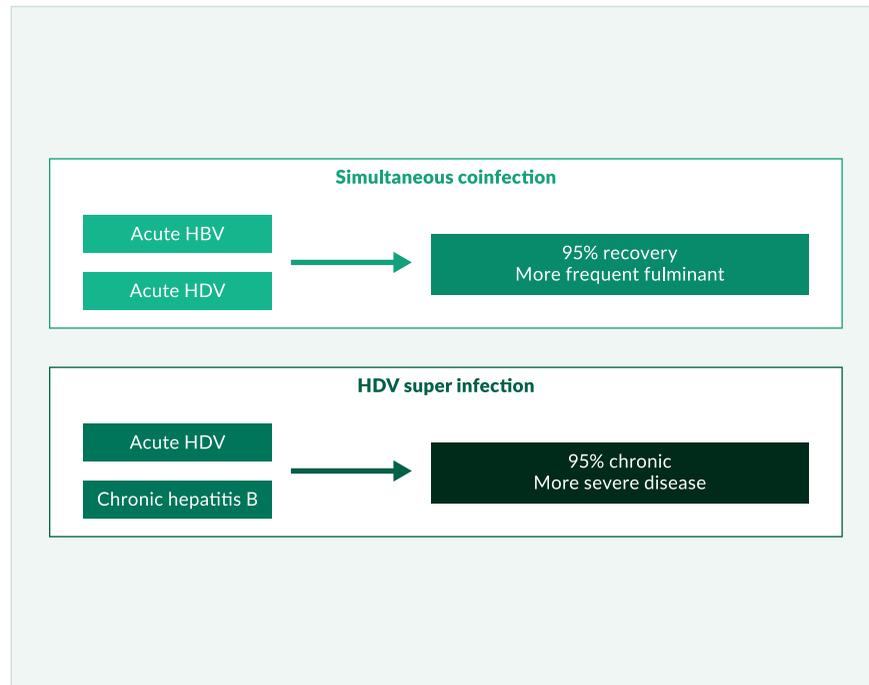


Figure 1. Courses of hepatitis delta

Virology of HDV

The hepatitis D virion is approximately 36 nm in size, containing HDV RNA and delta antigen. HDV RNA is single-stranded, highly base-paired, circular and by far the smallest known genome of any animal virus, containing close to 1700 nucleotides (Taylor 2012, Sureau 2016). It is coated with the envelope protein derived from the pre-S and S antigens of HBV. Other enveloped viruses including HCV and VSV can also propagate HDV infection, both in vitro as well in humanized mice (Peres-Vargas 2019). Still, it is currently unclear if viruses distinct from HBV induce dissemination of HDV also in patients. The HDV RNA has six open reading frames (ORFs), three on the genomic and three on the antigenomic strand. One ORF codes for the hepatitis delta antigen (HDAg), while the other ORFs do not appear to be actively transcribed. Two HDAgs exist: the small HDAg (24 kD) is 155 amino acids long and the large HDAg (27 kD) is 214 amino acids long. A single nucleotide change (A-G) in the small HDAg sequence leads to the synthesis of the large HDAg. The small HDAg accelerates genome synthesis, while the large HDAg that inhibits HDV RNA synthesis is necessary for virion morphogenesis (Taylor 2012). Replication of HDV RNA occurs through a 'double rolling circle' model in which the genomic strand is replicated by a host RNA polymerase to yield a multimeric linear structure that is then

autocatalytically cleaved to linear monomers and ligated into the circular HDV RNA viral progeny (Sureau 2016). Recent work showed that the host RNA polymerase II is coactivated by S-HDAg using a histone mimicry strategy (Abeywickrama-Samarakoon 2020).

Genetic analysis has revealed the presence of at least eight HDV genotypes (Le Gal 2017) (Figure 2). Genotype 1 is the most frequently seen and is distributed throughout the world, especially in Europe, the Middle East, North America and North Africa. Genotype 2 is seen in East Asia and the Yakutia region of Russia, and genotype 3 is seen exclusively in the northern part of South America, especially in the Amazon basin. Genotype 4 is seen in Taiwan and Japan while genotypes 5–8 are found in Africa (Deny 2006). HDV genotype 1 is associated with both severe and mild disease whereas genotype 2 causes a milder disease over a long-term course (Su 2006). HDV genotype 5 may also take a milder course and a better response to PEG-IFN α treatment compared to genotype 1 (Spaan 2020).

HDV quasispecies evolution declines over time during HDV infection even though a continuous adaptation of HDV occurs indicating ongoing immune pressure in chronic HDV (Homs 2016).

HBV genotypes may also contribute to distinct clinical courses of HDV. There is no evidence that specific HDV genotypes may infect patients with one specific HBV genotype exclusively. However, recent data indicate that distinct HDV mutations may facilitate association of certain HDV genotypes with different HBV genotypes (Kay 2014). The global distribution of HBV and HDV genotypes is shown in Table 1.

Table 1. HBV and HDV genotypes

Region	HDV genotype	HBV genotype
Europe	1	D/A
Brazil	1/3	F/A/D
China, Taiwan, Japan	1/2/4	B/C
Turkey, Iran, Pakistan, India	1	D
Western Pacific	1/2	B/C/D
Africa	1, 5–8	D/A/E



Figure 2. Prevalence of HDV genotypes

Epidemiology of HDV

HDV is not an uncommon disease. Being linked to HBV, HDV is spread in the same way as HBV, mainly through parenteral exposure (Niro 1999). It is highly endemic in Mediterranean countries, the Middle East, Central Africa, and northern parts of South America (Hughes 2011) (Figure 2). In high-income countries, high anti-HDV prevalence is found in people who inject drugs (PWID) who are HBsAg positive, both in Europe (Gaeta 2000, Heidrich 2009, Erhardt 2010) and North America (Kurcirka 2010). Worldwide, more than 240 million people are chronically infected with HBV and 15–25 million of those are estimated to be anti-HDV positive (Wedemeyer 2010). Two systematic reviews suggested that the prevalence of HDV may be even higher reaching up to 1% of the populations worldwide (Chen 2018, Miao 2019). This work has been criticized as systematic reviews can only be as good as studies included (Wedemeyer 2018). but HDV may still be more frequent than previously thought. HDV was endemic in Southern Europe. Several studies performed in the 1980s and 1990s showed a prevalence of anti-HDV of more than 20% among HBsAg positive individuals. As a result of the implementation of HBV vaccination programmes, the incidence of HDV infections significantly decreased in Southern Europe in the 1990s (Gaeta 2000, Degertekin 2008) (Figure 3). Other countries with a particularly high prevalence of HDV are Mongolia with up to one third of chronic hepatitis cases being caused by HDV (Tsatsralt-Od 2005), some Central Asian republics, Pakistan (Abbas 2012),

northwestern states of Brazil (Kay 2014, Braga 2014), distinct regions in Africa (Andernach 2014), and some Polynesian islands (Han 2014). Of note, prevalence rates of HBV and HDV are not linked - for example, HDV infections have been considered to be rather rare in most parts of mainland China despite very high frequencies of HBV. However, some studies revealed an HDV prevalence of up to 6.5%, suggesting that HDV may be more frequent in China than previously thought (Lia 2014). HBV/HDV coinfection was also associated with higher frequencies of end-stage liver disease in that study. In Taiwan, a country with a well-established national HBV vaccination program, the epidemiology of HDV changed over the last 20 years with PWID and HIV positive persons being particular risk groups and representing a main reservoir for HDV infection (Hung 2014, Lin 2015, Lee 2015).

One problem is that many HBsAg positive patients are not tested for HDV. A study from Greece even suggests that HDV testing declined over the last 10 years and only about one-third of people with HBV are currently assessed for the presence of HDV antibodies (Manesis 2013). Similarly, the HDV testing rate was low in four hospitals in London where people with HDV frequently had severe disease and patients were of very diverse ethnicity (El Bouzidi 2015). In the United States Veterans Affairs medical system, only 8.5% of more than 25,000 HBsAg positive patients were tested for HDV. Of those, 3.4% had evidence for HDV and HDV was associated with a 2.9 fold higher HCC incidence and a higher risk of all-cause mortality (Kushner 2015).

In our experience at a referral centre for liver disease, about 8–10% of HBsAg positive patients test positive for anti-HDV as chronic HDV still represents a significant health burden in Central Europe, which is a source of immigration (Wedemeyer 2007, Heidrich 2009, Erhardt 2003, Erhardt 2010) (Figure 3, Table 1). More than three quarters of these HDV patients were not born in Germany. However, the geographical origin of our patients has changed during the last decade. While until the mid-1990s the majority of HDV positive patients were born in Turkey, the proportion of Eastern European patients has significantly increased in recent years (Wedemeyer 2007). Similarly, high HDV prevalence in immigrant populations has been described in clinics in the UK (Cross 2008), France and Italy (Le Gal 2007, Mele 2007). HDV can also be found in high frequencies in people living with HIV who are also HBsAg positive with about 14.6% in different European regions (Soriano 2011). In France, the prevalence of HDV infection has increased during the last 15 years, again mainly in pre-infected newly arriving immigrants (Servant-Delmas 2013).

HDV prevalence is much lower in HBV patients without specific risk factors and cohorts excluding a referral bias. In this setting, less than 1–2% of HBsAg positive individuals test anti-HDV positive, even in countries

like Italy where HDV prevalence is thought to be higher than in Northern Europe (Ippolito 2011). Thus, even though HDV is a major problem in distinct regions and specific cohorts, HDV is overall a rare disease and has therefore been granted orphan designation both by the FDA and by the European Commission.

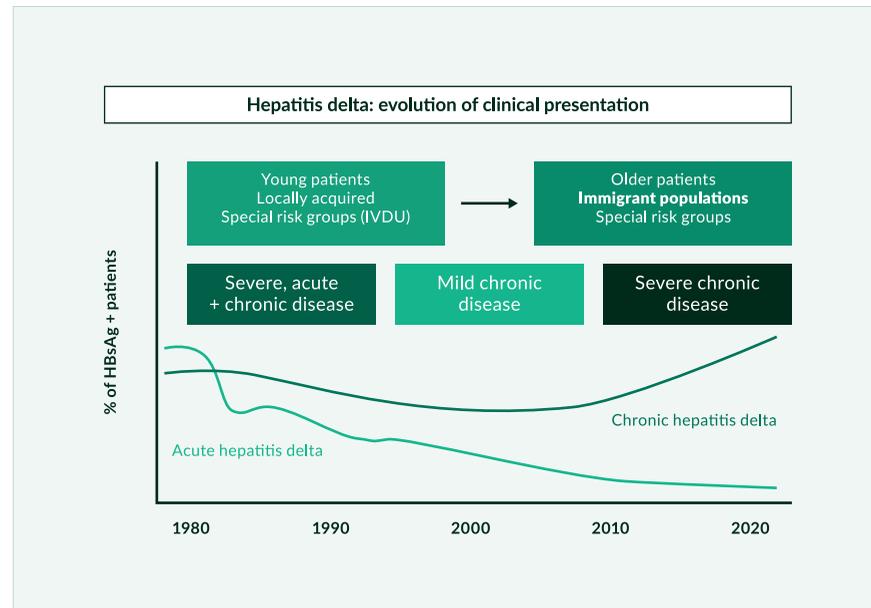


Figure 3. Hepatitis delta: evolution of clinical presentation

Pathogenesis of HDV

Knowledge about the pathogenesis of HDV infection is limited. Clinical observations have provided examples of mostly an immune-mediated process in HDV (Lunemann 2010). However, patterns suggesting a cytopathic viral disease have occasionally been observed. A typical example of this were outbreaks of severe hepatitis in the northern part of South America (Nakano 2001). These mostly fulminant hepatitis cases were induced by genotype 3 HDV. In HDV, liver histology is not different from a patient with HBV or HCV with accompanying necroinflammatory lesions. Importantly, HDV viraemia is not directly associated with the stage of liver disease in HDV genotype 1 infection (Zachou 2010) while in HDV genotype 3 infection higher viral loads were observed in patients with cirrhosis (Braga 2014). In both humanised chimeric mice as well as mice expressing the human HBV receptor (sodium taurocholate co-transporting polypeptide (NTCP)) HDV infection provoked a marked and broad induction of interferon

stimulated genes and cytokines which was more pronounced than in HBV mono-infection (Giersch 2015, He 2015) which may directly contribute to the more severe inflammation in patients with HDV. Another study showed that modification of three amino acids in mouse NTCP (H84R, T86K, and S87N) rendered mice susceptible to HDV (He 2016). In this respect it is important to note that distinct polymorphisms in the IL28B gene may be associated with HBsAg persistence also in HDV coinfecting patients (Karataylı 2015).

Cellular immune responses against the HDV have been described (Nisini 1997, Huang 2004, Grabowski 2011) suggesting that the quantity and quality of T cell responses may be associated with some control of the infection. The frequency of cytotoxic CD4⁺ T cells is higher in HDV patients than in individuals with HBV or HCV (Aslan 2006) and HDV-specific IFN gamma and IL-2 responses are more frequent in patients with low HDV viraemia (Grabowski 2011). Still, HDV-specific T cell responses are very weak in chronic infection. *In vitro*, the third signal cytokine IL-12 was able to restore the function of HDV-specific CD4⁺ and CD8⁺ T cells (Schirdewahn 2017). Still, HDV-specific T cells have been shown to induce immune pressure on HDV during leading to the development of T cell escape variants (Karimzadeh 2018; Karimzadeh 2019; Kefalakes 2019). However, in another study the breadth of HDV-specific T cell responses was not associated with the HDV replication status in patients (Landahl 2019). NK cells from patients with HDV have recently been investigated in more detail in comparison with other viral hepatitis infections (Lunemann 2014). Overall, NK cell frequencies increased but the cells were less activated and functionally impaired. HDV infection also did not alter NK cell differentiation, and the activity of liver disease reflected alterations in NK cell surface receptor expression. NK cell frequency may also be associated with early virological response to PEG-IFN α therapy although NK cells are severely functionally impaired during antiviral therapy (Lunemann 2015). Finally, mucosa-associated invariant T (MAIT) cells, which are innate-like T cells highly enriched in the human liver, are activated, functionally impaired and severely depleted in patients with chronic hepatitis D (Dias 2019). This loss of MAIT cells was associated with severity of liver disease. Collectively, this information suggests that HDV is mainly an immune-mediated disease, at least in HDV genotype 1 infection. Ideally, antiviral therapies should therefore also aim to enhance anti-HDV immunity to confer long-term control of the infection. Of note, chimpanzees that have recovered from HDV were successfully reinfected with HDV in one study performed in the 1980s (Negro 1988).

Coinfections with multiple hepatitis viruses are associated with diverse patterns of reciprocal inhibition of viral replication (Raimondo 2006, Wedemeyer 2010). HDV has frequently been shown to suppress HBV replication (Calle Serrano 2012). Between 70% and 90% of HDV patients are HBeAg negative with low levels of HBV DNA. Humanised HBeAg positive mice

that become superinfected with HDV also show a decrease in HBV replication (Lütgehetmann 2012). A molecular explanation for the suppression of HBV replication by HDV has been suggested via the HDV proteins p24 and p27 repressing HBV enhancers (Williams 2009). In addition, induction of a type-I interferon response by HDV may contribute to HBV repression. This hypothesis is supported by the induction of interferon stimulated genes in HBV cells which were superinfected with HDV which led to a decrease of HBV replication markers (Alfaiate 2016). Viral dominance may change over time (Wedemeyer 2010) and about half of the hepatitis delta patients showed significant HBV replication in one study (Schaper 2010). A recent study from Brazil reported similar viral loads for HBV and HDV in 40% of patients infected with HDV genotype 3 and HDV dominance in 56% (Braga 2014). HDV may facilitate the selection of distinct HBV mutants which can have major implications for the replicative capacity of both viruses (Shirvani-Dastgerdi 2016). HDV entry into hepatocytes via NTCP may also be altered by the bile acid pool (Yan 2014, Veloso Alves Pereira 2015) even though administration of chenodeoxycolic acid to three chronically infected patients did not lead to a change in serum HDV RNA levels (Veloso Alves Pereira 2015).

There is increasing evidence that HDV not only suppresses HBV replication but also HCV replication in triple-infected patients. In our experience, less than one fifth of anti-HCV/HBsAg/anti-HDV positive individuals are positive for HCV RNA (Heidrich 2009). We even observed a case where acute HBV/HDV superinfection led to clearance of chronic HCV infection (Deterding 2009). It is not clear how many anti-HCV positive/HCV RNA negative patients have recovered from HCV infection and how many of these patients just show a suppressed HCV replication in the context of viral coinfections. Repeated HCV RNA testing is suggested in this context. We did not observe HCV relapses after interferon-induced cure of HDV (Wedemeyer 2011).

HDV may also play a direct role in the development of hepatocellular carcinoma by altering DNA methylation events (Benegiamo 2013). However, to what extent HDV infection is associated with an increased HCC risk is a matter of debate. Even though liver cancer can be found more frequently in patients with HDV (Manesis 2013, Romeo 2014), this may be explained by earlier development of liver cirrhosis and may not necessarily be a consequence of the direct oncogenic effects of HDV.

HDV might also have a role in other autoimmune diseases. HDV has been detected in salivary gland tissue of patients with Sjögren's syndrome (SS) in the absence of HBsAg (Weller 2016). Interestingly, the expression of HDV antigens in salivary glands in mice resulted in the development of a SS-like phenotype. These findings have to be confirmed by others and it would be also interesting to investigate if HDV may cause similar pathology in other tissues.

Clinical course of HDV

Acute HBV/HDV coinfection

Acute HBV/HDV coinfection in adults leads to recovery in more than 90% of cases but frequently causes severe acute hepatitis with a high risk for developing a fulminant course (Rizzetto 2009). In contrast, HDV is cleared spontaneously only in a minority of patients with HDV superinfection of chronic HBsAg carriers (Figure 1). The observation that the histopathology of simultaneous HBV and HDV infection is more severe than in infection with HBV alone has also been documented in experiments with chimpanzees (Dienes 1990). Several outbreaks of very severe courses of acute HDV have been described in different regions of the world (Casey 1996, Flodgren 2000, Tsatsralt-Od 2006). Fortunately, acute HDV has become infrequent over the last two decades in high-income countries due to the introduction of vaccination programs (Figure 3).

Chronic HDV

Several early studies showed that chronic HDV leads to more severe liver disease compared to chronic HBV monoinfection, with an accelerated course of fibrosis progression, and early decompensation in the presence of cirrhosis (Farci 2012). HDV accounts for almost half of all cases of liver cirrhosis and hepatocellular carcinoma in southeast Turkey (Degertekin 2008). An observational study from Taiwan reported a cumulative survival of patients with HDV genotype 1 of as low as 50% after 15 years (Su 2006). Long-term follow-up data from Italy, Spain, Greece and Germany confirmed the particularly severe course of HDV (Romeo 2009, Niro 2010, Butí 2011, Manesis 2013, Calle-Serrano 2014). Characteristics of patients with HDV genotype 3 infection were reported in more detail recently (Braga 2014) confirming the severity of liver disease also for this specific HDV genotype. HDV infection has been associated with a particular high risk of developing liver cirrhosis in people who are living with HIV (Calle-Serrano 2012, Fernandez-Montero 2014). In one cross-sectional study from Spain, 66% of people coinfecting with HIV/HBV/HCV/HDV presented with liver cirrhosis compared to only 6% of people coinfecting with only HBV/HCV/HIV (Castellares 2008) and this translated to higher rates of liver decompensation and death (Fernandez-Montero 2014). Similarly, HDV was associated with poorer survival in HIV positive people in Taiwan (Sheng 2007, Lee 2013) and in the Swiss HIV cohort study (Beguelin 2017). The Swiss study showed a prevalence of HDV of 15.4% and showed a 2.3 fold increased risk of overall death for those coinfecting with HIV/HDV. Of note, the association of HDV with mortality and liver-related

complications including HCC was independent from ongoing drug injection or HCV coinfection.

An easy-to-apply clinical score, the baseline-event anticipation (BEA) score, has been suggested to predict the risk of developing liver-related morbidity and mortality (Calle-Serrano 2014). Factors associated with a poor long-term outcome included age above 40, male sex, low platelet counts, high bilirubin and INR values and southeast Mediterranean origin. The score differentiated patients with a benign (BEA-A), intermediate (BEA-B) and severe (BEA-C) mid-term course of HDV infection and is available on www.hepatitis-delta.org. Anti-HDV IgM testing may also be useful as anti-IgM levels are associated with activity of liver disease (Mederacke 2012). The majority of people with HDV test positive for HDV-specific IgM antibodies but IgM negative patients did not develop clinical complications in a retrospective-prospective follow-up study from Germany (Wranke 2014). Thus, clinical parameters may be used to decide if a patient should be considered for antiviral therapy with PEG-IFN α . Currently, treatment may be deferred for some time in individuals with a BEA-A score or in patients who are anti-HDV IgM negative.

Diagnosis of HDV

We recommend that everyone who is HBsAg positive be tested for anti-HDV antibodies at least once (Figure 4). There is currently no evidence that direct testing for HDV RNA in the absence of anti-HDV is of any use. A positive result for anti-HDV does not necessarily indicate active HDV, as HDV RNA can become negative indicating recovery from HDV infection. Also, over the long-term, anti-HDV antibodies can be lost after HDV recovery. However, anti-HDV may persist for years even when the patient has experienced HBsAg seroconversion and anti-HDV remains detectable in most patients even after liver transplantation when HBsAg and HDV RNA are cleared (Mederacke 2012).

Active replicative HDV should be confirmed by the detection of HDV RNA. If HDV RNA is positive, subsequent evaluation of grading and staging of liver disease, surveillance for hepatocellular carcinoma and consideration of antiviral treatment is indicated. HDV RNA quantification is offered by some laboratories. However, so far there is no consistent evidence that HDV RNA levels are strongly correlated with histological markers of liver disease (Zachou 2010) even though high HDV RNA levels may be predictive of developing cirrhosis and HCC in the long term (Romeo 2014). Another recent study in HDV genotype 3 infection also showed an association between HDV RNA levels and serum levels of liver enzymes (Braga 2014). HDV RNA quantification is useful in particular if antiviral treatment is

indicated. Stopping rules during antiviral treatment depending on the level of antiviral decline are currently being evaluated. A WHO standard for HDV has been released which allows comparison of performances of various PCR assays that have been published in recent years (Mederacke 2010, Niro 2011, Katsoulidou 2013, Bothelo-Souza 2014). Even commercial assays may show limited performance in detecting and quantifying HDV RNA (Brichler 2013). An international quality assessment study involving 28 laboratories globally revealed a very high heterogeneity of assay characteristics (Le Gal 2016). Less than half of the laboratories quantified all HDV RNA positive samples and reported quantitative values varied largely between the laboratories.

HDV genotyping is performed by some research labs and may help to identify patients with a higher or lower risk of developing end-stage liver disease (Su 2006). In high-income countries, almost all patients are infected with HDV genotype 1, thus genotyping may be considered mainly in immigrants or populations with mixed genotype prevalence.

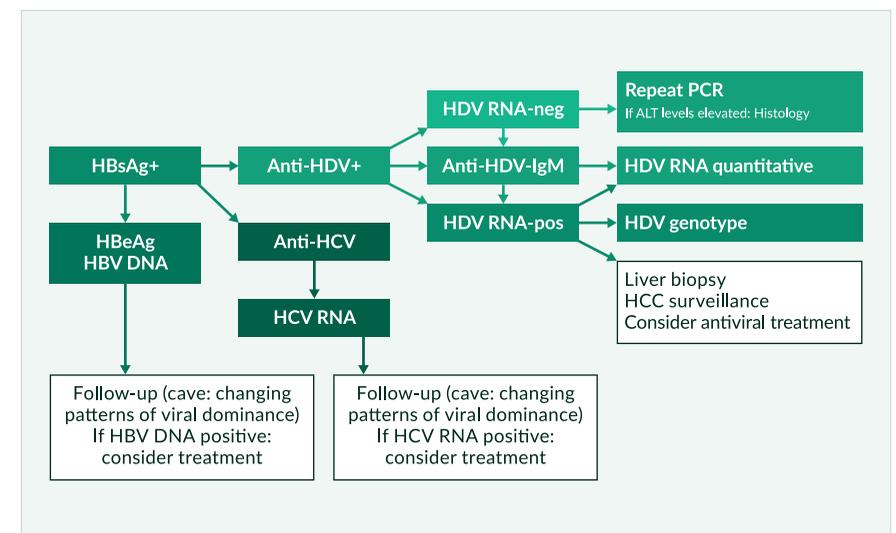


Figure 4. Diagnostic steps in HDV

During the 1980s and 1990s, the diagnosis of active HDV was dependent on anti-HDV IgM testing. Anti-HDV IgM testing might still be useful in patients who test HDV RNA negative but have evidence of liver disease, which cannot be explained by other reasons. Due to the variability of the HDV genome and the lack of standardisation of HDV RNA assays, HDV RNA may test false negative or be under the detection limit of the assay in the case of fluctuating viral load. In these cases, HDV RNA testing should be repeated and anti-HDV IgM testing might be performed, if available. Anti-HDV IgM levels also correlate with disease activity (Wranke 2014) and may be

predictive for response to IFN α -based antiviral therapy (Mederacke 2012).

As HDV occurs only in the context of HBV coinfection, a solid work-up of HBV infection including HBV DNA quantification and HBeAg/anti-HBe determination is warranted. Between 10% and 20% of HDV patients are HBeAg positive. Of note, HBV DNA is suppressed even in HBeAg positive hepatitis (Heidrich 2012) suggesting that the inhibitory effect of HDV on HBV is independent from the phase of HBV infection. The long-term clinical outcome of anti-HDV positive patients did not differ between HBeAg positive and HBeAg negative individuals in one study from Germany (Heidrich 2012). Most HDV patients in Europe are infected with HBV genotype D but infection with genotype A can also occur (Soriano 2011) which may have significant implications for treatment decisions, as HBV genotype A shows a better response to interferon α therapy – which however needs to be confirmed in the context of HDV coinfection. Similarly, testing for anti-HCV and anti-HIV is mandatory. Up to one third of anti-HDV positive patients can also test positive for anti-HCV (Heidrich 2009).

Quantitative HBsAg levels correlate with HDV RNA levels in HDV infection (Shih 2008). Higher HBsAg levels may also indicate more severe histological disease activity (Zachou 2010). Thus, a determination of quantitative HBsAg values also has some clinical relevance in patients with HDV. Monitoring of quantitative HBsAg levels should be performed in all patients undergoing antiviral therapies as long-term interferon therapy of HDV should be individualised until HBsAg is lost (Heller 2014, Guedj 2014).

Staging of liver disease is of particular importance in HDV as treatment options are limited and as the only possible therapy interferon α can lead to frequent and sometimes severe side effects. Various non-invasive serum markers have been developed to predict liver fibrosis and cirrhosis in HCV, HBV and NASH. However, scores such as APRI, FIB-4 or AST/ALT ratio have to be used with caution in HDV infection as they are of limited value in HDV (Lutterkort 2017, Takyar 2017). Novel score specifically developed for HDV have been proposed. One score is based on serum cholinesterase, gamma glutamyl transferase, albumin and age and has been validated in European patients (Lutterkort 2017). Another score included gamma-glutamyl transpeptidase, platelet count, alanine aminotransferase, and liver stiffness measurement (Da 2019). Transient elastography has been shown to be useful in staging HDV patients with similar cut-off values to determine liver cirrhosis as in HCV infection (e.g. 14 kPa) (Da 2019b).

Treatment of HDV

Nucleoside and nucleotide analogues

Several nucleoside and nucleotide analogues (NA) used for the treatment of HBV infection have no direct antiviral effects against HDV as HDV uses host polymerases for replication. Nevertheless, NAs have been studied in patients with HBV-HDV coinfection with the idea that beneficial effects may arise due to indirect mechanisms (Table 2).

Famciclovir, used in the 1990s to treat HBV (Wedemeyer 1999), had no significant antiviral activity against HDV in a Turkish trial (Yurdaydin 2002). Similarly, lamivudine was ineffective in trials of HDV (Wolters 2000, Niro 2005a, Yurdaydin 2008, Lau 1999b). Ribavirin alone or in combination with interferon also did not lead to increased rates of HDV RNA clearance (Niro 2005a, Gunsar 2005, Garripoli 1994). None of the patients treated with adefovir monotherapy for 12 months became HDV RNA negative in the HIDIT-1 trial (Wedemeyer 2011). Similarly, short-term entecavir treatment did not show significant activity against HDV (Kabacam 2012b). However, a long-term observational study of HIV positive people receiving antiretroviral therapy (ART) followed individuals coinfecting with HBV/HDV/HIV for a median of more than six years. Over this time, a decline of HDV RNA from 7 log₁₀ to 5.8 log₁₀ was observed and 3 out of 16 patients became HDV RNA negative (Sheldon 2008). Thus, very long treatment with HBV polymerase inhibitors may lead to beneficial effects in coinfecting, possibly due to a reduction of HBsAg levels (Figure 5). These earlier findings were confirmed in a recent report from the same group describing HDV RNA negativation in 10/19 patients after a median use of tenofovir-DF (TDF) of 58 months (Soriano 2014). It is interesting to note that HDV RNA declines were not associated with HBsAg declines in this analysis. Importantly, HDV RNA negative patients also showed improvements in liver stiffness values, while this was not the case in subjects who remained HDV RNA positive. A small case series seemed to confirm the observation that ART could modify the clinical course of HDV infection in HIV positive patients (Onali 2015). In the SWISS HIV cohort, tenofovir-containing ART was associated with relevant HDV RNA declines in 29% of patients and 14% had undetectable HDV RNA after 5 years (Beguelin 2017b). Future long-term trials will need to confirm these data in triple-infected individuals and potential mechanism need to be studied. One hypothesis is that tenofovir may induce interferon lambda (Murata 2019) which has been shown to exert also direct antiviral effects against HDV (Giersch 2017). Considering the favorable safety profile, TDF may be considered for patients with HDV in the absence of alternative treatment options - e.g., for interferon-intolerant patients. Still there is currently no evidence that nucleoside or nucleotide analogue therapy is

associated with a reduction of clinical complications of HDV as recently shown in a German single centre study (Wranke 2017).

Table 2. Treatment options in HDV

Nucleos(t)ide analogues	
Famciclovir ineffective	Yurdaydin 2002
Lamivudine ineffective	Wolters 2000, Lau 1999, Niro 2005a, Niro 2008, Yurdaydin 2008
Ribavirin ineffective	Niro 2006, Garripoli 1994, Gunsar 2005
Adefovir ineffective (12 months)	Wedemeyer 2011
Entecavir ineffective (12 months)	Kabacam 2012b
Tenofovir no evidence of short-term effect; long-term treatment associated with HBsAg and HDV RNA decline in some patients	Sheldon 2008, Soriano 2014, Beguelin 2017b
Interferon α	
Sustained biochemical responses in 0–36% of patients Few studies with virological endpoints Treatment >12 months may be required	Farci 1994, Di Marco 1996, Niro 2005b, Yurdaydin 2008
Higher IFN doses were associated with better survival in small study cohort	Farci 2004

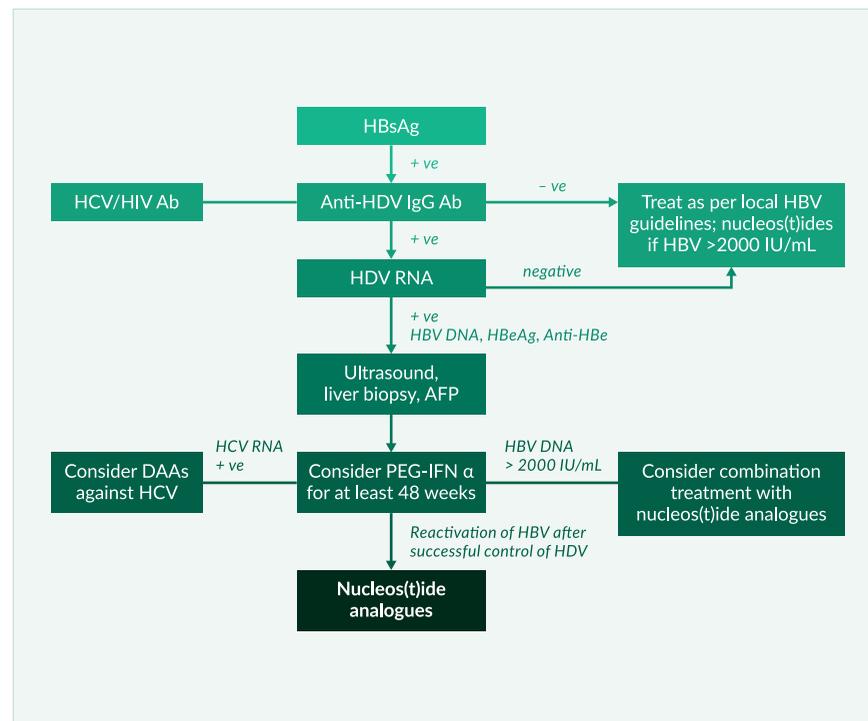


Figure 5. Treatment algorithm for HDV

One promising and surprising alternative to the currently approved HBV polymerase inhibitors may have been clevudine. Clevudine, a nucleoside analogue no longer in development for the treatment of HBV, was shown to inhibit HDV in woodchucks (Casey 2005). However, a first pilot trial showed no significant HDV RNA declines in humans (Yakut 2010).

Recombinant interferon α

Interferon α has been used for the treatment of HDV since the mid-1980s (Rizzetto 1986). Since then, many trials have explored different durations and doses of interferon α in people with HDV. However, data are difficult to compare as endpoints are different in the trials and few studies have followed HDV RNA levels over time (Niro 2005b).

An Italian study reported a beneficial long-term outcome in hepatitis delta patients randomised to high-dose interferon α (Farci 1994, Farci 2004). These findings were confirmed by a retrospective single centre study showing that interferon-based antiviral therapy was an independent factor associated with a lower frequency of liver-related clinical complications (Wranke 2017, Yurdaydin 2018). Some studies have used extended doses of interferon treatment and it seems that two years of treatment is superior in terms of HDV RNA clearance (Niro 2005b). In a case report from the US NIH, 12 years of interferon treatment led finally to resolution of both HDV infection and HBsAg clearance (Lau 1999a). However, only minority of people can tolerate high doses of extended treatment interferon and treatment options are very limited for the majority (Manns 2006, Deterding 2019).

Pegylated interferon α

PEG-IFN α has been used in small trials to treat HDV, with post-treatment virological response rates of about 20% (Castelnaud 2006, Niro 2006, Erhardt 2006) (Table 3).

Table 3. Pegylated interferon in hepatitis delta

Study	Course of therapy	Outcome*
Castelnaud, Hepatology 2006	12 months of PEG-IFN α -2b (n=14)	FU24R in 6 patients (43%)
Niro, Hepatology 2006	72 weeks of PEG-IFN α -2b (n=38) - Monotherapy: n=16 - PEG-IFN + ribavirin during first 48 weeks: n=22	FU24R in 8 patients (21%) Ribavirin had no additional effect
Erhardt, Liver Int 2006	48 weeks of PEG-IFN α -2b (n=12)	FU24R in 2 patients (17%)
Wedemeyer, NEJM 2011	a) 48 weeks PEG-IFN α -2a + adefovir (n=31) or b) PEG-IFN α -2a + placebo (n=29) or c) adefovir (n=30)	FU24R Group a) 26% Group b) 31% Group c) 0%
Ormeci, Hepatogastroenterology 2011	PEG-IFN α -2b 24 months (n=11) vs. 12 months (n=7)	No additional benefit of extended therapy
Karaca, Antivir Ther 2013	24 months PEG-IFN α -2a (n=32)	FU24R in 15 patients (47%)
Abbas, Antivir Ther 2014	48 weeks PEG-IFN α -2a (n=104)	FU24R in 24 patients (23%)
Wedemeyer, Lancet Infect Dis 2019	96 weeks PEG-IFN α -2a with or without tenofovir (n=120)	FU24R in 32 patients (27%)
Heller, AP&T 2014	PEG-IFN α -2a for up to 5 years (dose up to 270 μ g/week)	4/12 patients HDV RNA negative, 3/12 HBsAg loss
Niro, AP&T 2016	Treatment with PEG-IFN α . retrospective analysis of HBsAg kinetics	HBsAg and HDV RNA decline at month 6 predict long-term response

*FU24R: "Follow-up week 24 response" meaning HDV RNA negativity 24 weeks after the end of therapy. The term SVR should be avoided as late HDV RNA relapses may occur and thus an early off-treatment response may not necessarily be sustained.

Results of the Hep-Net International Delta hepatitis Intervention Trial (HIDIT-1) were published in 2011 (Wedemeyer 2011). 90 patients (42 in Germany, 39 in Turkey and 9 in Greece) with chronic HDV and compensated liver disease were randomised to receive either 180 μ g PEG-IFN α -2a QW plus 10 mg adefovir dipivoxil QD (group A, n=31), 180 μ g PEG-IFN α -2a QW plus placebo (group B, n=29) or 10 mg adefovir dipivoxil QD alone (group C, n=30) for 48 weeks. HBV DNA and HDV RNA were measured by real-time PCR. Ten patients did not complete 48 weeks of therapy because of disease progression (n=6) or interferon-associated side effects (n=4). Both PEG-IFN groups showed a significantly higher reduction in mean HDV RNA levels than the adefovir monotherapy group by week 48. HDV RNA was negative

24 weeks after the end of treatment in 28% of patients receiving PEG-IFN but in none of those treated with adefovir alone. While patients receiving PEG-IFN α -2a alone or adefovir monotherapy had similar mean HBsAg levels at week 0 and week 48, the PEG-IFN α -2a + adefovir combination group showed a 1.1 \log_{10} IU/mL decline of HBsAg levels by week 48 ($p < 0.001$) with 10/30 patients achieving a decline in HBsAg of more than 1 \log_{10} IU/mL. These data are similar to a report from Greece of a significant decline in HbsAg levels in patients with HDV receiving long-term treatment with interferon α (Manesis 2007).

Overall the HIDIT-1 study showed that (i) PEG-IFN α -2a displays a significant antiviral efficacy against HDV in more than 40% of patients with about one fourth becoming HDV RNA negative after 48 weeks; (ii) adefovir dipivoxil has little efficacy in terms of HDV RNA reduction but may be considered for patients with significant HBV replication; (iii) combination therapy of PEG-IFN α -2a plus adefovir has no advantages for HBV DNA or HDV RNA reduction; (iv) a combination therapy of PEG-IFN + adefovir was superior to either monotherapy in reducing HBsAg levels in patients with HBV (Wedemeyer 2011). However, adefovir treatment was associated with a decline in glomerular filtration rates (Mederacke 2012) and thus PEG-IFN α + adefovir combination treatment cannot be recommended as first-line treatment for all patients with HDV. Treatment was safe and effective in patients with compensated liver cirrhosis (Kabacam 2012a), however treatment is not recommended in individuals with more advanced liver disease as liver decompensation may occur (Heidrich 2013). Overall, findings of the HIDIT-1 trial were largely in line with other subsequent studies of patients treated in Pakistan (Abbas 2014), Turkey (Ormeci 2011) or at the NIH in the United States (Heller 2014) (Table 3). PEG-IFN α induces HDV RNA suppression in about one quarter of patients, which may last for some time after the end of therapy. However, a long-term follow-up study of the HIDIT-1 trial showed that late HDV RNA relapses can occur in more than half of patients with a post-treatment week 24 response even though these were not associated with the development of clinical hepatic events (Heidrich 2014). Thus, regular long-term follow-up is required for all interferon-treated HDV patients irrespective of virologic response to therapy.

A small study involving 32 patients explored whether interferon lambda 3 (IFNL3, also known as interleukin 28B) polymorphisms are associated with response to interferon alfa-based therapies of HDV (Yilmaz 2014). Of note, IFNL3 did not affect treatment responses as sustained responses were 27%, 27% and 50% in patients with CC, CT and TT genotypes at rs12979860, respectively.

Additional trials have been performed to investigate the efficacy of PEG-IFN α -2a in combination with TDF for the treatment of HDV. First

data of the HIDIT-2 trial were presented in 2013 showing that up to 47% of patients became HDV RNA negative after 96 weeks of PEG-IFN α -2a therapy irrespective of adding TDF or placebo (Wedemeyer 2019). In contrast to combination with adefovir, PEG-IFN α -2a plus TDF had no advantages in terms of HBsAg reduction after one year. However, relapses occurred after therapy and thus prolonged therapy may not necessarily prevent re-appearance of HDV and thus should not be considered in all patients unless pronounced HBsAg declines are observed – even though a smaller Turkish study reported rather high response rates of close to 50% after two years treatment (Karaca 2013). A long-term treatment study of up to five years in 13 patients at the NIH also observed a low virologic response rate despite prolonged therapy (Heller 2014). These findings suggest that therapy beyond one year is generally not beneficial although individual patients may benefit. If HBsAg kinetics can help to identify patients in whom longer treatment should be considered needs to be determined in future studies. Modelling data indicate that HBsAg-productive infected cells are the main source of HDV production (Guidj 2014) supporting the concept that treatment individualisation based on HBsAg levels during PEG-IFN α therapy is a reasonable approach. This is supported by a recent study which compared HDV patients who lost HBsAg during IFN α -based therapies compared to patients who were classified as partial responder (HBsAg positive, HDV RNA negative) or non-responder. A reduction of HBsAg at treatment month 6 was able to distinguish between the three groups (Niro 2016). Thus, determination of quantitative HBsAg is strongly recommended before and during PEG-IFN α therapy of HDV.

As PEG-IFN α therapy is of limited efficacy and as interferon-based therapies can cause significant side-effects, stopping rules would be helpful to avoid unnecessary PEG-IFN α exposure. Importantly, the HDV RNA level at week 24 of PEG-IFN α therapy can identify patients who will test HDV RNA negative during follow up after therapy (Keskin 2015). A decrease of HDV RNA of less than 1 log associated with no decline of HBsAg identified post-treatment non-responding patients with a positive predictive value of 83%. Another factor that is associated with response to PEG-IFN α therapy is the HBV-HDV dominance pattern before treatment. Interestingly, in the HIDIT-2 trial patients with higher HBV-DNA levels responded better to PEG-IFN α therapy compared to patients in whom HDV RNA levels were high and HBV DNA levels suppressed (Lutterkort 2018). Thus, the viral dominance pattern could be considered as a variable selecting patients for PEG-IFN α therapy.

New drugs against HDV in clinical development

Alternative treatment options for HDV are currently being explored in clinical trials (Wranke 2016; Deterding 2019). Treatment goals for new treatments against HDV have been suggested (Yurdaydin 2019). HDV RNA declines have been associated with improved clinical outcomes – even in the absence of complete negativation of HDV RNA. A preferred endpoint in clinical trials is a combination of HDV RNA decline with biochemical improvements of liver disease, e.g. normalization of ALT values (Yurdaydin 2019).

Prenylation inhibitors have been considered for quite some time (Bordier 2003). HDV replication depends on a prenylation step and prenylation inhibitors have already been developed for the treatment of malignancies. First proof-of-concept studies investigating the safety and efficacy of the prenylation inhibitor lonafarnib in patients with HDV have been initiated (www.clinicaltrials.gov) and indeed showed antiviral efficacy against HDV in patients (Koh 2015). Lonafarnib showed a dose-dependent reduction of HDV RNA levels of up to 2 log IU/mL after 28 days of therapy. Importantly, HDV RNA declines were associated with lonafarnib serum concentrations. While there was no evidence for viral resistance, higher doses of lonafarnib caused nausea and diarrhoea in most patients. Further trials on lonafarnib for HDV also exploring the potential of ritonavir boosting have been conducted suggesting that a better tolerated dose of lonafarnib may be possible (Yurdaydin 2018b). Furthermore, combination with PEG-IFN α -2a gave also promising results. A phase 3 trial is currently exploring both lonafarnib monotherapy as well as combination treatment with PEG-IFN α -2a in patients with HDV infection.

The HBV entry inhibitor bulevirtide (myrcludex-B) is also being developed for HDV. Bulevirtide is a lipopeptide derived from the preS1 domain of the HBV envelope and has been shown to hinder HDV infection in uPA/SCID mice transplanted with human hepatocytes (Lütgehetmann 2012). The molecular target of bulevirtide is the bile acid transporter sodium taurocholate cotransporting peptide (Ni 2013). The compound is also currently being tested in phase 1 and phase 2a trials in healthy volunteers and patients with HBV. 24 weeks of bulevirtide monotherapy was associated with an HDV RNA decline in the majority of patients in the first HDV study (Bogomolov 2016). Of note, patients receiving bulevirtide also showed a marked decline in ALT levels suggesting that blocking infection of cells can lead to a reduction in hepatitis activity. Additional trials exploring this compound in HDV alone or in combination with PEG-IFN α have already been completed. Overall, these studies confirmed a continuous and dose dependent linear HDV RNA decline during bulevirtide monotherapy associated with a marked improvement of biochemical disease activity

(Deterding 2019). Of note, liver elastography values also improved in several patients. However, none of the patients experienced HBsAg losses when treated with bulevirtide alone. The safety profile of bulevirtide was excellent. By mode of action, bile acids increased during treatment, in particular in patients receiving the high dose of bulevirtide without causing any clinical symptoms. Combination treatment of bulevirtide with PEG-IFN α -2a led to synergistic effects against HDV and some patients also achieved HBsAg declines or even losses (Deterding 2019). A phase 3 program exploring the safety and efficacy of bulevirtide in patients with HDV infection is currently ongoing.

Finally, preliminary data have been presented for distinct nucleic acid polymers to treat patients with HDV (Bazinet 2017). Rep 2139-Ca is believed to block release of subviral HBsAg particles from hepatocytes. The compound was injected once weekly and induced a marked decline of HBsAg in some but not all patients with HDV treated in a centre in Moldova. Of note, all patients treated (n=12) showed an HDV RNA decline after 15 weeks of monotherapy when PEG-IFN α was added. Responses were maintained in seven patients one year after completing treatment. A transient ALT increase was observed in patients with low HBsAg levels after REP 2139 monotherapy when PEG-IFN α was introduced. Future studies will need to determine the efficacy and safety of REP 2139 in a larger group of patients with HDV infection.

Interferon lambda is also explored in patients with HDV infection, both as a monotherapy or in combination with lonafanib (Deterding 2019). In vitro and in humanized mice, an antiviral effect comparable to interferon alpha has been observed (Giersch 2017). The potential advantage of interferon lambda is the lower frequency of systemic side effects as compared to interferon alpha.

Liver transplantation for HDV

Liver transplantation remains the ultimate treatment option for many people with HDV with end-stage liver disease. HDV patients have lower risk for reinfection after transplantation than patients with HBV mono-infection (Samuel 1993). If prophylaxis by passive immunisation with anti-HBs antibodies and administration of HBV polymerase is applied, HBV/HDV reinfection can be prevented in all individuals (Rosenau 2007) leading to an excellent long-term outcome after transplantation. HDV RNA levels rapidly decline during the first days after transplantation (Mederacke 2012) but HDVAg may persist in the transplanted liver for several years (Smedile 1998, Mederacke 2012). The possibility of reactivation of latent HDV infection by HBV superinfection has also been confirmed experimentally in a mouse

model with transplanted human hepatocytes (Giersch 2014). Mice infected with HDV lacking HBV could be rescued by HBV superinfection after 2–6 weeks leading to a productive coinfection. Long-term prophylaxis to prevent HBV reinfection is therefore generally recommended in patients transplanted for HDV as reinfection may lead to HDV reactivation for which treatment options are very limited. Still, a recent report suggested that prophylaxis with nucleos(t)ides alone may be feasible as only 2 out of 34 patients had HBV/HDV recurrence when administration of HBV immunoglobulins was stopped after transplantation (Cholongitas 2016).

More information on HDV for physicians and patients can be found on the website of the Hepatitis Delta International Network: www.hepatitis-delta.org

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11. Hepatitis C: diagnostic tests

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Common symptoms of hepatitis C like fatigue, muscle ache, loss of appetite or nausea are non-specific and, in many cases, mild or not present. Consequently, hepatitis C is often diagnosed accidentally and, unfortunately, remains heavily under-diagnosed. It is estimated that only 30–50% of individuals infected with HCV are aware of their disease and can take advantage of treatment options and avoid the risk of further transmission of the virus (Deuffic-Burban 2010). Untreated hepatitis C advances to a chronic state in up to 80% of people, which leads to liver cirrhosis in 20–40% with an accompanying risk of hepatic decompensation, hepatocellular carcinoma and death (Nature Outlook 2011). In light of these facts, HCV diagnostics should be performed thoroughly in all patients presenting with increased aminotransferase levels, with chronic liver disease of unclear aetiology and with a history of enhanced risk of HCV transmission (i.e., past IV or nasal drug dependency, transfusion of blood or blood products before the year 1990, major surgery before 1990, needle stick injuries, non-sterile tattoos or piercings, enhanced risk of sexual transmission).

For the diagnosis of hepatitis C both serologic and nucleic acid-based molecular assays are available (Scott 2007). Serologic tests are sufficient when chronic hepatitis C is expected, with a sensitivity of more than 99% in the 3rd generation assays. Positive serologic results require HCV RNA or with slightly reduced sensitivity HCV core antigen measurement in order to differentiate between chronic hepatitis C and resolved HCV infection from the past. When acute hepatitis C is considered, serologic screening alone is insufficient because anti-HCV antibodies may develop late after transmission of the virus. In contrast, HCV RNA is detectable within a few days of infection, making nucleic acid-based tests mandatory in diagnosing acute hepatitis C. HCV RNA measurement may be for some DAA regimens furthermore important in the determination of treatment indication, duration and success (Sarrazin 2010). Traditionally, HCV RNA measurement should be repeated 24 weeks after treatment completion to assess whether a sustained virologic response (SVR) has been achieved. However, as the probability of virologic relapse is similar after 12 and 24 weeks the new time point for assessment of final virologic treatment outcome is 12 weeks after the end-of-treatment (Yoshida 2014). Both qualitative and quantitative HCV RNA detection assays are available. Qualitative tests are highly sensitive and are used for diagnosing hepatitis C for the first time, for the screening of blood and organ donations and for confirming SVR

after treatment completion. Quantitative HCV RNA detection assays offer the possibility of measuring the viral load exactly and may help treatment monitoring. Qualitative and quantitative HCV RNA assays have now been widely replaced by real-time PCR-based assays that can detect HCV RNA over a very wide range, from low levels of approximately 10 IU/mL up to 10 million IU/mL. In case of lack of availability or financial restrictions, HCV core antigen testing can be used to confirm ongoing HCV infection and to monitor treatment outcome.

After diagnosing hepatitis C, traditionally the HCV genotype should be determined by nucleic acid-based techniques in every patient considered for HCV therapy. However, for selected easy to treat patients without advanced cirrhosis and without previous treatment failure in the past two years pangenotypic DAA regimens have been approved (velpatasvir/sofosbuvir, glecaprevir/pibrentasvir) for whom the knowledge of HCV genotype is not required. This may facilitate antiviral therapy especially in highly endemic countries. In patients with cirrhosis the HCV genotype is still required because for the use of Velpatasvir/Sofosbuvir in patients with HCV genotype 3 the addition of ribavirin is recommended. In addition, for patients with previous treatment failure to IFN-based therapies the HCV genotype is also required for the use of glecaprevir/pibrentasvir as longer treatment durations are recommended.

Morphological methods like immunohistochemistry, in situ hybridisation or PCR from liver specimens play no relevant role in the diagnosis of hepatitis C because of their low sensitivity, poor specificity and low efficacy compared to serologic and nucleic acid-based approaches.

Serologic assays

In current clinical practice, antibodies against multiple HCV epitopes are detected by commercially available 2nd and 3rd generation enzyme-linked immunoassays (EIAs). In these tests, HCV-specific antibodies from serum samples are captured by recombinant HCV proteins and are then detected by secondary antibodies against IgG or IgM. These secondary antibodies are labelled with enzymes that catalyse the production of coloured, measurable compounds.

The first applied EIAs for the detection of HCV-specific antibodies were based on epitopes derived from the NS4 region (C-100) and had a sensitivity of 70–80% and a poor specificity (Scott 2007). C-100-directed antibodies occur approximately 16 weeks after viral transmission. 2nd generation EIAs additionally detect antibodies against epitopes derived from the core region (C-22), NS3 region (C-33) and NS4 region (C-100), which leads to an increased sensitivity of approximately 95% and to a lower rate of false

positive results. With these assays HCV-specific antibodies can be detected approximately 10 weeks after HCV infection (Pawlotsky 2003). To narrow the diagnostic window from viral transmission to positive serological results, a 3rd generation EIA has been developed with an antigen from the NS5 region and/or the substitution of a highly immunogenic NS3 epitope. This innovation allows the detection of anti-HCV antibodies approximately four to six weeks after infection with a sensitivity of more than 99% (Colin 2001). Anti-HCV IgM measurement can narrow the diagnostic window in only a minority of patients. Anti-HCV IgM detection is also not sufficient to discriminate between acute and chronic hepatitis C because some chronically infected patients produce anti-HCV IgM intermittently and not all patients respond to acute HCV infection by producing anti-HCV IgM.

The specificity of serologic HCV diagnostics is difficult to define since an appropriate gold standard is lacking. It is evident, however, that false positive results are more frequent in patients with rheumatoid factors and in populations with a low hepatitis C prevalence, i.e., in blood and organ donors. Although several immunoblots for the confirmation of positive HCV EIA results are available, these tests have lost their clinical importance since the development of highly sensitive methods for HCV RNA detection. Immunoblots are mandatory to make the exact identification of serologically false positive-tested individuals possible. Importantly, the sensitivity of immunoblotting is lower compared to EIAs, which bears the risk of false negatively classifying HCV-infected individuals.

False negative HCV antibody testing may occur in patients on hemodialysis or in severely immunosuppressed patients like in HIV infection or in hematological malignancies.

HCV core antigen assays

In principle, detection of the HCV core antigen in serum could be a cheaper alternative to nucleic acid testing for the diagnosis and management of hepatitis C. The first HCV core antigen detection system (trak-C, Ortho Clinical Diagnostics) became commercially available in the US and Europe several years ago. This HCV core antigen assay proved highly specific (99.5%), genotype independent, and had a low inter- and intra-assay variability (coefficient of variation 5–9%) (Veillon 2003). HCV core antigen is measurable 1–2 days after HCV RNA becomes detectable. The limit of detection is 1.5 pg/mL (approximately 10,000–50,000 IU/mL HCV RNA). In a study of anti-HCV antibody and HCV RNA positive patients presenting in an outpatient clinic, 6/139 people (4%) were HCV core antigen negative. In these patients, HCV RNA concentrations were 1300–58,000 IU/mL, highlighting the limitations of the HCV core antigen assay as confirmation

of ongoing hepatitis C in anti-HCV positive patients. As a consequence, this first HCV core antigen assay was withdrawn from the market.

More recently, another quantitative HCV core antigen assay (Architect HCV Ag, Abbott Diagnostics), a further development of the previous assay, was approved by the EMA. This assay comprises 5 different antibodies to detect HCV core antigen, is highly specific (99.8%), equally effective for different HCV genotypes, and shows a relatively high sensitivity for determination of chronic hepatitis C (corresponding to 600-1000 IU/mL HCV RNA). However, HCV core antigen correlated well but not fully linearly with HCV RNA serum levels, and false negative results were obtained in patients with impaired immunity (Mederacke 2009, Medici 2011). Another study has shown that HCV core antigen quantification could be an alternative to HCV RNA quantification for on-treatment antiviral response monitoring (Vermehren 2012). Here, HCV core antigen below the limit of quantification at treatment week 1 was strongly predictive of RVR, whereas patients with a less than 1 log₁₀ decline in HCV core antigen at treatment week 12 had a high probability of achieving non-response.

The new HCV core antigen assay could be a cheaper, though somewhat less sensitive, alternative for nucleic acid testing. For careful monitoring of older treatment modalities which depend on response-guided treatment algorithms, proper rules for the application of the HCV core antigen assay have not been developed, but the clinical relevance of such regimens has substantially declined. Yet, a recent large study has shown that for highly effective all oral combination therapies without the need of on-treatment assessment of virologic response, the HCV core antigen assay can be an alternative for assessment of active HCV infection before initiation of antiviral therapy if an HCV RNA assay is not available or not affordable. But HCV RNA testing remains the gold standard to prove SVR as three out of 148 patients with SVR had a positive HCV core assay testing result (van Tilborg 2018).

Nucleic acid testing for HCV

Until 1997, HCV quantitative results from various HCV RNA detection systems did not represent the same concentration of HCV RNA in a clinical sample. Because of the importance of an exact HCV RNA determination for patient management, the World Health Organization (WHO) established the HCV RNA international standard based on international units (IU) which is used in all clinically applied HCV RNA tests. Other limitations of earlier HCV RNA detection assays were the false negative results due to polymerase inhibition, for example by drug interference, false positive results due to sample contamination because the reaction tubes had to be

opened frequently, or due to under- and over-quantification of samples of certain HCV genotypes (Morishima 2004, Pawlotsky 2003, Pawlotsky 1999). Currently, several HCV RNA assays are commercially available (Table 1). These assays are used in clinical practice to reliably quantify HCV RNA concentration before, during and after antiviral therapy in order to identify candidates for antiviral therapy, to choose optimal treatment regimens and durations and to prove treatment success.

However, with the implementation of highly effective and easy antiviral regimens, current goals in the management of hepatitis C are changing. While successful treatment of an individual patient is much easier, current efforts aim to globally eliminate HCV infection. For this purpose, easy diagnostic tools which can be widely distributed even in low income countries to diagnose active HCV infection would be of high value. Hence, several point-of-care assays which can rapidly and cheaply detect HCV RNA or HCV core antigen in a small drop of blood are in clinical evaluation, with which diagnostic sensitivities and specificities of >95% can be achieved.

Table 1. Commercially available HCV RNA detection assays

Assay	Distributor	Technology	Approval status
Qualitative HCV RNA detection assays			
Cobas Amplior/ Cobas® TaqMan qual	Roche Molecular Systems	PCR	FDA, CE
Quantitative HCV RNA detection assays			
Cobas AmpliPrep/ High pure system / Cobas® TaqMan®+	Roche Molecular Systems	Real-time PCR	FDA, CE
Abbott RealTime™ HCV	Abbott Diagnostics	Real-time PCR	FDA, CE
Artus HCV QS-RGQ assay	Qiagen	Real-time PCR	CE
Versant™ HCV 1.0 kPCR assay	Siemens	Real-time PCR	CE
Veris HCV Assay	Beckman Coulter	Real-time PCR	CE
Xpert HCV Viral Load	Cepheid	Real-time PCR	CE
Aptima HCV Quant Dx Real Time TMA	Hologic	TMA	CE
Cobas HCV Assay 4800, 6800, 8800	Roche Molecular Systems	Real-time PCR	CE, FDA

Qualitative assays for HCV RNA detection

Until recently, qualitative assays for HCV RNA had substantially lower limits of detection in comparison to quantitative HCV RNA assays. The costs of a qualitative assay are also lower compared to a quantitative assay. Therefore, qualitative HCV RNA tests are used for the first diagnosis of acute hepatitis C, in which HCV RNA concentrations are fluctuating and may be very low, as well as for confirmation of chronic hepatitis C infection in patients with positive HCV antibodies. In addition, they are used for the confirmation of virologic response during, at the end of, and after antiviral therapy, as well as in screening blood and organ donations for presence of HCV.

Qualitative RT-PCR

In reverse transcriptase-PCR- (RT-PCR-) based assays HCV RNA is used as a matrix for the synthesis of a single-stranded complementary cDNA by reverse transcriptase. The cDNA is then amplified by a DNA polymerase into multiple double-stranded DNA copies. Qualitative RT-PCR assays are expected to detect 50 HCV RNA IU/mL or less with equal sensitivity for all genotypes.

The Amplicor™ HCV 2.0 was an FDA- and CE-approved RT-PCR system for qualitative HCV RNA testing that allowed detection of HCV RNA concentrations down to 50 IU/mL of all genotypes (Table 1) (Nolte 2001). The DNA polymerase of *Thermus thermophilus* used in this assay provides both DNA polymerase and reverse transcriptase activity and allows HCV RNA amplification and detection in a single-step, single-tube procedure.

The below described Cobas Ampliprep / Cobas TaqMan system is available as a real-time PCR-based assay for the qualitative and highly sensitive detection of HCV genotype 1–6 samples, but also as a quantitative assay which allows precise quantification of HCV viral loads (see below).

Transcription-mediated amplification (TMA) of HCV RNA

TMA-based qualitative HCV RNA detection has a very high sensitivity (Hendricks 2003, Sarrazin 2002). TMA is performed in a single tube in three steps: target capture, target amplification and specific detection of target amplicons by a hybridisation protection assay. Two primers, one of which contains a T7 promoter, one T7 RNA polymerase and one reverse transcriptase, are necessary for this procedure. After RNA extraction from 500 µl serum, the T7 promoter-containing primer hybridises the viral RNA with the result of reverse transcriptase-mediated cDNA synthesis. The

reverse transcriptase also provides an RNase activity that degrades the RNA of the resulting RNA/DNA hybrid strand. The second primer then binds to the cDNA that already contains the T7 promoter sequence from the first primer, and a DNA/DNA double-strand is synthesised by the reverse transcriptase. Next, the RNA polymerase recognises the T7 promoter and produces 100–1000 RNA transcripts, which are subsequently returned to the TMA cycle leading to exponential amplification of the target RNA. Within one hour, approximately 10 billion amplicons are produced. The RNA amplicons are detected by a hybridisation protection assay with amplicon-specific labelled DNA probes. The unhybridised DNA probes are degraded during a selection step and the labelled DNA is detected by chemiluminescence.

A commercially available TMA assay was the Versant™ HCV RNA Qualitative Assay. This system is accredited by the FDA and CE and provides an extremely high sensitivity, superior to RT-PCR-based qualitative HCV RNA detection assays (Hofmann 2005, Sarrazin 2001, Sarrazin 2000). The lower detection limit is 5–10 IU/mL with a sensitivity of 96–100%, and a specificity of more than 99.5%, independent of the HCV genotype.

More recently, a novel TMA-based assay, the Aptima HCV Quant Dx assay, has been approved which allows automated quantitative and qualitative HCV RNA measurement in a single step (Chevaliez 2017). This assay is highly sensitive with a lower limit of detection of 2.8 IU/mL. Furthermore, the Aptima HCV Quant Dx assay is highly specific and HCV RNA test results are highly concordant to the real-time PCR-based Abbott RealTime HCV and Cobas AmpliPrep/Cobas TaqMan HCV Test, version 2.0, assays.

Quantitative HCV RNA detection

HCV RNA quantification can be achieved either by target amplification techniques (competitive and real-time PCR) or by signal amplification techniques (branched DNA (bDNA) assay) (Table 1). Several FDA- and CE-approved standardised systems are commercially available. The Cobas Amplicor™ HCV Monitor is based on a competitive PCR technique whereas the Versant™ HCV RNA Assay is based on a bDNA technique. More recently, the Cobas® TaqMan® assay and the Abbott RealTime™ HCV test, both based on real-time PCR technology, have been introduced. The technical characteristics, detection limits and linear dynamic detection ranges of these systems are summarised below. Due to their very low detection limit and their broad and linear dynamic detection range, they have already widely replaced the previously used qualitative and quantitative HCV RNA assays.

Competitive PCR: Cobas® Amplicor™ HCV 2.0 monitor

The Cobas® Amplicor™ HCV 2.0 monitor was a semi-automated quantitative detection assay based on a competitive PCR technique. Quantification is achieved by the amplification of two templates in a single reaction tube, the target and the internal standard. The latter is an internal control RNA with nearly the same sequence as the target RNA with a clearly defined initial concentration. The internal control is amplified by the same primers as the HCV RNA. Comparison of the final amounts of both templates allows calculation of the initial amount of HCV RNA. The dynamic range of the Amplicor™ HCV 2.0 monitor assay is 500 to approximately 500,000 IU/mL with a specificity of almost 100%, independent of the HCV genotype (Konnick 2002, Lee 2000). For higher HCV RNA concentrations pre-dilution of the original sample is required.

Branched DNA hybridisation assay (Versant™ HCV RNA 1.0 quantitative assay)

Branched DNA hybridisation assay was based on signal amplification technology. After reverse transcription of the HCV RNA, the resulting single-stranded complementary DNA strands bind to immobilised captured oligonucleotides with a specific sequence from conserved regions of the HCV genome. In a second step, multiple oligonucleotides bind to the free ends of the bound DNA strands and are subsequently hybridised by multiple copies of an alkaline phosphatase-labelled DNA probe. Detection is achieved by incubating the alkaline phosphatase-bound complex with a chemiluminescent substrate (Sarrazin 2002). The Versant™ HCV RNA assay is at present the only FDA- and CE-approved HCV RNA quantification system based on a branched DNA technique. The lower detection limit of the current version 3.0 is 615 IU/mL and linear quantification is ensured between 615–8,000,000 IU/mL, independent of the HCV genotype (Morishima 2004). The bDNA assay only requires 50 µl serum for HCV RNA quantification and is currently the assay with the lowest sample input.

Real-time PCR-based HCV RNA detection assays

Real-time PCR technology provides optimal features for both HCV RNA detection and quantification because of its very low detection limit and broad dynamic range of linear amplification (Sarrazin 2006) (Figure 1). Distinctive for real-time PCR technology is the ability to simultaneously amplify and detect the target nucleic acid, allowing direct monitoring of

the PCR process. RNA templates are first reverse-transcribed to generate complementary cDNA strands followed by a DNA polymerase-mediated cDNA amplification.

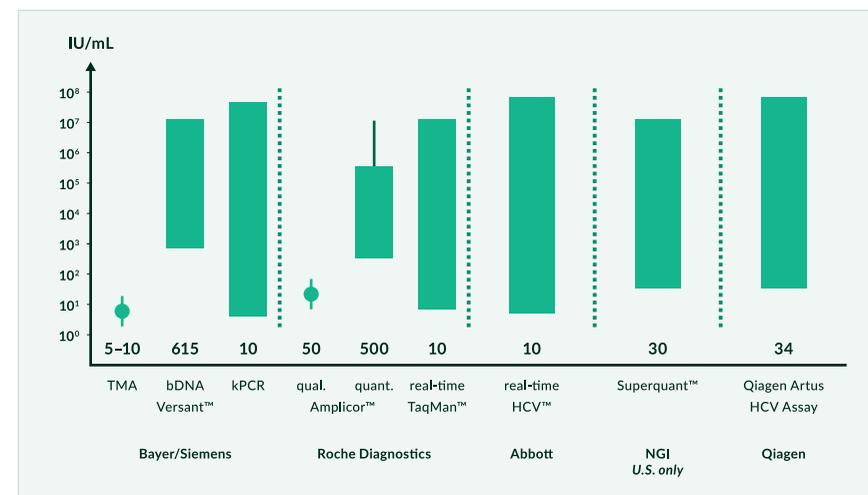


Figure 1. Detection limits and linear dynamic ranges of commercially available HCV RNA detection assays

DNA detection simultaneous to amplification is preferentially achieved by the use of target sequence-specific oligonucleotides linked to two different molecules, a fluorescent reporter molecule and a quenching molecule. These probes bind the target cDNA between the two PCR primers and are degraded or released by the DNA polymerase during DNA synthesis. In case of degradation the reporter and quencher molecules are released and separated, which results in the emission of an increased fluorescence signal from the reporter. Different variations of this principle of reporter and quencher are used by the different commercially available assays. The fluorescence signal, intensified during each round of amplification, is proportional to the amount of RNA in the starting sample. Quantification in absolute numbers is achieved by comparing the kinetics of the target amplification with the amplification kinetics of an internal control of a defined initial concentration.

Highly effective and almost completely automated real-time PCR-based systems for HCV RNA measurement have been introduced.

All commercially available HCV RNA assays are calibrated to the WHO standard based on HCV genotype 1. Significant differences between different RT-PCR assays and other quantitative HCV RNA tests have been reported – in the case of the real-time PCR-based assays a slight under-quantification by one assay and a slight over-quantification by the other, in comparison to the WHO standard by Cobas® TaqMan®. In addition, it

has been shown that results may vary significantly between assays with different HCV genotypes despite standardisation to IU (Chevaliez 2007, Vehrmeren 2008).

Cobas® TaqMan® HCV test

The FDA- and CE-accredited Cobas® TaqMan® (CTM) assay uses reporter- and quencher-carrying oligonucleotides specific to the 5' UTR of the HCV genome and to the template of the internal control, a synthetic RNA for binding the same primers as for HCV RNA. Reverse transcription and cDNA amplification is performed by the Z05 DNA polymerase. For HCV RNA extraction from serum or plasma samples, a Cobas® TaqMan® assay was developed either in combination with the fully automated Cobas® AmpliPrep (CAP) instrument using magnetic particles, or in combination with manual HCV RNA extraction with glass fibre columns using the High Pure System (HPS) viral nucleic acid kit. The current versions of both combinations have a lower detection limit of approximately 10 IU/mL and a linear amplification range of HCV RNA from approximately 40 to 10,000,000 IU/mL. Samples from HCV genotypes 2-5 have been shown to be under-quantified by the first version of the HPS-based Cobas® TaqMan® assay. The second version of this assay has now demonstrated equal quantification of all HCV genotypes (Colucci 2007). For the Cobas® AmpliPrep/Cobas® TaqMan® (CAP/CTM) assay, significant under-quantification of HCV genotype 4 samples has been shown. In the meanwhile, a second version CAP/CTM assay (CAP/CTM HCV Test, v2.0) was evaluated. Based on a dual-probe design, this assay was able to accurately quantify HCV RNA samples from patients infected with all HCV genotypes, including HCV genotype 4 transcripts with rare sequence variants that had been under-quantified by the first generation assay (Vermehren 2011). Furthermore, this assay has a lower limit of detection and quantification of approximately 15 IU/mL across all HCV genotypes, and a linear amplification range of HCV RNA from approximately 15 to 10,000,000 IU/mL (Zitzer 2013). Taken together, the Cobas® TaqMan® assay makes both highly sensitive qualitative and linear quantitative HCV RNA detection feasible with excellent performance in one system with complete automation.

Yet, the Cobas® TaqMan® assay has been replaced by Cobas 6800/8800 HCV and Cobas 4800 HCV assays for high and medium-throughput demands, respectively. It was shown that the Cobas 6800/8800 HCV and Cobas 4800 HCV assays were able to quantify HCV genotype 1 RNA concentrations in a linear range from lower limits of detection of 8.2 IU/ml and 11.7 IU/ml, respectively. The concordances using a cutoff of 6 million IU/ml was at least 90% with previously established assays (Vermehren 2017).

RealTime HCV test

The CE-accredited RealTime HCV test also uses reporter- and quencher-carrying oligonucleotides specific for the 5'UTR. HCV RNA concentrations are quantified by comparison with the amplification curves of a cDNA from the hydroxypyruvate reductase gene from the pumpkin plant *Cucurbita pepo*, which is used as an internal standard. This internal standard is amplified with different primers from those of the HCV RNA, which may be the reason for the linear quantification of very low HCV RNA concentrations. The RealTime HCV test provides a lower detection limit of approximately 10 IU/mL, a specificity of more than 99.5% and a linear amplification range from 12 to 10,000,000 IU/mL independent of the HCV genotype (Michelin 2007, Sabato 2007, Vehrmeren 2008). In a multi-centre study, its clinical utility to monitor antiviral therapy of patients infected with HCV genotypes 1, 2 and 3 was proven and the FDA approved the RealTime HCV test (Vermehren 2011). In this study, highly concordant baseline HCV RNA levels as well as highly concordant data on rapid and early virologic response were obtained compared to reference tests for quantitative and qualitative HCV RNA measurement, the Versant® HCV Quantitative 3.0 branched DNA hybridisation assay and the Versant® HCV RNA Qualitative assay.

Artus hepatitis C virus QS-RGQ assay

Qiagen has developed a novel real-time based HCV RNA assay, the artus HCV QS-RGQ assay. The artus HCV RNA assay has a lower limit of quantification of 30 IU/mL and a linear range of quantification up to 10⁸ IU/mL. Compared to the Cobas® TaqMan® assay, the artus HCV assay had a slightly lower sensitivity (Paba 2012).

Versant HCV 1.0 kPCR assay

For replacement of the qualitative TMA and the quantitative bDNA-based assays, a real-time-based PCR test (Versant® kPCR Molecular System) has been introduced recently. While little is known for the use of this assay in response-guided conventional dual and triple therapies in HCV genotype 1-infected patients, a limitation of this assay seems to be a substantial underquantification of HCV RNA concentrations in certain HCV subtypes (2a, 3a, 4a) (Kessler 2013).

Cepheid Xpert HCV Viral Load Assay and Beckman Coulter DxN Veris HCV Assay

In the meanwhile, Cepheid has developed the RT-PCR-based Xpert HCV Viral Load assay, which – according to the manufacturer's instructions – quantifies viral load in a linear range from 10 to 100,000,000 IU/mL for HCV genotypes 1-6, with a lower limit of detection of 4.0 IU/mL. These excellent performance characteristics have been confirmed in an independent validation study (Mc Hugh 2017). Of note, the Xpert HCV Viral Load assay is also suitable to detect and quantify HCV RNA from finger-stick capillary blood samples (Grebely 2017). Another RT-PCR-based assay is the Beckman Coulter DxN Veris HCV Assay.

Furthermore, the novel TMA-based Aptima HCV Quant Dx assay has been approved which allows highly specific and sensitive HCV RNA detection and quantification (details above).

HCV genotyping

HCV is heterogeneous with an enormous genomic sequence variability due to a rapid replication cycle with the production of 10¹² virions per day and the low fidelity of the HCV RNA polymerase. Six genotypes (1-6), multiple subtypes (a, b, c...) and most recently a seventh HCV genotype have been characterised. These genotypes vary in approximately 30% of their RNA sequence with a median variability of approximately 33%. HCV subtypes are defined by differences in their RNA sequence of approximately 10%. Within one subtype, numerous quasispecies exist and may emerge during treatment with specific antivirals. These quasispecies are defined by a sequence variability of less than 10% (Simmonds 2005). Because the currently recommended treatment durations can depend on the HCV genotype, HCV genotyping is mandatory in every patient who considers antiviral therapy (Lange 2014). For several DAA-based therapies, determination of HCV genotypes and even subtypes is important because of significantly distinct barriers to resistance on the HCV subtype level. Furthermore, rarely viral recombinants exist of different HCV sub- or genotypes. The most frequent viral chimera is the so named St. Petersburg variant consisting of a HCV genotype 2k/1b recombinant. Proper diagnosis by routine HCV genotyping assays and treatment of viral chimeras may be challenging (see below). However, the importance for HCV genotyping declined with the availability of highly and broadly effective all oral combination therapies. In fact for treatment-naïve patients without liver cirrhosis antiviral therapy with velpatasvir/sofosbuvir for 12 weeks or glecaprevir/pibrentasvir for 8 weeks is possible without the need for HCV

genotyping (Feld 2015, Zeuzem 2018).

Both direct sequence analysis and reverse hybridisation technology allow HCV genotyping. Initial assays were designed to analyse exclusively the 5' untranslated region (5'UTR), which is burdened with a high rate of misclassification especially on the subtype level. Current assays were improved by additionally analysing the coding regions, in particular the genes encoding the non-structural protein NS5B and core protein, both of which provide non-overlapping sequence differences between the genotypes and subtypes (Bowden 2006).

Reverse hybridising assay (Versant® HCV Genotype 2.0 System (LiPA))

In reverse hybridising, biotinylated cDNA clones from HCV RNA are produced by reverse transcriptase and then transferred and hybridised to immobilised oligonucleotides specific to different genotypes and subtypes. After removing unbound DNA by a washing step, the biotinylated DNA fragments can be detected by chemical linkage to coloured probes.

The Versant® HCV Genotype 2.0 System is suitable for identifying genotypes 1-6 and more than 15 different subtypes and is currently the preferred assay for HCV genotyping. By simultaneous analyses of the 5' UTR and core region, a high specificity is achieved to differentiate the genotype 1 subtypes. In a study evaluating the specificity of the Versant® HCV Genotype 2.0 System, 96.8% of all genotype 1 samples and 64.7% of all genotype samples were correctly subtyped. No misclassifications at the genotype level were observed. Difficulties in subtyping occurred in particular in genotypes 2 and 4. Importantly, none of the misclassifications would have had clinical consequences, which qualifies the Versant® HCV Genotype 2.0 System as highly suitable for clinical decision-making (Bouchardeau 2007).

However, the recent discovery of intergenotypic chimeras, which cannot be classified accurately by the current version of the LiPA assay, has shown that exclusive usage of the LiPA-assay for HCV genotyping can in rare cases result in the selection of inadequate all-oral treatment regimens (details below).

Direct sequence analysis (TruGene® HCV 5'NC genotyping kit)

The TruGene® assay determines the HCV genotype and subtype by direct analysis of the nucleotide sequence of the 5'UTR region. Incorrect

genotyping rarely occurs with this assay. However, the accuracy of subtyping is poor (approx. 20% misclassifications according to a recent study) because of the exclusive analyses of the 5'UTR (Sarrazin 2015).

Real-time PCR technology (RealTime™ HCV Genotype II assay)

The current RealTime HCV Genotype II assay is based on real-time PCR technology, which is less time consuming than direct sequencing. Preliminary data revealed a 96% concordance at the genotype level and a 93% concordance on the genotype I subtype level when compared to direct sequencing of the NS5B and 5'UTR regions. Nevertheless, single genotype 2, 3, 4, and 6 isolates were misclassified at the genotype level, indicating a need for assay optimisation (Ciotti 2010). A more recent study has shown that the RealTime HCV Genotype II assay fails to correctly classify HCV genotype 6n and 6e genotypes (Yang 2014). Furthermore, misclassifications on the subtype level have been reported for HCV genotype 1a/1b (Liu 2015). The diagnostic performance of this assay for viral recombinants is unclear but theoretically, due to amplification of areas in the structural and non-structural HCV genome, viral chimeras may be recognised.

Cobas® HCV genotyping test

The Cobas® HCV genotyping test is a novel PCR-based assay using genotype-specific primers for three different regions of the HCV genome (Stelzl 2016). Compared to direct sequencing analysis, the Cobas® HCV genotyping test produced concordant results in 95.7% for genotyping of HCV genotype 2-6 and in 99.2% for subtyping of HCV genotype 1a/1b. No misgenotyping was observed (Nieto-Aponte 2016).

Implications for diagnosing and managing acute and chronic hepatitis C

Diagnosing acute hepatitis C

When acute hepatitis C is suspected, the presence of both anti-HCV antibodies and HCV RNA should be tested. For HCV RNA detection, sensitive qualitative techniques with a lower detection limit of 50 IU/mL or less are required, for example TMA, qualitative RT-PCR or the newer real-time

PCR systems. Testing for anti-HCV alone is insufficient for the diagnosis of acute hepatitis C because HCV specific antibodies appear only weeks (up to 6 months) after viral transmission. In contrast, measurable HCV RNA serum concentrations emerge within the first days after infection. However, HCV RNA may fluctuate during acute hepatitis C, making a second HCV RNA test necessary several weeks later in all negatively tested patients with a suspicion of acute hepatitis C. When HCV RNA is detected in seronegative patients, acute hepatitis C is very likely. When patients are positive for both anti-HCV antibodies and HCV RNA, it may be difficult to discriminate between acute and acutely exacerbated chronic hepatitis C. Anti-HCV IgM detection will not clarify this because its presence is common in both situations. In rare cases and especially in association with low amounts of inoculum, HCV infection may be only associated with transient HCV RNA detectability or exclusively by markers of innate immune response (Heller 2013).

Diagnosing chronic hepatitis C

Chronic hepatitis C should be considered in every patient presenting with clinical, morphological or biological signs of chronic liver disease. When chronic hepatitis C is suspected, screening for HCV antibodies by 2nd or 3rd generation EIAs is adequate because their sensitivity is >99%. False negative results may occur rarely in immunosuppressed patients (i.e., HIV) and in patients on dialysis. When anti-HCV antibodies are detected, the presence of HCV RNA (or alternatively HCV core antigen, details above) has to be determined in order to discriminate between chronic hepatitis C and resolved HCV infection. The latter cannot be distinguished by HCV antibody tests from rarely occurring false positive serological results, the exact incidence of which is unknown. Serological false positive results can be identified by the additional performance of an immunoblot assay. Many years after disease resolution, anti-HCV antibodies may become undetectable on commercial assays in some patients.

Diagnostic tests in the management of hepatitis C therapy

The current treatment recommendations for acute and chronic hepatitis C are based on HCV genotyping and on HCV RNA determination before, (during) and after antiviral therapy. When HCV RNA has been detected, exact genotyping and HCV RNA determination is recommended in patients considered for antiviral therapy. Exact geno- and subtyping appears to be highly important for therapies in pre-treated patients, patients with cirrhosis and for some directly acting antiviral (DAA) agents because some

subtypes (especially HCV genotype 1a vs. 1b) behave differently regarding treatment response and the development of resistance. In this regard, it is important that conventional genotyping (based on reverse hybridisation) can miss the detection of intergenotypic chimeras and misclassify them as easy-to-treat HCV genotype 2 strains (details below). Low HCV RNA concentration (<600,000–800,000 IU/mL) is a positive predictor of SVR for some treatment regimens, including dual combination therapy with PEG-IFN and ribavirin, conventional triple therapies with one DAA in combination with pegylated interferon and ribavirin, and all-oral therapy with grazoprevir, elbasvir and ribavirin in patients infected with HCV genotype 1a or 4 (Sarrazin 2010, Komatsu 2016). Furthermore, for treatment-naïve, non-cirrhotic patients shortening treatment duration with the all-oral, interferon-free combination therapy of sofosbuvir and ledipasvir to 8 weeks is possible based on a new baseline viral load cut-off of 6 million IU/mL according to the EMA and FDA labels. Genotyping is mandatory for the selection of the optimal treatment regimen and duration of therapy, since some DAA regimens are selectively effective for only some HCV genotypes (Lange 2014).

Interferon-based therapies

Here we summarise treatment algorithms for the previously used interferon-based therapies, which might be of (historical) interest for some readers.

For HCV genotype 1 (and 4) treatment could be shortened to 24 weeks in patients with low baseline viral load (<600,000–800,000 IU/mL) and rapid virologic response (RVR) with undetectable HCV RNA at week 4 of treatment (Sarrazin 2010). In slow responders with a 2 log₁₀ decline but still detectable HCV RNA levels at week 12 and undetectable HCV RNA at week 24, treatment was extended to 72 weeks (Sarrazin 2010). In patients with complete early virologic response with undetectable HCV RNA at week 12 (cEVR), standard treatment was continued to 48 weeks. Genotypes 5 and 6 were treated the same as genotype 1-infected patients due to the lack of adequate clinical trials, whereas genotypes 2 and 3 generally allowed treatment duration of 24 weeks, which was shortened to 16 weeks (depending on RVR and [low] baseline viral load) or extended to 36–48 weeks depending on the initial viral decline (Sarrazin 2010).

Independent of the HCV genotype, proof of HCV RNA decrease was necessary to identify patients with little chance of achieving SVR. HCV RNA needs to be quantified before and 12 weeks after treatment initiation and antiviral therapy was usually discontinued if a decrease of less than 2 log₁₀ HCV RNA was observed (negative predictive value 88–100%). All patients on sofosbuvir-based combination therapies with PEG-IFN α and ribavirin

achieved undetectable HCV RNA concentrations on antiviral therapy and no response-guided therapy approaches have been developed (Jacobson 2013, Lawitz 2013, Lawitz 2013). Therefore, on-treatment monitoring of HCV RNA was not necessary for determination of treatment duration or early stopping rules.

All-oral IFN-free therapies

So far, no response-guided treatment-algorithms have been established for approved all-oral DAA combination therapies.

Furthermore, it was shown in a large retrospective analysis of the ION studies, that the initial viral load decline during sofosbuvir and ledipasvir therapy had no relevant impact on treatment outcome in general (Welzel 2014). In another study, no correlation between early viral kinetics and outcome of treatment with paritaprevir/r, ombitasvir and dasabuvir was observed (Sulkowski 2014). Another study has shown that on-treatment HCV RNA levels ≥ 45 IU/mL (assessed with the Cobas® TaqMan® assay) were associated with high relapse rates in HCV genotype 3 patients who were treated with sofosbuvir and ribavirin (Massoumy 2016). However, this was not the case in patients treated with more potent regimens such as sofosbuvir and daclatasvir.

It is important to know that viral load monitoring during the approval studies of sofosbuvir-based IFN-free regimens has been performed with the HPS-based Cobas® TaqMan® assay. However, if on-treatment viral load monitoring is performed with other assays (e.g., the RealTime HCV test), positive HCV RNA detection below the limit of quantification (i.e., <12 IU/mL positive) has been observed on an IFN-free regimen without any negative impact on treatment outcome, despite detectable residual HCV RNA until the end-of-treatment in individual patients (Cloherty 2015). Another study has performed repetitive early HCV RNA measurements in 11 HCV genotype 1 patients who were treated with combination therapy of paritaprevir/r, ombitasvir, dasabuvir and ribavirin for 12 weeks (Sarrazin 2015). HCV RNA quantification results were compared for the RealTime HCV (ART) and the High-Pure-System/Cobas® TaqMan® (HPS) assays. On-treatment HCV RNA was detectable in a relevant number of samples when assessed by the ART but not when assessed by the HPS assay, while the converse has rarely been reported. However, residual HCV RNA detection even at late points of antiviral therapy did not correlate with treatment failure in this study. More data are required to fully understand this phenomenon. However, for the time being it is very important not to consider these test results as treatment failure but to continue antiviral therapy for the originally planned duration in such a scenario. Further studies should also better define whether very early HCV RNA kinetics may have an impact on treatment outcome and

on determination of optimal treatment duration of such potent all-oral regimens: it has been shown that the usage of different assays for HCV RNA quantification may have a profound impact on results of on-treatment HCV viral load monitoring. For example, a recent study showed that the Roche / High-Pure-System Cobas® TaqMan® V2 measures HCV RNA levels that are 0.46 log IU/mL higher than those determined by the Abbott RealTime HCV test before and during all-oral therapy with paritaprevir, ombitasvir and dasabuvir (Wiesmann 2014).

A viral load <6 million IU/mL at baseline with sofosbuvir plus ledipasvir allows shortening treatment duration from 12 weeks to 8 weeks, according to the ledipasvir label in the US. These recommendations were derived from on-treatment monitoring with the HPS-based Cobas® TaqMan® assay. Therefore, when using other commercially available assays such as the Cobas AmpliPrep Cobas® TaqMan® assay or the RealTime HCV test for viral load quantification, rates of patients with a viral load <6 million IU/mL may be much higher. In fact, a recent study has shown that HCV-RNA levels were significantly higher when measured with the Cobas AmpliPrep Cobas® TaqMan® assay versus the RealTime HCV assay in the same sample. According to this study, treatment-naïve, non-cirrhotic HCV genotype 1 patients, 95% or 78% had HCV-RNA viral load <6 million IU/mL, when measured with the RealTime HCV assay compared to the Cobas AmpliPrep Cobas® TaqMan® assay, respectively (Vermehren 2016). It may therefore be relevant to assess whether the recommendation to shorten treatment with sofosbuvir and ledipasvir in patients with viral load <6 million IU/mL is valid for test results from assays other than the HPS-based Cobas® TaqMan®. Calculation of conversion factors revealed a viral load cut-off between 2 and 3 million IU/mL as suitable for RealTime HCV and Cobas AmpliPrep Cobas® TaqMan® (Feverly 2014, Kessler 2015). However, data from recent real-world studies showed that application of the 6 Million IU/mL HCV RNA cut-off rule based on different commercially available assays was associated with high SVR rates (97–98%) (Kowdley 2016).

It is important to note that neither baseline viral load nor on-treatment viral kinetics play a role in determining treatment durations with the newer DAA-combination regimens sofosbuvir plus velpatasvir, sofosbuvir plus velpatasvir plus voxilaprevir, or glecaprevir plus pibrentasvir. In contrast, HCV genotype, failure of previous DAA therapy, and the presence of liver cirrhosis or decompensated liver cirrhosis are important determinants of required treatment durations with these regimens (*see Chapter 12 for details*). An important exception is therapy with grazoprevir plus elbasvir of patients infected with HCV genotype 1a or 4. In these patients, treatment extension to 16 weeks (versus 12 weeks) and additional administration of ribavirin is required in case of a baseline antiviral load >800,000 IU/mL, at least in the absence of reliable resistance testing (Zeuzem 2017). In contrast, no

treatment extension is required in patients infected with HCV genotype 1b or in patients with HCV genotype 1a in whom baseline resistance mutations have been excluded.

Detection of intergenotypic recombinant strains (chimeras)

Recent reports have described the occurrence of intergenotypic recombinant strains (chimeras), in which the 5' part of the genome corresponded to HCV genotype 2 sequences and the 3' part to HCV genotype 1 sequences (the recombination breakpoint was located between NS2 and NS3). Of note, the widely used INNO-LiPA 2.0 assay has classified these variants as HCV genotype 2 isolates, though they clinical behave like HCV genotype 1 isolates (i.e. lower responsiveness to sofosbuvir/ribavirin, but not to currently preferred sofosbuvir / velpatasvir and glecaprevir / pibrentasvir therapy than one would expect for HCV genotype 2). Intergenotypic chimeras, which were misclassified as HCV genotype 2 isolates, were observed in 2.5% of all HCV genotype 2 isolates, based on genotyping results using the INNO-LiPA (Hedskog 2015). Of note, in some geographic regions (e.g. Georgia), such chimeras may occur much more frequently (Karchava 2015). Due to migration, high frequencies of HCV genotype 2k/1b chimeras have been observed in cohorts from Israel and Germany as well (14% and 25% of HCV genotype “2” samples, respectively) (Susser 2017, Hostager 2019). A correct clinical classification of these variants as viral recombinants can be achieved by sequencing the 5' NTR or part of the structural HCV genes together with an area within the non-structural genes (NS3, NS5A or NS5B). In this regard, a recent study has shown that the real-time-PCR-based assays Cobas HCV GT and Abbott RealTime HCV Genotype II Assay) could classify HCV 2/1 chimeras correctly in 90% and 65% of all cases, respectively, whereas the Versant HCV Genotype 2.0 (LiPA 2.0) hybridization assay failed to identify chimeras in 100% of cases (Peiffer 2019).

Resistance testing during DAA therapies

HCV variants resistant to DAAs can emerge during antiviral therapy and result in treatment failure. Resistance testing prior to antiviral therapy can help select the optimal treatment regimen for individual patients (Schneider 2014). For example, before initiating simeprevir-based triple therapy, patients should be screened for the presence of the frequent Q80K variant in NS3, because in HCV genotype 1a patients with Q80K variants, the addition of simeprevir did not improve SVR rates (Jacobson 2013). The presence of resistance variants at baseline of IFN-free therapy with a first generation NS3 plus NS5A inhibitor like daclatasvir plus asunaprevir or

daclatasvir plus simeprevir also strongly reduced the chance of achieving an SVR (approx. 40% vs. 84% in patients without resistance variants) (Manns 2014, Zeuzem 2015).

Furthermore, it was shown that the presence of NS5A resistance-associated substitutions (RAS) at baseline with sofosbuvir + ledipasvir resulted in reduced SVR rates, especially in patients who were treated for only 8 weeks instead of 12 weeks, or in patients with previous failure to antiviral therapy (Sarrazin 2016). Similar results have been obtained for combination regimens of sofosbuvir plus another NS5A inhibitor like daclatasvir or velpatasvir. Furthermore, baseline NS5A RAS negatively impact on outcome of treatment with grazoprevir, elbasvir and ribavirin in patients infected with HCV genotypes 1a and 4. The underlying principle seems to be the combination of several negative treatment predictors. While the importance of the pre-existence of RAS alone is limited a combination of RAS plus another stress factor like cirrhosis or shortened treatment duration is associated with markedly reduced SVR rates (Sarrazin 2015).

A report has also described a variant in the HCV NS5B polymerase (C316N), which, if detectable at baseline, was associated with lower SVR rates after treatment with sofosbuvir in combination with ribavirin with and without interferon alfa (Vermehren 2015). Of note, the C316N variant was detected almost exclusively in baseline serum samples of HCV genotype 1b patients compared to HCV genotype 1a patients. For combination regimens of sofosbuvir with another highly active DAA like ledipasvir no importance of C316N variant was observed (Sarrazin 2014).

Baseline resistance variants were detected in 20,5% (NS3), 11,9% (NS5A) and 22,1% (NS5B) of patients infected with HCV genotype 1 infection (Dietz 2015). Yet, it has been shown, that baseline resistance testing allows a selection of approved interferon-free regimens for which 98,6% and 100% of HCV genotype 1b and 1a patients are wildtype, respectively. Even more important, resistance testing allows selection of appropriate re-treatment regimens after failure of IFN-free all oral combination therapies. A recent study has identified RAS in 90% and 39% in NS3, NS5A or NS5B in HCV genotype 1 and genotype 3 patients, respectively, who had experienced treatment-failure after prior IFN-free therapy (Vermehren 2016). Re-treatment was performed with DAA combinations for which no RAS were detected and resulted in SVR in approx. 90% of patients, if a new DAA class was used for rescue treatment (Dietz 2019). In addition, an association of a major NS5A RAS (Y93N) with the presence of the beneficial IL28B (IFN-L3) CC genotype was reported. This observation explains the unexpected low SVR rates in patients with IL28B CC genotype after several IFN-free DAA combination regimens (Peiffer 2016).

The so far largest study of the emergence of RAS after DAA failure has shown that after simeprevir or paritaprevir failure, R155K and D168E/V in

NS3 are typically observed, whereas Q80K/R is a typical RAS selected after treatment with simeprevir. Typical RAS after failure with NS5A inhibitors were Y93H and L31M in NS5A. L159F and S282T RAS in NS5B were observed in patients with failure of sofosbuvir-containing regimens (Dietz 2018).

However, it is important to note that so far very limited impact of the presence of RAS on treatment outcome with the newest DAA combinations glecaprevir/pibrentasvir, velpatasvir/sofosbuvir or sofosbuvir/velpatasvir/voxilaprevir has been shown (Krishnan 2017; Bourlière 2017). Here, only for patients infected with HCV genotype 3 lower SVR rates have been described: For glecaprevir / pibrentasvir this was true for shortening treatment duration to 8 weeks and RASs especially within the NS5A region (i.e. A30K) (Zeuzem 2018). For velpatasvir / sofosbuvir patients with cirrhosis and treatment for 12 weeks without ribavirin had lower SVR rates in the presence of Y95H variants (Esteban 2018). This applies also for re-treatment of patients with prior DAA-failure with sofosbuvir / velpatasvir / voxilaprevir. Baseline RASs seem to have no impact on virologic treatment outcome. However, the majority of virologic failure patients had HCV genotype 3 and the importance of RAS in this subgroup of patients is not completely clear (Sarrazin 2018). Generally, very few patients failed during approval studies of these second generation regimens and further data from real-world experience have to be awaited.

Commercially available assays for resistance testing are available in the US and are currently being established in other countries. However, currently no validated and standardised assay for HCV resistance testing is available and correspondingly results of resistance testing in different experienced laboratories will vary substantially. In summary, resistance testing should be performed – if possible – before treatment of HCV genotype 1a or 4 patients with grazoprevir, elbasvir and ribavirin (the presence of NS5A RAS requires extended treatment duration of 16 weeks), before treatment of HCV genotype 3 patients with sofosbuvir and velpatasvir (if NS5A RAS are detected, patients should be treated with additional ribavirin), before treatment with simeprevir (which should be avoided in the presence of NS3 Q80K variants), and perhaps in selected cases before re-treatment with older regimens after failure of IFN-free DAA combination therapies.

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12. Standard therapy of chronic hepatitis C virus infection

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Preface

In the next years we will hopefully see a dramatic and universal impact on end-stage liver disease due to the introduction of potent oral drug regimens against hepatitis C virus (HCV) infection. Thanks to a colossal and decade-long effort by medical researchers and pharmaceutical companies around the world, the vast majority of the 64-103 million people living with chronic HCV infection (Gower 2014, Cornberg, 2011, Polaris, 2017) can now potentially be cured by the oral anti-HCV drugs that have been approved over the last five years. One remaining obstacle that has to be solved is the global access to these therapies. The following chapter gives you an overview of today's standard of care.

Goal of antiviral therapy

The prevalence of HCV has already peaked or is starting to decline in some countries due to the implementation of blood-donor screening and treatment uptake; however, globally, HCV-related complications such as cirrhosis, hepatic decompensation, and hepatocellular carcinoma (HCC) are expected to increase in several countries over the course of the next decade with today's treatment paradigm (Razavi 2014). In 2015, approximately 400,000 people died from HCV associated diseases (<http://www.who.int/hepatitis/publications/global-hepatitis-report2017/en/>). Importantly, chronic HCV infection not only increases liver-related mortality but also mortality from extrahepatic diseases (Negro 2015, EASL 2018).

The goal of antiviral therapy is to cure hepatitis C via a sustained elimination of the virus. A sustained elimination of HCV is achieved, if the HCV RNA remains negative three to six months after the end of treatment (sustained virologic response, SVR-12 or SVR-24). Follow-up studies documented that more than 99% of patients who achieved an SVR-24 after interferon alfa (IFN) based therapies remain HCV RNA negative 4-5 years after the end of treatment and no signs of hepatitis have been documented

(Swain 2010, Manns 2013, EASL 2018). In 2011, the FDA accepted SVR12 (HCV RNA negativity 12 weeks after end of treatment) as endpoint for future trials because HCV relapse usually occurs within the first 12 weeks after the end of treatment. The first long-term follow-up studies after therapy with direct-acting antiviral agents (DAA) confirm the durability of SVR-12 in more than 99% of treated patients (Reddy 2018). For DAA treatment regimens even HCV RNA negativity four weeks after therapy has been shown to be highly predictive for achieving long term viral clearance (positive predictive value >98%) (Yoshida 2015). Late virologic relapses at time points beyond 24 weeks after the end of therapy may appear in rare cases but reinfection should always be considered in this situation (Midgard 2016).

Importantly, long-term benefits of SVR are the reduction of HCV-related hepatocellular carcinoma (HCC) and overall mortality (Veldt 2007, Backus 2011, van der Meer 2012). Most data are available after IFN based therapy but first data confirm that eradication of HCV with DAA reduces the risk of HCC by more than 70% (Ioannou 2017). Mathematical modeling forecasts that an increase in SVR by new DAAs and increase in treatment uptake will result in a decline of HCC, decompensated and compensated cirrhosis and consecutive liver-related deaths by 75% in the next 15 years (Wedemeyer 2014). It has been shown that patients with SVR (treated with IFN) have a similar life expectancy compared with the general population (van der Meer 2014, Bruno 2016). In patients with advanced and decompensated cirrhosis, SVR can lead to improvement of liver function (Deterding 2015) and may reduce the need for liver transplantation (Pascasio 2017, Belli 2016). However, the risk to develop HCC is not zero in patients achieving SVR if cirrhosis is already present (El-Serag 2016). In addition to liver disease, several other hepatic manifestations such as cryoglobulinaemia, non-Hodgkin's lymphoma, membranoproliferative glomerulonephritis or porphyria cutanea tarda have been reported in the natural history of HCV infection. Antiviral therapy with IFN can reduce extrahepatic manifestations related to HCV, especially when SVR is achieved (Cacoub 2018a). First data for the newer IFN-free DAA regimens show similar results (Saadoun 2017) (*see also Chapter 13*).

Therapeutic concepts and medication

Development of antiviral treatment

Before the identification of HCV as the infectious agent for non-A, non-B hepatitis (Choo 1989), interferon alfa (IFN) led to a normalisation of transaminases and an improvement of liver histology in some patients (Hoofnagle 1986). After the identification of HCV it became possible to

measure success of therapy as the long-lasting disappearance of HCV RNA from serum, the SVR. Since then, SVR rates have increased from 5-20% with IFN monotherapy, and up to 40-50% with the combination of IFN + ribavirin (RBV) to now close to 100% with direct-acting antiviral agents (DAA) (Figure 1). In between, the development and approval of pegylated interferon alfa (PEG-IFN) improved the pharmacokinetics of IFN, allowing more convenient dosing intervals and resulting in higher SVR, especially for HCV genotype 1 (GT1). Two PEG-IFNs were available: PEG-IFN α -2b (PEG-Intron[®], Merck) and PEG-IFN α -2a (PEGASYS[®], Roche). Although smaller trials from southern Europe have suggested slightly higher SVR rates in patients treated with PEG-IFN α -2a (Ascione 2010, Rumi 2010), a large US multicentre study did not detect any significant difference between the two PEG-IFNs + RBV regarding SVR (McHutchison 2009). For further details regarding pegylated interferons, see Hepatology 2015.

The development of DAA against HCV has revolutionised the treatment of chronic hepatitis C. The main targets for DAAs are the NS3/4A protease, NS5B polymerase and the NS5A replication complex. Combinations of different DAAs from these different classes allow very potent treatments. In 2011, the first selective protease inhibitors (PI) were approved for patients with HCV GT1. Boceprevir (BOC) (Victrelis[®]) and telaprevir (TLV) (Incivek[®]; Incivo[®]) improved SVR rates to up to 75% in naïve HCV GT1 patients and 29-88% in treatment-experienced HCV GT1 patients (Manns 2012, Sarrazin 2012). However, both PIs required combination with PEG-IFN + RBV because monotherapy would result in rapid emergence of drug resistance. Also, these two PIs cannot be combined as they have the same target and cross-resistance. Either of the two PIs can be combined with PEG-IFN α -2a or PEG-IFN α -2b (Sarrazin 2012). TLV had to be administered at least twice daily (Buti 2012) and BOC three times daily and both PIs are associated with severe side effects, especially anaemia (Maasoumy 2013b, Hezode 2014a). In 2014, new DAAs were approved. Simeprevir (SMV) (Olysio[®], Sovriad[®]) was the first once-daily PI. The SVR rates for treatment-naïve GT1 patients increase to 80-81% with PEG-IFN+RBV plus SMV (Jacobson 2014, Manns 2014). However, this was not a major improvement over BOC or TLV triple therapy (Reddy 2015b). However, with SMV more patients achieve an early treatment response and qualify for shorter treatment duration of 24 weeks compared with the first wave PIs. Importantly, SMV also has significantly less side effects (Reddy 2015b).

Sofosbuvir (SOF) (Sovaldi[®]) was the first available once-daily NS5B polymerase inhibitor (approved 12/2013 by FDA and 1/2014 by EMA). For genotype 1, PEG-IFN+RBV + SOF for just 12 weeks leads to 89% SVR in treatment-naïve patients (Lawitz 2013). The resistance barrier of SOF is much higher compared to the available PIs. Very few individuals have developed a confirmed selection of SOF-resistant variants. Thus, a combination of only

SOF + RBV was sufficient for a substantial proportion of patients. Valid data were first published for genotypes 2 and 3 (Zeuzem 2014a) with SVR rates of 85–100% for treatment-naïve GT2/3 patients. SOF can also be combined with a PI or a NS5A inhibitor, i.e. treatment with SOF+SMV resulted in 92% SVR in GT1 (Lawitz 2014) also later confirmed in large real-world cohorts (Sulkowski 2016).

The combination of SOF with the NS5A inhibitor daclatasvir (DCV, Daklinza®) or ledipasvir (LDV) were the first NS5A based IFN free combination therapies that have also shown >90% SVR (Sulkowski 2014, Kowdley 2014, Afdhal 2014a; Afdhal 2014b). Importantly, the combination SOF+DCV (approved by EMA in 8/2014) and the fixed dose single tablet combination of SOF/LDV (Harvoni® approved in 10/2014 by FDA and 11/2014 by EMA) showed >95% SVR in GT1 patients with treatment failure on PEG-IFN+RBV/PI triple therapies (Sulkowski 2014, Afdhal 2014a). SOF in combination with DCV or LDV also has some activity against other genotypes including GT3 (Cornberg 2017). The so-called 3D regimen, ombitasvir (OBV), paritaprevir/r (PTV/r) (Viekirax®), and dasabuvir (DSV, Exviera®) (approved in 12/2014 by FDA and 1/2015 by EMA for GT1 and GT4 patients) was the first combination that includes DAAs against all three targets (Ferenzi 2014, Poordad 2014, Zeuzem 2014b, Feld 2014). In 2016, the fixed dose combinations elbasvir (EBR) plus grazoprevir (GZR) (Zepatier®) was approved and was the first single tablet regimen for GT1 and GT4 patients with chronic kidney disease (CKD) (Zeuzem 2015, Kwo 2017, Roth 2015). The first pangenotypic DAA combinations SOF plus velpatasvir (VEL) (Curry 2015b, Feld 2015, Foster 2015) and glecaprevir (GLE) plus pibrentasvir (Zeuzem 2018, Puoti 2018) were approved in 2016 and 2017. Finally, the triple fixed dose combination SOF/VEL plus voxilaprevir (VOX) was approved in 8/2017 and allows retreatment of patients who failed DAA therapy (Bourlière 2017). In 2020 all patients can be treated with DAA therapy, only non-GT1/4 patients with decompensated cirrhosis plus CKD may remain challenging. SVR rates are above 95% for all patients and RBV is only necessary in decompensated cirrhosis.

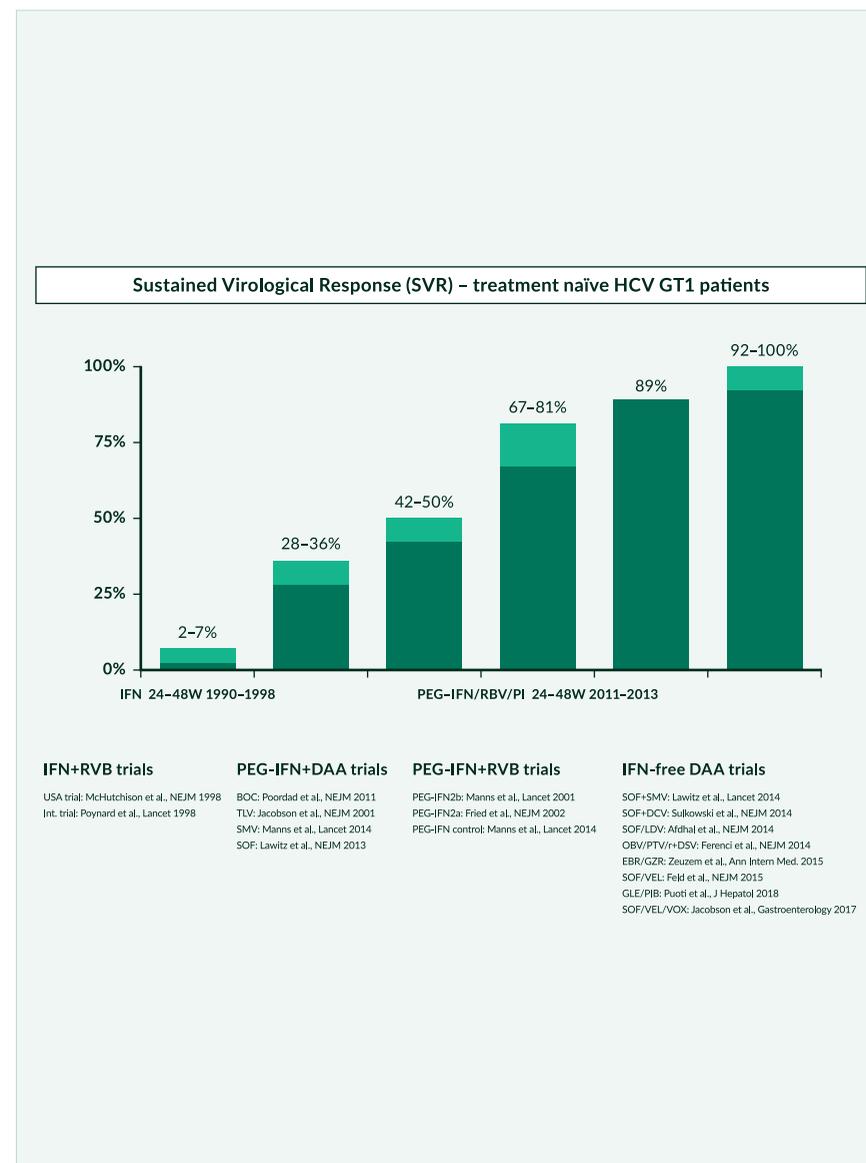


Figure 1. Development of chronic hepatitis C therapy. The sustained virologic response rates have improved from around 5% with interferon monotherapy in the early 90s to >95% today with DAA combinations (data for treatment-naïve GT1 patients). Indicated trials are not head-to-head and it is difficult to compare SVR between different studies because the populations had significant differences in genetic and socioeconomic backgrounds.

Table 1. Approved medication for the treatment of chronic hepatitis C (5/2018). Not all medication is still available or recommended. Generics are not considered.

Medication	Dosing	Comment
Type I interferons	Subcutaneous injection	IFNs are not recommended if DAA combinations are available (EASL, 2018)
Pegylated interferon α -2a (Pegasys®)	180 μ g once weekly	
Pegylated interferon α -2b (PEG-Intron®)	1.5 μ g/kg once weekly	
Interferon α -2a (Roferon®)	3 - 4.5 Mill IU three times weekly	
Interferon α -2b (Intron A®)	3 Mill IU three times weekly	
Consensus Interferon (Infergen®)	9 μ g three times weekly	
Ribavirin	Oral	Ribavirin should be avoided if possible (EASL, 2018)
Ribavirin (Copegus®)	800 - 1200 mg daily (200 mg or 400 mg tablets)	
Ribavirin (Rebetol®)	600 - 1400 mg daily (200 mg tablets or solution)	
HCV NS3/4A protease inhibitors	Oral	
Boceprevir (Victrelis®)	800 mg (4 x 200 mg tablets) every 7-9 hours	Boceprevir and Telaprevir are no longer available since 2014/2015
Telaprevir (Incivek®, Incivo®)	750 mg (2 x 375 mg tablets) every 7-9 hours* *3 x 375 mg every 12 hours in treatment-naïve patients	
Simeprevir (Olysio® (US, EU), Sovriad® (Japan), Galexos® (Canada))	150 mg (1 x 150 mg tablets) once daily 100 mg in Japan	Olysio® is no longer available since 5/2018
Paritaprevir (coformulated with ritonavir and ombitasvir as Viekirax®)	150 mg once daily (2 x 75 mg, 2 tablets once daily)	Paritaprevir is no longer available in some countries such as Germany since 2018
Asunaprevir (Sunvepra® (Japan))	100 mg (1 tablet) twice daily	Asunaprevir is only available in Japan in combination with Daclatasvir
Grazoprevir (coformulated with elbasvir as Zepatier®)	100 mg (1 tablet) once daily	
Glecaprevir (coformulated with pibrentasvir as Maviret® or Mavyret®)	300 mg once daily (3 tablets a 100 mg) once daily	
Voxilaprevir (coformulated with sofosbuvir and velpatasvir as Vosevi®)	100 mg once daily (1 tablet) once daily	

Medication	Dosing	Comment
HCV NS5B polymerase inhibitors	Oral	
Sofosbuvir (Sofaldi®) (Nucleotide analogue)	400 mg (1 tablet) once daily	
Dasabuvir (Exviera®) (Non-Nucleoside analogue)	250 mg (1 tablet) twice daily	Dasabuvir is no longer available in some countries such as Germany since 2018
HCV NS5A replication complex inhibitor	Oral	
Daclatasvir (Daklinza®)	60 mg (1 tablet) once daily (dose adjustments if coadministered with CYP3A4 inhibitor (30 mg/d) or inducer (90 mg/d))	
Ledipasvir (coformulated with sofosbuvir as Harvoni®)	90 mg (1 tablet) once daily	
Ombitasvir (coformulated with paritaprevir/ritonavir as Viekirax®)	25 mg once daily (2 x 12.5 mg, 2 tablets once daily)	Ombitasvir is no longer available in some countries such as Germany since 2018
Elbasvir (coformulated with grazoprevir as Zepatier®)	100 mg (1 tablet) once daily	
Velpatasvir (coformulated with sofosbuvir as Eplclusa® or with sofosbuvir and voxilaprevir as Vosevi®)	100 mg (1 tablet) once daily	
Pibrentasvir (coformulated with glecaprevir as Maviret® or Mavyret®)	120 mg (3 tablets a 40 mg) once daily	

Table 2. Fixed dose DAA combinations used* for the treatment of chronic hepatitis C in 2018 and discussed in this document. Generics are not considered.

Name	DAA	Dosing
Harvoni®	sofosbuvir/ledipasvir (SOF/LDV)	1 tablet once daily (with or without food)
Zepatier®	grazoprevir/elbasvir (GZR/EBR)	1 table once daily (with or without food)
Eplclusa®	sofosbuvir/velpatasvir (SOF/VEL)	1 tablet once daily (with or without food)
Maviret®	glecaprevir/pibrentasvir (GLE/PIB)	3 tablets once daily (with food)
Vosevi®	sofosbuvir/velpatasvir/voxilaprevir (SOF/VEL/VOX)	1 tablet once daily (with food)

Treatment indication

In general, every patient with chronic hepatitis C should receive antiviral therapy, because patients who are cured of their HCV infection benefit as indicated above, i.e., reduction in the risk of hepatocellular carcinoma (HCC), liver-related mortality and even all-cause mortality. DAA regimens, ideally IFN-free and RBV-free regimens, should be preferred (EASL 2018). However, if resources are limited and DAA therapies are not easily accessible, treatment should not be delayed in patients with advanced fibrosis and high risk for liver-related complications. These patients should be treated with high priority with the best available treatment option. Also, patients with severe extrahepatic hepatitis C manifestations should be given high priority for immediate treatment. The timing of treatment in patients with mild liver disease can be individualised; waiting for IFN-free therapies with low risk for side effects - if so far not available - should be considered.

Another reason for early treatment is the prevention of further transmission of the virus in individuals at high risk of transmitting HCV (PWIDs, men who have sex with men (MSM) with high-risk sexual practices, women of childbearing age, hemodialysis patients, prison inmates) (EASL 2018). However, the risk of re-infection is high in risk groups (PWIDs and MSM) (Midgard 2016, Ingiliz 2017) and preventive measures to reduce this risk after successful treatment should be implemented.

Patients with decompensated cirrhosis and an indication for liver transplantation with a MELD score above 18-20 may be treated after transplantation, because the probability of significant improvement in liver function and delisting is low (EASL 2018). Treatment is generally not recommended in patients with limited life expectancy because of non-HCV-related comorbidities (EASL 2018).

Predictors of treatment response and pre-therapeutic assessment

Over the last decade, tailoring treatment duration and dosing with interferon-based therapies according to individual parameters associated with response has improved SVR. Predicting SVR before the start of antiviral treatment helps in making treatment decisions. Important baseline factors associated with SVR to PEG-IFN+RBV are the HCV genotype, the degree of liver fibrosis and steatosis, baseline viral load, presence of insulin resistance, age, gender, body mass index, ethnicity, and HIV coinfection (see Hepatology 2015). Many of these factors may have less relevance for DAA therapy. For IFN-free therapies other parameters seem to be more important such as HCV subtypes 1a and 1b or antiviral resistance

(for antiviral resistance see extra section below) and in many countries the cost of therapy. Still, liver disease severity is important to assess.

Genotype

The HCV genotype (GT), including GT1 subtype (1a or 1b) is still important to tailor the treatment regimen (duration or decision to add RBV). GT3 is now the most difficult to treat genotype and not all available regimens (e.g. grazoprevir/elbasvir) are effective (EASL 2018). Patients with HCV GT1a have a higher risk of developing resistance on a first wave PI-based therapy compared to HCV GT1b because HCV GT1a requires an exchange of only one nucleotide versus two for HCV GT1b at position 155 in order to develop resistance. For SMV, a GT1a variant with the Q80K mutation is important (reviewed in Sarrazin and Zeuzem 2010). GT1a versus GT1b also plays a role with NS5A inhibitor-based therapies. Efficacy of daclatasvir (DCV) in combination with PEG-IFN+RBV was significantly higher in GT1b compared with GT1a patients (Hezode 2014b). The IFN-free regimen of the protease inhibitor (PI) asunaprevir with DCV is approved in Japan but only for GT1b patients as success rates in GT1a patients were rather low (Lok 2012). Also for the “3D” regimen ombitasvir/paritaprevir/r + dasabuvir there are notable differences between HCV GT1a and GT1b. While the addition of RBV seems to be necessary for all GT1a patients, GT1b does not require RBV (Ferenci 2014). For grazoprevir/elbasvir there seem to be slightly lower SVR rates in genotype 1a. This is mainly due to baseline NS5A resistant associated substitutions (RAS) specific to elbasvir in genotype 1a (Zeuzem 2015). In contrast, no obvious difference has been documented with SOF/LDV or SOF/VEL therapy (Kowdley 2014, Feld 2015). Although SOF has a high barrier for resistance, low-frequency NS5B substitutions may be potentially associated with reduced response rates in HCV GT1b but not GT1a patients (Donaldson 2014). Interestingly, 8 weeks SOF/VEL/VOX was not as effective in GT1a versus GT1b because of the Q80K variant, which were more prevalent in US patients (Jacobson 2017).

However, for the easy to treat non-cirrhotic patients determination of the genotype may be dispensable because pangenotypic therapies are available. This simplified treatment algorithm would be important to accelerate treatment uptake.

Genotype 2k / genotype 1b hybrid

Patients can be infected with hepatitis C viruses that are hybrids of different genotypes. Some patients that are infected with such hybrids can be misclassified as GT2a/c with standard genotype assays, which is specific for the structural HCV GT2 proteins. However, the virus has a

GT2k sequence in the structural HCV proteins and a GT1b sequence in the non-structural (NS) HCV proteins. This can only be detected by sequencing or with certain assays that also analyses this region (De Keukeleire 2015b, De Keukeleire 2015a). This specific subtype is predominantly prevalent in patients from Eastern Europe (i.e. Georgia) (Karchava 2015). However, the GT2k/GT1b can also be found in other countries due to immigration. If GT2k/GT1b is present, patients can be successfully treated with a GT1 specific therapy because DAA target the NS proteins (Susser 2017, Todt 2017). Thus, we recommend testing for GT2k/GT1b in patients with origin from Eastern Europe and other parameter, which could hint towards this issue, such as treatment failure in a GT2 patient, if patients have no access to pangenotypic therapies.

HCV RNA

Quantitative HCV RNA kinetics during treatment was (and is) the strongest on-treatment SVR predictor for most PEG-IFN+RBV-based regimens. However, nowadays this issue is less relevant if potent DAA combinations are being used. Due to the excellent tolerance and the rare cases of virological breakthroughs a response guided strategy or stopping criteria have not been implemented for IFN-free regimens. According to the prescribing information all patients are treated for a fixed treatment duration and SVR rates are in general high. However, the current AASLD/IDSA guidance still recommend testing for quantitative HCV RNA at week 4 of DAA therapy mainly to monitor patient compliance (<https://www.hcvguidelines.org/evaluate/monitoring>). However, so far data are rather limited on how on-treatment HCV RNA levels have to be interpreted. For the majority of patients on treatment HCV RNA during IFN-free DAA therapy does not seem to have any predictive value (Maasoumy 2016). Some studies demonstrated that detectable HCV RNA may be found frequently even at the end of therapy, in particular if highly sensitive assays are used for HCV RNA quantification. However, the vast majority of these patients still achieve SVR (Maasoumy 2016). Therefore, treatment extension cannot be recommended in these cases.

In an Asian proof-of-concept study with GT1b patients without cirrhosis, all patients who achieved an ultrarapid virological response on triple direct-acting antiviral regimens by day 2 and received only 3 weeks of treatment achieved SVR. This study suggests that HCV RNA measurements at very early points in time during treatment could guide treatment duration (Lau 2016). Despite this interesting data, on-treatment HCV RNA monitoring is not recommended to shorten or prolong treatment with modern IFN-free DAA therapies and should only be used to monitor patient compliance (<https://www.hcvguidelines.org/evaluate/monitoring>). EASL

has diminished the need for any viral load testing for the first time in their current version of HCV treatment recommendations. Viral load testing is only recommended before and 12 or 24 weeks after the end of antiviral therapy. Instead of HCV RNA also HCV core antigen can be performed if HCV RNA tests are not available or affordable (EASL 2018).

Genetic polymorphisms

Genome-wide association studies have identified host genetic polymorphisms (i.e., rs12979860, rs8099917) located on chromosome 19 upstream of the region coding for IL28B (or IFN λ 3) associated with spontaneous HCV clearance and SVR to treatment with PEG-IFN+RBV (Ge 2009, Rauch 2010, Tanaka 2009, Suppiah 2009). Recently, a new dinucleotide variant ss469415590 (TT or Δ G) upstream of IL28B (or IFN λ 3), which is in high linkage disequilibrium with IL28B rs12979860 was discovered (Prokunina-Olsson 2013). Compared to the IL28B SNP, the IFN λ 4 DNP is more strongly associated with HCV clearance in individuals of African ancestry, although it provides comparable information in Europeans and Asians (Prokunina-Olsson 2013). So far, screening for genetic variants has not been shown to be useful for modern IFN-free DAA regimens. Given the high overall response rates of DAA combination therapies it is in general difficult to identify statistically significant predictive markers.

Others

For SOF plus RBV or LDV, female sex and to a lesser degree baseline viraemia of $<6 \log_{10}$ IU/mL and a body weight $<30 \text{ kg/m}^2$ are associated with numerically higher SVR rates. As discussed above, GT1b patients generally respond better to some of the approved DAA regimens (i.e. GZR/EBR). An important response predictor remains the stage of liver disease and interestingly previous treatment with PEG-IFN+RBV.

Antiviral resistance

The development of DAA leads to the emerging problem of drug resistance due to so-called resistance-associated amino acid substitutions (RAS) of the virus. Patients who received monotherapy with certain DAAs, i.e., the 1st generation PIs BOC or TLV developed resistance within a few days (Sarrazin 2007). If RAS emerge, it is not completely known for how long they persist and if this has any significant consequences for future therapies. Some studies suggest that the majority of PI resistant variants revert to wild type within 1-2 years after the end of therapy. This may be

different for NS5A RASs (reviewed in Sarrazin 2016).

At this stage there is no recommendation to routinely analyse HCV sequences either before therapy or during DAA treatment, because it has no practical consequence up to now. One exception was the testing for the Q80K variant in GT1a patients treated with PEG-IFN+RBV plus SMV. The combination of different DAA classes should overcome the problem of resistance and allow IFN-free combinations. SOF has a very high resistance barrier and even SOF plus the weak antiviral RBV lead to high SVR rates and treatment failure is mainly related to relapse and not breakthrough (Osinusi 2013). SOF combined with a PI (SOF+SMV) or an NS5A inhibitor (SOF+DCV or SOF/LDV or SOF/VEL) shows SVR rates >90%. However, based on some studies NS5A RASs may become an issue in clinical practice. The frequency of baseline NS5A RAS was approximately 16% in the SOF/LDV studies (reviewed in (Sarrazin 2016) and 20% in GZR/EBR studies (Jacobson et al., 2015) based on population Sanger sequencing (PopSeq) with a threshold of >25% for minor variant detection. With next generation deep sequencing (NGS) and a sensitivity threshold of 1%, the frequency of detectable NS5A RASs is much higher but minor populations that are now detected may have less clinical relevance (Jacobson et al., 2015). Drug specific NS5A variants detected with PopSeq have the highest impact on SVR but these RASs are not frequent. This has been systematically analysed for GZR/EBR (Table 3).

Table 3. Relevance of baseline NS5A RASs. Efficacy of 12 weeks GZR/EBR in genotype 1a patients with baseline NS5A RASs (Jacobson et al., 2015).

Sequencing method	All NS5A RAVs		EBR specific RAVs		No NS5A RAVs	
	RAS prevalence	SVR12	RAS prevalence	SVR12	Prevalence	SVR12
PopSeq n=438	86/438 (20%)	74/86 (86%)	24/438 (5%)	14/24 (58%)	352/438 (80%)	389/396 (98%)
NGS 1% sensitivity n=439	150/439 (34%)	136/150 (91%)	43/439 (10%)	31/43 (72%)	289/439 (66%)	284/289 (98%)

In the case of GZR/EBR, NS5A RASs had no impact on SVR in GT1b patients. The NS5A RASs may be of more importance in GT1a and especially if other negative predictors (previous non-responder, advanced cirrhosis) are present. Baseline NS5A RAS testing may therefore be important in certain patient groups (i.e. GT1a and GT3) to optimise treatment, especially because NS5A RAS do not vanish over time (reviewed in (Sarrazin 2016).

In the case of potent NS5A regimens, baseline RAS testing may not be necessary prior to first-line therapy (EASL 2018). However, this topic may deserve more attention in the future when we need to select the ideal salvage therapy for patients after treatment failure on DAA combinations.

Treatment in 2020

We will only review the fixed dose combinations that are listed in table 2. For previous DAA therapies (i.e. sofosbuvir + daclatasvir or ombitasvir/paritaprevir/r + dasabuvir see Hepatology 2016). In countries where the DAA combinations listed in table 2 are available and reimbursable, these therapies replace the older regimens. As a consequence of the rapid development of new DAAs, the marketing and production of boceprevir and telaprevir was terminated in the US by the respective pharmaceutical company in 2014/2015. In 2018, Janssen Pharmaceuticals Inc. (Janssen) has decided to terminate the license that it holds for simeprevir due to their assessment of market demand. Daclatasvir is not used anymore in several countries such as Germany because it has to be combined with sofosbuvir and this combination is more expensive than any other DAA regimen listed in table 2. However, the combination of sofosbuvir + daclatasvir is still frequently used in countries where these drugs are generic. Due to the approval of Maviret® (glecaprevir/pibrentasvir), Abbvie decided to withdraw the 3D combination (ombitasvir/paritaprevir/r + dasabuvir) in some countries (e.g. Germany). The EASL recommendations do not recommend interferon alfa anymore and suggest avoiding ribavirin if possible (EASL 2018). The combination of at least two of the three major drug classes (protease inhibitors, polymerase inhibitors and NS5A inhibitors) results in SVR ≥95% with just 8-12 weeks treatment. Most of efficacy data of the DAA combinations have been confirmed in real-world by large registries.

However, treatment options can be different around the world as not all new treatment options will be accessible in all countries at the same time and in some countries, generics are available (Zeng 2017). Thus, for detailed information regarding older treatment options such as dual treatment with PEG-IFN+RBV or triple treatment regimens including PEG-IFN+RBV plus protease inhibitors we refer you to the previous edition of the textbook dating from 2015. For DAA combinations sofosbuvir + ribavirin, sofosbuvir + simeprevir, sofosbuvir + daclatasvir and for the 3D combination (ombitasvir/paritaprevir/r + dasabuvir) we refer you to the textbook dating from 2016.

All approved IFN-free DAA regimens have an excellent safety profile and a similar efficacy. Table 4 & 5 give an overview of the treatment schedules with SOF/LDV, GZR/EBR, SOF/VEL, GLE/PIB and SOF/VEL/VOX based on the data discussed below. Due to the high efficacy, good tolerability and wider eligibility it is very likely that these regimens will also prove to have a high population-based effectiveness, which was in the end disappointing for the first-generation PI-based triple therapies (Maasoumy 2014).

Table 4. Treatment of patients with chronic hepatitis C without cirrhosis.

GT	Pretreatment	SOF/LDV	GZR/EBR	GLE/PIB	SOF/VEL	SOF/VEL/VOX
1a	No (naïve)	8 weeks ⁶	12 weeks ²	8 weeks	12 weeks	8# weeks
	PEG-IFN+RBV ± SOF or SOF+RBV	12 weeks ^{3,5}	12 weeks ^{2,3}	8 weeks ⁹	12 weeks ³	8* [#] -12 weeks
	DAA-Tx with NS5A Inhibitor	no	no	16 weeks ⁷	no	12 weeks
1b	No (naïve)	8 weeks ⁶	12 weeks ⁴	8 weeks	12 weeks	8 weeks
	PEG-IFN+RBV ± SOF or SOF+RBV	12 weeks ³	12 weeks ³	8 weeks ⁸	12 weeks ³	8*-12 weeks
	DAA-Tx with NS5A Inhibitor	no	no	16 weeks ⁷	no	12 weeks
2	No (naïve)	no	no	8 weeks	12 weeks	8 weeks
	PEG-IFN+RBV ± SOF or SOF+RBV	no	no	8 weeks	12 weeks	8*-12 weeks
	DAA-Tx with NS5A Inhibitor	no	no	no	no	12 weeks
3	No (naïve)	no	no	8 weeks	12 weeks	8 weeks
	PEG-IFN+RBV ± SOF or SOF+RBV	no	no	16 weeks ¹	12 weeks	8*-12 weeks
	DAA-Tx with NS5A Inhibitor	no	no	no	no	12 weeks
4	No (naïve)	12 weeks	12 weeks ²	8 weeks	12 weeks	8 weeks
	PEG-IFN+RBV ± SOF or SOF+RBV	12 weeks ^{3,5}	12 weeks ^{2,3,5}	8 weeks	12 weeks ³	8*-12 weeks
	DAA-Tx with NS5A Inhibitor	no	no	no	no	12 weeks
5, 6	No (naïve)	12 weeks	no	8 weeks	12 weeks	8 weeks
	PEG-IFN+RBV ± SOF or SOF+RBV	no	no	8 weeks	12 weeks	8*-12 weeks
	DAA-Tx with NS5A Inhibitor	no	no	no	no	12 weeks

■ SVR_≥95%, RBV free

■ Ribavirin may be required in some patients

■ SVR _≥95% but not recommended as first line treatment

■ SVR <95%, or no sufficient data.

PEG-IFN: pegylated Interferon alfa; **RBV:** ribavirin; **GLE:** Glecaprevir; **PIB:** Pibrentasvir; **GZR:** Grazoprevir; **EBR:** Elbasvir; **SOF:** Sofosbuvir; **VEL:** Velpatasvir; **LDV:** Ledipasvir; **VOX:** Voxilaprevir

* DAA naïve. # lower response in GT1a patients in the US (Q80K). ¹ EASL recommends 12 weeks. ² HCV RNA \leq 800,000 IU/mL at baseline or exclusion of NS5A RAS. ³ Treatment experience with PEG-IFN+RBV+PI (Simeprevir, Telaprevir or Boceprevir) was analysed. ⁴ 8 weeks if FO-2 (fibroscan \leq 9.5kPA). ⁵ not recommended as RBV free treatment option by EASL 2018. ⁶ Female and male with HCV RNA <6 million IU/mL at baseline. ⁷ prior therapy with NS5A-Inhibitor but without protease inhibitor (FDA label, not recommended by EMA). ⁸ 12 weeks if pretreatment with protease-Inhibitor but without NS5A inhibitor (FDA label, not recommended by EMA).

Table 5. Treatment of patients with chronic hepatitis C with compensated cirrhosis.

GT	Pretreatment	SOF/LDV	GZR/EBR	GLE/PIB	SOF/VEL	SOF/VEL/VOX
1a	No (naïve)	12 weeks ⁵	12 weeks ¹	8 weeks ⁸	12 weeks	12 weeks
	PEG-IFN+RBV ± SOF or SOF+RBV	12 weeks ^{3,5}	12 weeks ^{1,2}	12 weeks ⁷	12 weeks ²	12 weeks
	DAA-Tx with NS5A Inhibitor	no	no	16 weeks ⁶	no	12 weeks
1b	No (naïve)	12 weeks ⁵	12 weeks	8 weeks ⁸	12 weeks	12 weeks
	PEG-IFN+RBV ± SOF or SOF+RBV	12 weeks ^{3,5}	12 weeks ²	12 weeks ⁷	12 weeks ²	12 weeks
	DAA-Tx with NS5A Inhibitor	no	no	16 weeks ⁶	no	12 weeks
2	No (naïve)	no	no	8 weeks ⁸	12 weeks	12 weeks
	PEG-IFN+RBV ± SOF or SOF+RBV	no	no	12 weeks	12 weeks	12 weeks
	DAA-Tx with NS5A Inhibitor	no	no	no	no	12 weeks
3	No (naïve)	no	no	8 weeks ⁸	12 weeks ^{3,4}	8 weeks
	PEG-IFN+RBV ± SOF or SOF+RBV	no	no	16 weeks	12 weeks ^{3,4}	8*-12 weeks
	DAA-Tx with NS5A Inhibitor	no	no	no	no	12 weeks
4	No (naïve)	12 weeks	12 weeks ¹	8 weeks ⁸	12 weeks	12 weeks
	PEG-IFN+RBV ± SOF or SOF+RBV	12 weeks ^{3,5}	12 weeks ^{1,2,3}	12 weeks	12 weeks ²	12 weeks
	DAA-Tx with NS5A Inhibitor	no	no	no	no	12 weeks
5, 6	No (naïve)	12 weeks	no	8 weeks ⁸	12 weeks	12 weeks
	PEG-IFN+RBV ± SOF or SOF+RBV	no	no	12 weeks	12 weeks	12 weeks
	DAA-Tx with NS5A Inhibitor	no	no	no	no	12 weeks

■ SVR_≥95%, RBV free

■ Ribavirin may be required in some patients

■ SVR _≥95% but not recommended as first line treatment

■ SVR <95%, or no sufficient data.

PEG-IFN: pegylated Interferon alfa; **RBV:** ribavirin; **GLE:** Glecaprevir; **PIB:** Pibrentasvir; **GZR:** Grazoprevir; **EBR:** Elbasvir; **SOF:** Sofosbuvir; **VEL:** Velpatasvir; **LDV:** Ledipasvir; **VOX:** Voxilaprevir

* DAA naïve. ¹ HCV RNA \leq 800,000 IU/mL at baseline or exclusion of NS5A RAS. ² Treatment experience with PEG-IFN+RBV+PI (Simeprevir, Telaprevir or Boceprevir) was analysed. ³ not recommended as RBV free treatment option by EASL 2018. ⁴ If NS5A RAS (Y93H) detected additional RBV recommended. ⁵ If platelets <75,000/ μ l ribavirin should be added or/and treatment may be extended to 24 weeks. ⁶ pretreatment with NS5A-Inhibitor but without protease inhibitor (FDA label, not recommended by EMA). ⁷ prior therapy with protease-Inhibitor but without NS5A inhibitor (FDA label, not recommended by EMA). ⁸ based on the The Expedition-8 Trial (FDA approved for all genotypes; EMA approval for GT3 pending).

Sofosbuvir and Ledipasvir (SOF/LDV)

The combination of SOF and LDV is available as a single-tablet fixed-dose combination (Harvoni®, Gilead Sciences). The single pill contains the NS5B polymerase inhibitor SOF (400 mg) and the NS5A inhibitor LDV (90 mg). SOF/LDV is recommended for patients infected with genotype 1, 4-6. Some data (phase 2 and real-world) are available for GT3 patients (Cornberg 2017), but as better treatment options for GT3 are available, SOF/LDV is not recommended for GT3 (EASL 2018). SOF/LDV is not primarily recommended for patients with chronic kidney disease (CKD with GFR < 30 mL/min) but can be used in these patients when no other relevant treatment options are available, e.g. in patients with decompensated cirrhosis where protease inhibitors are not recommended. The FDA has updated the label and now state that that no dosage adjustment is recommended in patients with any degree of renal impairment including patients on dialysis based on pharmacokinetic data obtained from studies involving HCV-infected patients with renal impairment including dialysis patients.

Genotype 1

SOF/LDV was studied in the ION-1 (Afdhal 2014b) and ION-3 (Kowdley 2014) trials in treatment-naïve patients (Table 6). ION-1 studied 12 vs. 24 weeks SOF/LDV in 865 patients, including cirrhotic patients, and ION-3 investigated 8 vs. 12 weeks in 647 non-cirrhotic patients. In non-cirrhotic patients, SOF/LDV demonstrated an SVR₁₂ of >99.5% irrespective of the use of RBV or a 12 or 24-week treatment duration (Afdhal 2014b). Shortening treatment duration to 8 weeks was evaluated in the ION-3 trial, which showed an SVR of 93% and 94% with and without RBV, respectively (Kowdley et al., 2014). Relapse occurred more frequently in patients with baseline viral load >6 million IU/mL (relapse 10% versus 2% without RBV) and male patients (relapse 8% versus 1%). In real-world data, excellent SVR rates are confirmed with 8 weeks SOF/LDV in patients who fall into this category but the cut-off of 6 million IU/mL may not be so important (Buggisch 2017). However, SVR was slightly diminished to 90% in patients taking proton pump inhibitors (PPI) (Terrault 2016). The timing of PPI dosing needs consideration. Based on the approvals of FDA and EMA, treatment can be shortened to 8 weeks in treatment-naïve non-cirrhotic patients with a baseline viral load <6 million IU/mL (Table 4). AASLD/IDSA does only recommend 8 weeks SOF/LDV in naïve GT1 patients without cirrhosis who are non-black, HIV-uninfected, and whose HCV RNA level is <6 million IU/mL (<https://www.hcvguidelines.org/treatment-naive/gt1a/no-cirrhosis>).

Cirrhotic patients had an SVR of 100% if SOF/LDV was combined with RBV for 12 or 24 weeks. Without the concomitant use of RBV, an SVR of

97% was achieved with 12 or 24 weeks of treatment (Afdhal 2014b). Based on these results, the FDA recommends 12 weeks of treatment in treatment-naïve patients with cirrhosis, while the EMA recommends 24 weeks of treatment, which may be shortened to 12 weeks in patients with a slow disease progression and the option for retreatment. The concomitant use of RBV is not recommended in naïve patients with compensated cirrhosis. A retrospective analysis of >500 patients with cirrhosis confirmed that naïve patients with compensated cirrhosis can be treated for 12 weeks with SOF/LDV without RBV (Reddy 2015a). SOF/LDV plus RBV over 12 or 24 weeks has been investigated in patients with decompensated liver cirrhosis (Child-Pugh B and C) before or after liver transplantation. SVR₁₂ was around 75% in Child-C patients and more than 80% in Child-B patients. Some patients died during the study period mainly because of complications related to hepatic decompensation (Charlton 2015, Manns 2016) (see section cirrhosis below). Due to limited data at the time of approval in patients with advanced or decompensated cirrhosis EMA recommended 24 weeks SOF/LDV + RBV for decompensated cirrhosis pre-/post liver transplant.

The use of IFN-free SOF/LDV in PEG-IFN+RBV treatment-experienced patients was investigated in the ION-2 trial in cirrhotic and non-cirrhotic patients (Afdhal 2014a). Previous treatment was either with PEG-IFN+RBV or PEG-IFN+RBV + TLV or BOC. Overall, no marked difference could be shown between the treatment duration of 12 or 24 weeks and the addition of RBV to the SOF/LDV combination in non-cirrhotic patients. 12 weeks of SOF/LDV achieved an SVR of 95%, whereas 24 weeks achieved 99%. The addition of RBV to 12 weeks of SOF/LDV demonstrated an SVR of 100% and 99% for 24 weeks of treatment. In cirrhotic patients the SVR rates decreased to 86% for 12 weeks of SOF/LDV and 82% for SOF/LDV+RBV. Treatment for 24 weeks achieved an SVR of 100% regardless of the use of RBV (Table 7). However, each study arm consisted of only 22 treatment-experienced cirrhotic patients. Based on these findings the FDA recommended a duration of 12 weeks for treatment-experienced non-cirrhotic patients and 24 weeks for treatment-experienced cirrhotic patients with SOF/LDV. Of note, a retrospective analysis of >500 patients with cirrhosis treated within all Gilead SOF/LDV Phase 2 and Phase 3 trials revealed that SVR after 12 weeks SOF/LDV was 5-9% lower in the treatment-experienced patients compared to naïve patients (Reddy 2015a). The addition of RBV to a 12 weeks regimen of SOF/LDV demonstrates SVR rates of 96% and is comparable with the 24 weeks regimen in treatment-experienced patients with compensated cirrhosis. Although the addition of RBV is not part of the EMA or FDA label, it may be considered in treatment-experienced patients with compensated cirrhosis as an option to shorten treatment, while maintaining a reasonable SVR rate. The EASL IFN and RBV free recommendations recommend 12 weeks SOF/LDV only in treatment-experienced GT1b patients (EASL 2018).

The efficacy of SOF/LDV in patients with prior exposure to a PI has been investigated in the ION-2 and the SIRIUS trial (Afdhal 2014a, Bourlière 2015). Overall, response rates were similar to the response rates in patients who were treated with PEG-IFN+RBV. In total, 231 patients in the ION-2 trial had previous exposure to a PI. LDV/SOF for 12 weeks led to an SVR of 94%, and for 24 weeks 97%. The addition of RBV resulted in SVR rates of 97% and 100%, respectively (Afdhal 2014a). In the SIRIUS trial 155 GT1 patients with previous PEG-IFN+RBV+PI non-response and compensated cirrhosis have been treated either with SOF/LDV + RBV for 12 weeks or SOF/LDV + placebo for 24 weeks. 12 weeks SOF/LDV + RBV or SOF/LDV for 24 weeks provided similarly high SVR12 rates of 96-97% (Bourlière 2015).

Genotypes 2 and 3

SOF/LDV is not recommended for GT2 (EASL 2018). There are limited data for SOF/LDV in naïve patients with GT3. 51 patients were treated either with SOF/LDV or with SOF/LDV+RBV for 12 weeks in the ELECTRON-2 study. 64% of patients treated with SOF/LDV achieved SVR while 100% of patients achieved SVR with SOF/LDV plus RBV. SOF/LDV+RBV for 12 weeks has been studied in 50 treatment-experienced patients with GT3. SVR was 89% for patients without cirrhosis and 73% for patients with cirrhosis (Gane 2015). EMA (not FDA) approved SOF/LDV + RBV for 24 weeks for GT3 patients with cirrhosis. Thus, some real-world data are available. For example, in Germany SOF/LDV + RBV for 24 weeks achieved 89-93% in cirrhotic patients (Cornberg 2017). Despite these data we will not recommend SOF/LDV + RBV for 24 weeks for GT3 because alternative treatment options are available (see below).

Genotype 4

SOF/LDV is approved for GT4 although data were limited at that time. SOF/LDV given for 12 weeks resulted in 95% SVR in 21 GT4 patients (Kapoor 2014). A phase 3 study from Egypt has investigated SOF/LDV in 255 patients. Treatment-naïve patients showed 95% and 90% SVR with 8 weeks of SOF/LDV and SOF/LDV + RBV, respectively, and 98% for 12 weeks SOF/LDV ± RBV. Among PEG-IFN-experienced patients, SVR rates were 94% for 12 weeks SOF/LDV and 100% for SOF/LDV + RBV (Shiha 2018) (Table 8).

Real-world studies have shown >90% SVR in GT4 infected patients (Ahmed 2018).

Genotype 5-6

Data with IFN free regimens are still rare for GT5 and 6. SOF/LDV for 12

weeks has been studied in 41 GT5 and 25 naïve GT6 patients. SVR for GT5 and GT6 are 95-96% (Abergel 2016, Gane 2015) (Table 8).

Table 6. Pivotal phase 3 studies with SOF/LDV treatment regimens in treatment-naïve patients with HCV genotype 1. Studies are not head-to-head and it is difficult to compare SVR between different studies because the populations had significant differences in genetic and socioeconomic backgrounds.

Study	Dosing	SVR
ION-1 (Afdhal 2014b) N=865	a) 400/90 mg SOF/LDV 12 weeks	No cirrhosis: 100% Cirrhosis: 97%
	b) 400/90 mg SOF/LDV + 1000-1200 mg RBV 12 weeks	No cirrhosis: 100% Cirrhosis: 100%
	c) 400/90 mg SOF/LDV 24 weeks	No cirrhosis: 99.5% Cirrhosis: 96.9%
	d) 400/90 mg SOF/LDV + 1000-1200 mg RBV 24 weeks	No cirrhosis: 100% Cirrhosis: 100%
ION-3 (Kowdley 2014) N=647 No cirrhosis	a) 400/90 mg SOF/LDV 8 weeks	94%
	b) 400/90 mg SOF/LDV + 1000-1200 mg RBV 8 weeks	93%
	c) 400/90 mg SOF/LDV 12 weeks	95%

SOF: sofosbuvir, **LDV:** ledipasvir, **RBV:** ribavirin

Table 7. Pivotal phase 2-3 studies with DAA treatment regimens in PEG-IFN+RBV based treatment-experienced patients infected with HCV genotype 1. Studies are not head-to-head and SVR between studies are difficult to compare because they had significant differences in genetic and socioeconomic backgrounds.

Study	Dosing	SVR
ION-2 (Afdhal et al., 2014a) n=440 (treatment-experienced, incl. n=231 pts with failure to previous PI -based therapy)	a) 400/90 mg SOF/LDV 12 weeks	No cirrhosis: 95% Cirrhosis: 86%
	b) 400/90 mg SOF/LDV + 1000-1200 mg RBV 12 weeks	No cirrhosis: 100% Cirrhosis: 82%
	c) 400/90 mg SOF/LDV 24 weeks	No cirrhosis: 99% Cirrhosis: 100%
	d) 400/90 mg SOF/LDV + 1000-1200 mg RBV 24 weeks	No cirrhosis: 99% Cirrhosis: 100%
SIRIUS (Bourlière et al., 2015) n=155 pts with failure to previous PI -based therapy and compensated cirrhosis 63% HCV GT1a	a) 400/90 mg SOF/LDV + 1000-1200 mg RBV 12 weeks	96%
	b) 400/90 mg SOF/LDV 24 weeks	97%

SOF: sofosbuvir, **LDV:** ledipasvir, **RBV:** ribavirin

Table 8. Pivotal phase 2-3 studies with DAA treatment regimens in HCV GT4-6 infection.

Studies are not head-to-head and SVR between studies are difficult to compare because they had significant differences in genetic and socioeconomic backgrounds.

Study	Dosing	SVR
(Kapoor 2014) n=21 GT4	400/90 mg SOF/LDV 12 weeks	95%
(Shiha et al., 2018) n=255 GT4	400/90 mg SOF/LDV 8 weeks	95% (naïve)
	400/90 mg SOF/LDV + RBV 8 weeks	90% (naïve)
	400/90 mg SOF/LDV 12 weeks	94% (IFN-Exp.)
	400/90 mg SOF/LDV + RBV 12 weeks	100% (IFN-Exp.)
	400/90 mg SOF/LDV + RBV 12 weeks (SOF exp.)	100% (SOF-Exp.)
(Abergel et al., 2016) n=41 GT5	400/90 mg SOF/LDV 12 weeks	95% Cirrhosis 89%
(Gane et al., 2015) n=25 GT6, 92% naïve	400/90 mg SOF/LDV 12 weeks	96%

SOF: sofosbuvir, **LDV:** ledipasvir, **RBV:** ribavirin, **Exp.:** treatment experienced patients

Grazoprevir and Elbasvir (GZR/EBR)

The combination of GZR and EBR is available as a single-tablet fixed-dose combination (Zepatier[®], MSD). GZR is a second-generation PI (Summa 2012). EBR is a selective inhibitor of the HCV NS5a replication complex (Coburn 2013). The combination of GZR/EBR (Zepatier[®]) is a fixed dose single tablet regimen. The FDA and EMA approved GZR/EBR (Zepatier[®]) in 2016 for genotype 1 and 4. GZR/EBV can be used in patients with CKD including hemodialysis. GZR/EBR is not recommended for patients with decompensated cirrhosis based on pharmacokinetic data (EASL 2018).

Genotype 1

Treatment naïve GT1 patients have been treated in phase 2 (C-WORTHY) and phase 3 (C-EDGE) trials (Table 9) (Sulkowski 2015, Zeuzem 2015, Cornberg and Manns 2015). Based on this data, naïve patients with GT1 with or without cirrhosis should receive 12 weeks GZR/EBR (Table 4 & 5). Patients with GT1a (naïve as well as PEG-IFN+RBV experienced) who have baseline NS5A RASs have demonstrated lower SVR rates (Table 3). However, this was only relevant for patients with baseline HCV RNA <800,000 IU/mL. It is recommended that GT1a patients with baseline NS5A RASs or a baseline viral load >800,000 IU/mL should be treated for 16 weeks plus RBV. One study analysed if 8 weeks GZR/EBR with or without RBV is sufficient in naïve GT1b patients without cirrhosis. The SVR rate was 93-94% (Vierling

2015). Interim data from a phase 3 study (STREAGER) indicate even higher SVR rates of 98% in GT1b patients with Fo-2 fibrosis (Abergel 2018). Thus, the EASL recommendations suggested using 8 weeks GZR/EBR in treatment naïve GT1b patients with Fo-2 fibrosis (EASL 2018). However, 8 weeks GZR/EBR is so far only approved in Switzerland and Canada.

GZR/EBR has also been evaluated in PEG-IFN+RBV treatment-experienced GT1 patients in the C-EDGE study (Kwo 2017). 35% of the study cohort had cirrhosis. Patients were randomised to receive 12 weeks GZR/EBR, 12 weeks GZR/EBR plus RBV, 16 weeks GZR/EBR or 16 weeks GZR/EBR plus RBV (SVR by intention-to-treat analysis is shown in Table 10). All patients who had a previous relapse and all patients with GT1b achieved SVR with 12 weeks GZR/EBR in the per protocol analysis. GT1a patients with previous non-response to PEG-IFN+RBV had lower SVR rates with 12 weeks GZR/EBR (91%) and may benefit from 16 weeks GZR/EBR plus RBV (100% SVR). However, the reason for relapse was most likely due to baseline NS5A RASs.

The open-label C-SALVAGE study investigated 12 weeks GZR/EBR plus RBV in 79 patients with GT1 and failure to PEG-IFN+RBV plus either BOC, TLV or SMV (Forns 2015). Overall 96% of patients achieved SVR12. There was no significant difference between GT1a and GT1b with 93% versus 96%, respectively. Patients with baseline NS3 RASs had 91% SVR.

A retrospective pooled analysis of all GT1b patients from 11 trials conformed the high efficacy of 12 weeks GZR/EBR. Only 15 out of 1077 (1.4%) treated patients experienced a virological failure (Zeuzem 2018).

Several real-world cohorts confirm the excellent safety and efficacy profile of 12 weeks GZR/EBR with 95-99% SVR rates in GT1 (Flamm 2018, Kramer 2018). Interestingly, in real world patients with GT1a have been treated with 12 weeks GZR/EBR without RAS testing. The SVR in those patients was 98% in the TRIO network analysis (Flamm 2018).

Genotype 3

GZR/EBR was investigated in combination with SOF in treatment naïve and experienced patients with GT3 and compensated liver cirrhosis. Treatment naïve patients were treated for 8 or 12 weeks and achieved 91% and 96% SVR, respectively, Treatment experienced patients were treated 12 weeks, 16 weeks and 12 weeks plus RBV and achieved 100%, 94% and 94% SVR, respectively (Foster 2018). SVR was 100% in the per protocol analysis. Thus, 12 weeks GRZ/EBR + SOF could be an option for GT3. Actually this is recommended by AASLD as one of two first line treatment options in interferon treatment experienced GT3 patients with cirrhosis (<https://www.hcvguidelines.org/treatment-experienced/gt3/p-r/compensated-cirrhosis>). However, this combination is not approved and other options are available.

Genotype 4

GZR/EBR is also effective against genotype 4. The integrated analysis of the phase 2-3 trials showed SVR₁₂ rates of 96% (97/101) for treatment-naïve patients treated with 12 weeks and 100% (8/8) in treatment-experienced participants treated with 16 weeks GZR/EBR plus ribavirin (Asselah 2018b) (Table 11). Baseline NS5A RAS did not impact SVR in this analysis. Based on the data treatment naïve GT4 patients without cirrhosis may be treated with 12 weeks GZR/EBR. However, EASL recommends 12 weeks GZR/EBR in GT4 (no cirrhosis and cirrhosis) if baseline HCV RNA is <800,000 IU/mL.

Other genotypes

The phase 2 C-SCAPE study evaluated GZR/EBR, with or without ribavirin (RBV), in participants with HCV genotype 2, 4, 5 or 6 infections. GT2 patients received 12 weeks GZR + RBV ± EBR. SVR was suboptimal with 73-80%. Those with genotype 4, 5 or 6 infections were randomised to receive EBR/GZR ± RBV for 12 weeks. SVR in GT4 was 90-100%. GT5 SVR was 25% without and 100% with RBV. GT 6 SVR was 75% irrespective of RBV (Brown 2018). Thus, GZR/EBR is not recommended for GT 2, 5 and 6 (EASL 2018).

Table 9. Pivotal phase 2-3 studies with GZR/EBR/ treatment regimens in treatment-naïve patients with HCV genotype 1. Studies are not head-to-head and it is difficult to compare SVR between different studies because the populations had significant differences in genetic and socioeconomic backgrounds.

Study	Dosing	SVR
C-EDGE TN (Zeuzem et al., 2015) n=288 / 94 (treatment-naïve GT1, 22% cirrhosis)	a) 50/100 mg GZR/EBR 12 weeks (n=288 GT1)	GT1a: 92% No NS5A RAS: 98%
	b) Deferred / placebo (n=94 GT1)	GT1b: 99%
C-WORTHY, part C (Vierling et al., 2015) n=61 (treatment-naïve GT1b, no cirrhosis)	a) 50/100 mg GZR/EBR 8 weeks	94%
	b) 50/100 mg GZR/EBR + 1000- 1200 mg RBV 8 weeks	93%
STREAGER (Abergel et al., 2018) n=90 (treatment-naïve GT1b, no cirrhosis, fibrosan <9.5kPa)	a) 50/100 mg GZR/EBR 8 weeks	97%

RBV: ribavirin, GZR: grazoprevir, EBR: elbasvir

Table 10. Pivotal phase 2-3 studies with GZR/EBR in PEG-IFN+RBV based treatment-experienced patients infected with HCV genotype 1. Studies are not head-to-head and SVR between studies are difficult to compare because they had significant differences in genetic and socioeconomic backgrounds.

Study	Dosing	SVR
C-EDGE TE (Kwo et al., 2017) n=374 (PEG-IFN+RBV treatment-experienced, 35% cirrhosis GT1)	a) 50/100 mg GZR/EBR 12 weeks	GT1a: 90.2% GT1b: 100%
	b) 50/100 mg GZR/EBR + 1000-1200 mg RBV 12 weeks	GT1a: 93.3% GT1b: 96.6%
	c) 50/100 mg GZR/EBR 16 weeks	GT1a: 93.8% GT1b: 95.8%
	d) 50/100 mg GZR/EBR + 1000-1200 mg RBV 16 weeks	GT1a: 94.8% (mITT 100%) GT1b: 100%
C-SALVAGE (Forns et al., 2015) n=79 pts. with failure to previous PI based therapy, 43% cirrhosis	50/100 mg GZR/EBR + 1000- 1200 mg RBV 12 weeks	96.2%, cirrhosis 94.1% GT1a: 93.3% GT1b: 95.5% NS3 RASs: 91.2% NS5A RASs: 75%

RBV: ribavirin, GZR: grazoprevir, EBR: elbasvir, mITT: modified ITT excluding virological failures

Table 11. Pivotal phase 2-3 studies with GZR/EBR/ treatment regimens in patients with HCV genotype 4. Studies are not head-to-head and SVR between studies are difficult to compare because they had significant differences in genetic and socioeconomic backgrounds.

Study	Dosing	SVR
C-EDGE TN (Zeuzem et al., 2015) n=18 GT4	50/100 mg GZR/EBR 12 weeks	GT4: 100%
Pooled analysis (Asselah et al., 2018) n=155, N=111 naïve (13.5% cirrhosis), n=44 exp (41% cirrhosis).	50/100 mg GZR/EBR 12 weeks (naïve, n=101)	96%
	50/100 mg GZR/EBR + RBV 12 weeks (naïve, n=10)	100%
	50/100 mg GZR/EBR 12 weeks (exp., n=16)	89%
	50/100 mg GZR/EBR + RBV 12 weeks (exp., n=15)	93%
	50/100 mg GZR/EBR ± RBV 16 weeks (exp., n=13)	60-100%

RBV: ribavirin, GZR: grazoprevir, EBR: elbasvir, Exp.: treatment (PEG-IFN + RBV) experienced patients

Glecaprevir and Pibrentasvir (GLE/PIB)

The combination of GLE and PIB is available as a fixed-dose combination (Maviret[®], Mavyret[®], Abbvie). GLE is a NS₃/4A protease inhibitor. PIB is a selective second-generation inhibitor of the HCV NS₅a replication complex.

The combination of GLE/PIB (Maviret[®], Mavyret[®]) was approved in 2017. All genotypes can be treated with GLE/PIB. GLE/PIB can be used in patients with CKD and hemodialysis but is not recommended in patients with decompensated cirrhosis.

Genotype 1

The integrated analysis of all phase 2 and 3 studies showed that 8 or 12 weeks GLE/PIB resulted in ≥99% SVR in GT₁ patients without cirrhosis. There was only one treatment failure in the 8-week group (Puoti 2018). Thus, there were no differences in naïve or treatment-experienced patients. The EXPEDITION-1 trial investigated 12 weeks GLE/PIB in 87 GT₁ patients with compensated cirrhosis. Only one GT_{1a} patients had a relapse (Forns 2017) (Table 12). International guidelines recommend 8 weeks GLE/PIB for non-cirrhotic GT₁ and until recently 12 weeks for patients with compensated cirrhosis (EASL 2018). Based on the EXPEDITION-8 trial published in 2019 (Brown 2019), 8 weeks GLE/PIB can be recommended in treatment naïve GT₁ patients with cirrhosis. 231 GT₁ patients with compensated cirrhosis (95 GT_{1a} / 136 GT_{1b}) showed 100% (PP) SVR₁₂ after 8 weeks GLE/PIB. Thus, 8 weeks GLE/PIB can be recommended in naïve GT₁ patients with compensated cirrhosis.

Genotype 2

The integrated analysis of all phase 2 and 3 studies showed that 8 or 12 weeks GLE/PIB resulted in ≥98% SVR in GT₂ patients without cirrhosis. There were two treatment failure in the 8-week group (Puoti et al., 2018). Thus, there were no differences in naïve or treatment-experienced patients. The EXPEDITION-1 trial investigated 12 weeks GLE/PIB in 31 GT₂ patients with compensated cirrhosis. SVR was 100% (Forns 2017) (Table 13). A Japanese study (CERTAIN-2) confirmed 100% SVR in 18 patients (Toyoda 2017). International guidelines recommend 8 weeks GLE/PIB for non-cirrhotic GT₂ and until recently 12 weeks for patients with compensated cirrhosis (EASL 2018). The EXPEDITION-8 trial (Brown 2019) included 26 naïve GT₂ patients with compensated cirrhosis. 100% achieved SVR₁₂ after 8 weeks GLE/PIB. Thus, 8 weeks GLE/PIB can be recommended in naïve GT₂ patients with compensated cirrhosis.

Genotype 3

The ENDURANCE-3 study analysed >500 naïve GT₃ patients without cirrhosis. 12 weeks GLE/PIB were compared with 12 weeks SOF + DCV. In addition, the study contained an additional arm testing 8 weeks GLE/PIB. SVR was ≥95% in all treatment arms (Table 14). There were numerically more treatment failures in the eight week group (Zeuzem 2018). Most of the patients treated in the phase 3 ENDURANCE-3 trial had mild Fo-F2 fibrosis and only 17% had F3 fibrosis. The integrated analysis of all phase 2 and 3 GT₃ studies showed that 8 or 12 weeks GLE/PIB resulted in 95% SVR in treatment-naïve GT₃ patients without cirrhosis, respectively (Puoti 2018). The SURVEYOR-II - Part 3 trial investigated 12 or 16 weeks GLE/PIB in treatment-experienced (PEG-IFN + RBV ± SOF) without cirrhosis. In addition, treatment-naïve patients with cirrhosis were treated for 12 weeks and treatment-experienced patients with cirrhosis for 16 weeks. SVR was 91-98% (Wyles 2017) (Table 14). Based on the results, the EMA label recommends 8 weeks GLE/PIB for naïve non-cirrhotic GT₃ patients and 12 weeks for treatment naïve patients with cirrhosis. For treatment experienced patients with or without cirrhosis, 16 weeks are recommended. EASL recommends for treatment-experienced patients without cirrhosis only 12 weeks GLE/PIB (EASL 2018), while AASLD/IDSA recommends 16 weeks (<https://www.hcvguidelines.org/treatment-experienced/gt3/p-r/without-cirrhosis>). The EXPEDITION-8 trial (Brown 2019) demonstrated in 63 GT₃ treatment naïve patients with compensated cirrhosis a 98.4% SVR₁₂ after 8 weeks GLE/PIB. Thus, 8 weeks GLE/PIB can be recommended in naïve GT₃ patients with compensated cirrhosis. However, EMA approval is pending (1/2020).

Genotype 4-6

The integrated analysis of all phase 2 and 3 studies showed that 8 or 12 weeks GLE/PIB resulted in 92-100% SVR in GT₄₋₆ patients without cirrhosis. However, no virological failure was documented (Puoti 2018) (Table 15). So far there are only limited data for GT₄₋₆ in patients with cirrhosis. However, no relapse has been documented with 12 weeks GLE/PIB (Gane 2017b). Thus, the treatment recommendations for GT₄₋₆ are the same as for GT₁, which is 8 weeks for patients without cirrhosis and 12 weeks for patients with cirrhosis (Table 4 & 5). The EXPEDITION-8 trial (Brown 2019) demonstrated in 13 GT₄, 1 GT₅ and 9 GT₆ treatment naïve patients with compensated cirrhosis a 100% SVR₁₂ after 8 weeks GLE/PIB. Thus, 8 weeks GLE/PIB can be recommended in naïve GT₄₋₆ patients with compensated cirrhosis.

Table 12. Pivotal phase 2-3 studies with GLE/PIB in patients infected with HCV genotype 1.

Studies are not head-to-head and SVR between studies are difficult to compare because they had significant differences in genetic and socioeconomic backgrounds.

Study	Dosing	SVR
Integrated analysis of phase 2 and 3 studies (Puoti et al., 2018) N=875 GT1, no cirrhosis (naïve and exp.)	a) 300/120 mg GLE/PIB 8 weeks	99% ITT, 99.8% mITT
	b) 300/120 mg GLE/PIB 12 weeks	99.8% ITT, 100% mITT
EXPEDITION-1 (Forns et al., 2017) N=87 GT1, cirrhosis	300/120 mg GLE/PIB 12 weeks	GT1a: 98% GT1b: 100%
EXPEDITION-8 (Brown et al., 2019) N=231 GT1 (95 GT1a / 136 GT1b), naïve, cirrhosis	300/120 mg GLE/PIB 8 weeks	GT1: 97.8% ITT, 100% PP

GLE: glecaprevir, **PIB:** pibrentasvir, **Exp.:** treatment (PEG-IFN) experienced patients, **ITT:** intention-to-treat analysis, **mITT:** modified ITT excluding virological failures

Table 13. Pivotal phase 2-3 studies with GLE/PIB in patients infected with HCV genotype 2.

Studies are not head-to-head and SVR between studies are difficult to compare because they had significant differences in genetic and socioeconomic backgrounds.

Study	Dosing	SVR
Integrated analysis of phase 2 and 3 studies (Puoti et al., 2018) N=436 GT2, no cirrhosis (naïve and exp.)	a) 300/120 mg GLE/PIB 8 weeks	98% ITT, 99% mITT
	b) 300/120 mg GLE/PIB 12 weeks	99% ITT, 100% mITT
EXPEDITION-1 (Forns et al., 2017) N=31 GT2, cirrhosis	300/120 mg GLE/PIB 12 weeks	100%
CERTAIN-2 (Toyoda et al., 2017) N=18 GT2, cirrhosis	300/120 mg GLE/PIB 12 weeks	100%
EXPEDITION-8 (Brown et al., 2019) N=26 GT2, naïve, cirrhosis	300/120 mg GLE/PIB 8 weeks	100%ITT, 100% PP

GLE: glecaprevir, **PIB:** pibrentasvir, **Exp.:** treatment (PEG-IFN + RBV) experienced patients, **mITT:** modified intention-to-treat excluding non-virological failures

Table 14. Pivotal phase 2-3 studies with GLE/PIB in patients infected with HCV genotype 3.

Studies are not head-to-head and SVR between studies are difficult to compare because they had significant differences in genetic and socioeconomic backgrounds.

Study	Dosing	SVR
ENDURANCE-3 (Zeuzem et al., 2018) N=505 GT3, naïve, no cirrhosis	a) 300/120 mg GLE/PIB 8 weeks	95% (149/157)
	b) 300/120 mg GLE/PIB 12 weeks*	95% (222/233)
	c) 300/120 mg SOF + DCV 12 weeks*	97% (111/115)
	* 2:1 randomisation	
SURVEYOR-2, part 3 (Wyles et al., 2017) N=131 GT3, Exp. without cirrhosis, naïve and exp. with cirrhosis	a) Naïve cirrhosis: 300/120 mg GLE/PIB 12 weeks	98%
EXPEDITION-8 (Brown et al., 2019) N=63 GT3, naïve, cirrhosis	300/120 mg GLE/PIB 8 weeks	95.2% ITT, 98.4% PP, 1 relapse

GLE: glecaprevir, **PIB:** pibrentasvir, **Exp.:** treatment (PEG-IFN + RBV ± SOF) experienced patients

Table 15. Pivotal phase 2-3 studies with GLE/PIB in patients infected with HCV genotype 4-6.

Studies are not head-to-head and SVR between studies are difficult to compare because they had significant differences in genetic and socioeconomic backgrounds.

Study	Dosing	SVR
Integrated analysis of phase 2 and 3 studies (Puoti et al., 2018) N=174 GT4 n=30 GT5 n=43 GT6, no cirrhosis (naïve and exp.)	a) 300/120 mg GLE/PIB 8 weeks	GT4: 95% ITT, 100% PP GT5: 100% ITT, 100% PP GT6: 92% ITT, 100% PP
	b) 300/120 mg GLE/PIB 12 weeks	GT4: 99% ITT, 100% PP GT5: 100% ITT, 100% PP GT6: 100% ITT, 100% PP
Integrated analysis of phase 2 and 3 studies (Gane et al., 2017b) N=22 GT4 n=2 GT5, n=7 GT6, cirrhosis (naïve and exp.)	300/120 mg GLE/PIB 12 weeks	GT4: 100% ITT, 100% PP GT5: 100% ITT, 100% PP GT6: 100% ITT, 100% PP
EXPEDITION-8 (Brown et al., 2019) N=13 GT4, n=1 GT5 and n=9 GT6, naïve, cirrhosis	300/120 mg GLE/PIB 8 weeks	100%ITT, 100% PP

GLE: glecaprevir, **PIB:** pibrentasvir, **Exp.:** treatment (PEG-IFN + RBV) experienced patients, **PP:** per-protocol population

Sofosbuvir and velpatasvir (SOF/VEL)

The combination of SOF and VEL is available as a fixed-dose combination (Epclusa®, Gilead Sciences). VEL is a selective inhibitor of the HCV NS5a replication complex. SOF/VEL was approved in 2016 as the first regimen that is effective in all genotypes. Based on the phase 3 studies (Feld 2015, Curry 2015b), treatment duration of 12 weeks is the standard for all GT1,2,4-6 patients and GT3 patients without cirrhosis (Table 4 & 5). For genotype 3 patients with cirrhosis, baseline RASs may be relevant. SOF/VEL is not primarily recommended for patients with CKD (GFR < 30 mL/min), but can be used in these patients when no other relevant treatment options are available, e.g. in patients with decompensated cirrhosis where protease inhibitors are not recommended. The FDA has updated the label and now state that no dosage adjustment is recommended in patients with any degree of renal impairment including patients on dialysis based on pharmacokinetic data obtained from studies involving HCV-infected patients with renal impairment including dialysis patients.

Genotype 1

The efficacy of 12 weeks SOF/VEL in previously treated patients with GT1 with or without cirrhosis was investigated in the ASTRAL-1 trial. In the ASTRAL-1 trial, 32% of the SOF/VEL treated patients were treatment experienced. A small number of 56 SOF/VEL treated patients were PEG-IFN+RBV+PI treatment-experienced. As the SVR rate was 98-99% for all GT1 (Table 16), there was no obvious difference between treatment-experienced and naïve patients (Feld 2015).

An integrated analysis of patients with advanced fibrosis and cirrhosis showed 98% SVR and 99% SVR, respectively (Asselah 2018a). Patients with CHILD-B cirrhosis were treated in the ASTRAL-4 study. Here 12 weeks SOF/VEL was compared with 12 weeks SOF/VEL + RBV and 24 weeks SOF/VEL. SVR rates were 88-100%. There was a numerically higher SVR rate with 12 weeks SOF/VEL + RBV (Curry 2015b).

Thus, 12 weeks SOF/VEL is recommended for all GT1 patients, including cirrhosis or PEG-IFN+RBV+PI treatment-experienced. RBV may be added in patients with decompensated cirrhosis or treatment duration can be extended to 24 weeks if patients are ineligible for RBV (EASL 2018) (<https://www.hcvguidelines.org/unique-populations/decompensated-cirrhosis>).

Genotype 2

The ASTRAL-2 and 3 trials (Foster 2015) investigated 12 weeks SOF/VEL versus SOF+RBV in 266 GT2 patients. SVR was 99% with 12 weeks SOF/VEL

and 94% with 12 weeks SOF+RBV (Table 17). In addition, the ASTRAL-4 study analysed the responses to 12 weeks SOF/VEL in decompensated cirrhosis (Curry 2015b). However, the study enrolled only 12 decompensated patients with GT2. All patients achieved SVR. 12 weeks SOF/VEL is recommended for all GT2 patients, including compensated cirrhosis. RBV may be added in patients with decompensated cirrhosis or treatment duration can be extended to 24 weeks if patients are ineligible for RBV, as data are limited for this specific group of patients (EASL 2018) (<https://www.hcvguidelines.org/unique-populations/decompensated-cirrhosis>).

Genotype 3

The ASTRAL-2 and 3 trials (Foster 2015) investigated 12 weeks SOF/VEL versus SOF+RBV in 552 GT3 patients. For GT3, the additional benefit for SOF/VEL was higher (SVR 95% versus 80%) (Table 18). The integrated analysis of patients with fibrosis and cirrhosis showed 99% SVR in patients with fibrosis but only 91% SVR in patients with cirrhosis (Asselah 2018a). Treatment-experienced patients with advanced fibrosis and cirrhosis showed 90% SVR versus 97% in naïve patients (Asselah 2018a). Patients with baseline RAS in GT3 and advanced fibrosis and cirrhosis achieved SVR in only 79% with 12 weeks SOF/VEL (Asselah 2018a). This was one reason that EASL does not recommend 12 weeks SOF/VEL without RBV in GT3 cirrhosis, if RAS testing is not available (EASL 2018). In the POLARIS-3 study, 109 GT3 patients with cirrhosis received SOF/VEL. The only 2 virological treatment failures were prior PEG-IFN + RBV non-responder (Jacobson et al., 2017). AASLD/IDSA guidance recommends 12 weeks SOF/VEL in naïve GT3 patients with cirrhosis and 12 weeks SOF/VEL/VOX or SOF/VEL + RBV in treatment-experienced patients with cirrhosis, if RAS test is not available (<https://www.hcvguidelines.org/treatment-experienced/gt3/p-r/compensated-cirrhosis>). A randomised trial in 204 GT3 patients with cirrhosis showed 91% SVR with 12 weeks SOF/VEL and 96% with 12 weeks SOF/VEL + RBV. In this study, treatment-experienced GT3 patients with cirrhosis had even better responses with 12 weeks SOF/VEL compared with naïve patients. The impaired SVR was related to NS5A RAS (Table 18). Patients without NS5A RAS (Y93H) had 96% SVR with or without RBV (Buti 2018). The frequency of baseline NS5A RAS (Y93H) may impact the response to 12 weeks SOF/VEL in patients with GT3 cirrhosis. Thus, in areas with a high frequency of NS5A RAS, GT3 patients with cirrhosis should either receive additional RBV or RAS testing should be performed. Otherwise alternative treatment options should be preferred.

In contrast, patients with mild fibrosis may require only 8 weeks SOF/VEL. 8 weeks SOF/VEL in 90 patients receiving opiate substitution therapy (OST) showed 93% SVR but no patients had a virological failure (Boyle 2018)

(Table 18). However, in 2018 8 weeks SOF/VEL are not recommended by international guidelines.

The ASTRAL-4 study analysed the responses to 12 weeks SOF/VEL in decompensated cirrhosis (Curry 2015b). SVR with 12 weeks SOF/VEL in GT3 patients was low with 50%. The addition of RBV increased SVR rate to 85% (Table 18). Thus, RBV needs to be added in patients with decompensated cirrhosis and GT3 infection. For patients with decompensated cirrhosis who are RBV ineligible, SOF/VEL for 24 weeks is currently recommended, although this did not result in higher SVR rates compared to 12 weeks in the ASTRAL-4 trial. However, the number of patients analysed was quite small. (EASL 2018) (<https://www.hcvguidelines.org/unique-populations/decompensated-cirrhosis>).

Genotype 4-6

ASTRAL-1 included 116 GT4 treatment-naïve patients without cirrhosis or with compensated cirrhosis, all of whom achieved SVR12 (100%) (Feld 2015). In the POLARIS-2 study, 57 patients with GT4 received 12 weeks SOF/VEL and 98% achieved SVR (Table 19) (Jacobson 2017).

ASTRAL-1 included 35 GT5 and 41 GT6 treatment-naïve patients without cirrhosis or with compensated cirrhosis. 97% of GT5 and 100% of GT6 patients achieved SVR with 12 weeks SOF/VEL (Feld 2015).

Treatment recommendations are the same as for GT1 patients (EASL 2018).

Table 16. Pivotal phase 3 studies with SOF/VEL treatment regimens in treatment-naïve patients with HCV genotype 1. Studies are not head-to-head and it is difficult to compare SVR between different studies because the populations had significant differences in genetic and socioeconomic backgrounds.

Study	Dosing	SVR
ASTRAL-1 (Feld et al., 2015) n=393 (GT1, 19% cirrhosis, 68-72% naïve)	a) 400/100mg SOF/VEL 12 weeks (n=328)	GT1a: 98%
	b) placebo (n=65)	GT1b: 99%
ASTRAL-4 (Curry et al., 2015) n=207(GT1, decompensated cirrhosis, 36-53% naïve)	a) 400/100mg SOF/VEL 12 weeks (n=90)	GT1a: 88% GT1b: 89%
	b) 400/100mg SOF/VEL + 1000-1200 mg RBV 12 weeks (n=87)	GT1a: 94% GT1b: 100%
	c) 400/100mg SOF/VEL 24 weeks (n=90)	GT1a: 93% GT1b: 88%

GT: genotype, RBV: ribavirin, SOF: sofosbuvir, VEL: velpatasvir,
Exp.: treatment-experienced patients

Table 17. Pivotal phase 3 studies with SOF/VEL treatment regimens in HCV GT2 infection (naïve and treatment-experienced). Studies are not head-to-head and SVR between studies are difficult to compare because they had significant differences in genetic and socioeconomic backgrounds.

Study	Dosing	SVR
ASTRAL-2 (Foster et al., 2015) GT2: 266 14-30% cirrhosis	a) 400/100 mg SOF/VEL 12 weeks	99%
	b) 400 mg SOF + 1000-1200 mg RBV 12 weeks	94%
Pooled analysis (Asselah et al., 2018) n=155, N=111 naïve (13.5% cirrhosis), n=44 exp (41% cirrhosis).	a) 400/100mg SOF/VEL 12 weeks (n=90)	100%
	b) 400/100mg SOF/VEL + 1000-1200 mg RBV 12 weeks (n=87)	100%
	c) 400/100mg SOF/VEL 24 weeks (n=90)	75% (3/4)

GT: genotype, RBV: ribavirin, SOF: sofosbuvir, VEL: velpatasvir,
Exp.: treatment-experienced patients

Table 18. Pivotal phase 3 with SOF/VEL treatment regimens in HCV GT3 infection (naïve and treatment-experienced). Studies are not head-to-head and SVR between studies are difficult to compare because they had significant differences in genetic and socioeconomic backgrounds.

Study	Dosing	SVR
ASTRAL-3 (Foster et al., 2015) n=552 14-30% cirrhosis	a) 400/100 mg SOF/VEL 12 weeks	95% exp. cirrhosis 89%
	b) 400 mg SOF + 1000-1200 mg RBV 24 weeks	80% exp. cirrhosis 58%
ASTRAL-4 (Curry et al., 2015) n=39 with decompensated cirrhosis	a) 400/100mg SOF/VEL 12 weeks (n=90)	50%
	b) 400/100mg SOF/VEL + 1000-1200 mg RBV 12 weeks (n=87)	85%
	c) 400/100mg SOF/VEL 24 weeks (n=90)	50%
POLARIS-3 (Jacobson et al., 2017) n=109, cirrhosis, n=32 exp.	400/100 mg SOF/VEL 12 weeks (control group)	96%, mITT 98% Naïve: 100% mITT Exp.: 93.75% mITT
(Pianko et al., 2015) n=105 GT3 treated with 400/100 mg SOF/VEL, n=52 with cirrhosis	a) 400/100 mg SOF/VEL 12 weeks	100% no cirrhosis 92% (24/26) cirrhosis
	b) 400/100 mg SOF/VEL weeks + 1000-1200 mg RBV 12 weeks	100% no cirrhosis 96% (25/26) cirrhosis
(Buti et al., 2018) N=204 with cirrhosis, 27% exp., 19-22% NS5A RAS	a) 400/100 mg SOF/VEL 12 weeks	91% (5 relapser) Naïve 89%, exp. 96% NS5A-RAS 84%
	b) 400/100 mg SOF/VEL weeks + 1000-1200 mg RBV 12 weeks	96% (2 relapser) Naïve 96%, exp. 96% NS5A-RAS 96%
(Boyle et al., 2018) n=90 GT3, F0-F3 (31% F3), mainly OST patients	400/100 mg SOF/VEL 8 weeks	93% ITT, 100 mITT

GT: genotype, **RBV:** ribavirin, **SOF:** sofosbuvir, **VEL:** velpatasvir, **Exp.:** treatment-experienced patients, **mITT:** modified intention-to-treat excluding non-virological failures

Table 19. Pivotal phase 3 with SOF/VEL treatment regimen in HCV GT4-6 infection. Studies are not head-to-head and SVR between studies are difficult to compare because they had significant differences in genetic and socioeconomic backgrounds.

Study	Treatment	SVR
ASTRAL-1 (Feld et al., 2015) n=116 GT4 35 GT5 41 GT6	400/100 mg SOF/VEL 12 weeks	GT4: 100% GT5: 97% GT6: 100%
ASTRAL-4 (Curry et al., 2015) n=8 GT4 1 GT6 with decompensated cirrhosis	a) 400/100 mg SOF/VEL 12 weeks	GT4: 4/4
	b) 400/100 mg SOF/VEL weeks + 1000-1200 mg RBV 12 weeks	GT4: 2/2
	c) 400/100 mg SOF/VEL 24 weeks	GT4: 2/2, GT6: 1/1

GT: genotype, **RBV:** ribavirin, **SOF:** sofosbuvir, **VEL:** velpatasvir, **Exp.:** treatment-experienced patients

Sofosbuvir and velpatasvir and voxilaprevir (SOF/VEL/VOX)

Voxilaprevir (VOX) is an HCV N₃/4A protease inhibitor (Rodriguez-Torres 2016) that is combined with SOF/VEL in a single tablet (Vosevi®). SOF/VEL/VOX was approved in 2017. It is the first approved RBV free DAA therapy for patients who failed an NS5A-containing DAA regimen (see Treatment of patients with prior DAA treatment failure below). SOF/VEL/VOX has also been approved for DAA naïve patients by EMA (not FDA) but as other options are available SOF/VEL/VOX should be restricted to patients with DAA failure. SOF/VEL/VOX may be used with caution in patients with CKD (GFR<30 mL/min) and hepatic impairment due to the combination of sofosbuvir and the protease inhibitor voxilaprevir.

Treatment of DAA naïve patients

Two phase 3 studies investigated SOF/VEL/VOX in DAA naïve patients. POLARIS-2 compared the efficacy of 8 weeks of SOF/VEL/VOX to 12 weeks of SOF/VEL. The study included 941 patients infected with all HCV genotypes with or without cirrhosis, except patients with genotype 3 and cirrhosis. POLARIS-3 enrolled 219 GT₃ patients with cirrhosis (Jacobson 2017). 8 weeks SOF/VEL/VOX missed the pre-specified non-inferiority criteria, mainly because of a lower SVR in GT_{1a} patients (Table 20). The lower SVR in GT_{1a} patients was associated with the Q80K variant. However, 8 weeks SOF/VEL/VOX showed a similar SVR compared with 12 weeks SOF/VEL in GT₃ patients with and without compensated cirrhosis. At least, 29% of GT₃ patients treated with SOF/VEL/VOX had platelets <100/nl. Thus, 8 weeks SOF/VEL/VOX can be an option for all GT₃ patients according to the EMA label. However, EASL recommends 12 weeks SOF/VEL/VOX for GT₃ patients with cirrhosis (EASL 2018). AALSD/IDSA considers also 12 weeks SOF/VEL/VOX but specifically only for naïve GT₃ patients with cirrhosis if baseline Y93H is present (<https://www.hcvguidelines.org/treatment-naive/gt3/compensated-cirrhosis>) or in PEG-IFN+RBV experienced GT₃ patients with cirrhosis (<https://www.hcvguidelines.org/treatment-experienced/gt3/p-r/compensated-cirrhosis>).

Table 20. Pivotal phase 3 studies with SOF/VEL/VOX treatment regimens in DAA naive patients with HCV genotype 1-6. Studies are not head-to-head and it is difficult to compare SVR between different studies because the populations had significant differences in genetic and socioeconomic backgrounds.

Study	Dosing	SVR
POLARIS-2 (Jacobson et al., 2017) n=941 GT1-6, 23-24% exp., 18-19% cirrhosis (GT3 only no cirrhosis)	a) 400/100mg SOF/VEL 12 weeks (n=440)	GT1a: 99% GT1b: 97% GT2: 100% GT3: 97% GT4: 98% GT5: - GT6: 100%
	b) 400/100/100mg SOF/VEL/VOX 8 weeks (n=501)	GT1a: 95% GT1b: 97% GT2: 97% GT3: 99% GT4: 94% GT5: 94 GT6: 100%
POLARIS-3 (Jacobson et al., 2017) n=219 GT3 with cirrhosis	a) 400/100mg SOF/VEL 12 weeks (n=109)	GT3: 96%
	b) 400/100/100mg SOF/VEL/VOX 8 weeks (n=110)	GT3: 96%

GT: genotype, SOF: sofosbuvir, VEL: velpastasvir, VOX: voxilaprevir

Treatment of patients with prior DAA treatment failure

As more patients are treated, the size of the population of patients who fail to achieve SVR with DAA-including regimens might expand in the future. Retreatment of patients with previous treatment failure is one important topic in the treatment of chronic hepatitis C. RAS testing may be performed (Dietz 2018) to select the therapy based on susceptibility to the corresponding drug class.

However, the only EMA approved RBV free treatment for patients with DAA treatment failure in 2018 is SOF/VEL/VOX.

The POLARIS-4 study investigated patients with HCV genotype 1, 2, or 3 infection who had previously received a DAA regimen but not an NS5A inhibitor. Patients received either SOF/VEL/VOX or SOF/VEL for 12 weeks. An additional 19 GT4 patients were treated with SOF/VEL/VOX. Overall, SVR was 98% with SOF/VEL/VOX and 90% with SOF/VEL (Bourlière 2017) (Table 21).

NS5A inhibitors are part of all currently used DAA combinations. NS5A RAS, unlike NS3 and NS5B RAS, appear to maintain the viability of the virus after unsuccessful treatment with an NS5A inhibitor containing therapy. Thus, NS5A remain at high frequency in the majority of patients for more than

five years after the end of treatment (Pawlotsky 2016). As a result, retreatment of patients after NS5A failure is of special importance. The POLARIS-1 trial investigated 263 patients who failed previous NS5A based therapy. Overall, the SVR was 96% with 12 weeks SOF/VEL/VOX (Bourlière 2017) (Table 21). SVR was 99% in patients without cirrhosis and 93% in patients with cirrhosis. 147 of the 152 patients in the placebo group have been treated later with 12 weeks SOF/VEL/VOX and 97% achieved SVR (Bourlière 2018). The recommended treatment duration for SOF/VEL/VOX for DAA experienced patients is 12 weeks. However, GT3 patients with cirrhosis (especially those with NS5A RAS) may be considered for additional RBV to minimise the relapse risk (<https://www.hcvguidelines.org/treatment-experienced/gt3/daa>). In the POLARIS-4 study, all 4 GT3 patients who experienced a relapse had cirrhosis (Bourlière 2017).

In patients with contraindications for SOF/VEL/VOX (i.e. decompensated cirrhosis), SOF/VEL + RBV for 24 weeks could be an alternative and is not off-label use according to the EMA label.

However, not all patients with previous DAA therapy must be treated with SOF/VEL/VOX. Data for retreatment of patients with HCV GT1 infection and failure to previous therapy with PEG-IFN+RBV + TLV or BOC are available for SOF/LDV, GZR/EBR and SOF/VEL regimens (see above). Thus, these combinations can be used for these patients.

Also patients that failed a SOF + SMV retreatment do not necessarily require SOF/VEL/VOX.

Retreatment with GLE/PIB for 12 weeks could be an option. In the MAGELLAN-1 Part 2 study, GLE/PIB was investigated in patients who failed previous NS3/4A protease and/or NS5A inhibitor-containing therapy. SVR12 was achieved by 89% (39 of 44) and 91% (43 of 47) of patients who received 12 and 16 weeks GLE/PIB, respectively (Poordad 2018) (Table 22). Patients with failure to NS3/4A inhibitor-based therapy showed 100% SVR even with 12 weeks therapy. However, there were only 3 patients enrolled who failed SOF + SMV but all were treated for 16 weeks. In another study, patients who failed NS5A inhibitor-based therapy were treated 12 (with ribavirin in cirrhosis) or 16 weeks with GLEB/PIB. Based on the results, patients with cirrhosis require 16 weeks therapy (Lok 2018).

Of note, GLE/PIB is only approved for DAA failures in the FDA label and not in EMA.

Finally, the combination of GLE/PIB + SOF + RBV would be the most powerful therapy because it combines SOF with GLE and the second-generation NS5A inhibitor PIB, which has a higher barrier to NS5A resistance. This combination has been used in the MAGELLAN-3 study in patients who failed prior GLE/PIB therapy. Only one of 23 patients showed a relapse after 12 weeks or 16 weeks therapy (Wyles 2018). EASL recommends 12 weeks GLE/PIB + SOF for patients after DAA failure with complex NS5A RAS profiles or in combination with RBV for patients who failed multiple DAA therapies (EASL 2018).

Table 21. Phase 3 studies with SOF/VEL/VOX treatment regimens in DAA treatment-experienced patients with HCV genotype 1-6. Studies are not head-to-head and it is difficult to compare SVR between different studies because the populations had significant differences in genetic and socioeconomic backgrounds.

Study	Dosing	SVR
POLARIS-1 (Bourlière et al., 2017) n=388 NS5A failure 34-46% cirrhosis	a) 400/100/100mg SOF/VEL/VOX 12 weeks (n=236)	GT1a: 96%, GT1b: 100% GT2: 100% GT3: 95% GT4: 91% GT6: 100%
	b) Placebo (n=152)	
POLARIS-4 (Bourlière et al., 2017) n=333 non-NS5A failure 46% cirrhosis	c) 400/100mg SOF/VEL 12 weeks (n=151)	GT1a: 89%, GT1b: 95% GT2: 97% GT3: 85%
	d) 400/100/100mg SOF/VEL/VOX 12 weeks (n=182)	GT1a: 98% GT1b: 96% GT2: 100% GT3: 96% GT4: 100%
deferred treatment group of POLARIS-1 (Bourlière et al., 2018) n=147 NS5A failure	400/100/100mg SOF/VEL/VOX 12 weeks	97% (3% relapse, all GT1a)

GT: genotype, SOF: sofosbuvir, VEL: velpatasvir, VOX: voxilaprevir

Table 22. Phase 2-3 studies with GLE/PIB treatment regimens in DAA treatment-experienced patients with HCV genotype 1-6. Studies are not head-to-head and it is difficult to compare SVR between different studies because the populations had significant differences in genetic and socioeconomic backgrounds.

Study	Dosing	SVR
MAGELLAN-1 (Poordad et al., 2018) n=91 with GT1, 4	a) 300/120 mg GLE/PIB 12 weeks	PI failure: 100% (14/14) NS5Ai failure 88% (14/16) PI + NS5Ai failure 79% (11/14)
	b) 300/120 mg GLE/PIB 16 weeks	PI failure: 100% (13/13) NS5Ai failure 94% (17/18) PI + NS5Ai failure 81% (13/16)
(Lok et al., 2018) n=167 NS5A failure 27% cirrhosis	a) 300/120 mg GLE/PIB 12 weeks	No cirrhosis 96%
	b) 300/120 mg GLE/PIB 16 weeks	No cirrhosis 96%
	c) 300/120 mg GLE/PIB 12 weeks + RBV	Cirrhosis 86% (enrollment stopped)
	d) 300/120 mg GLE/PIB 16 weeks	Cirrhosis 100%
MAGELLAN-3 (Wyles et al., 2018) n=23 GT1-6, GLE/PIB failure 61% F4	a) 300/120 mg GLE/PIB + SOF + RBV 12 weeks	100%
	b) 300/120 mg GLE/PIB + SOF + RBV 16 weeks (prior NS5Ai and/or PI or cirrhosis or GT3)	95% (1 relapse GT1)

GT: genotype, GLE: glecaprevir, PIB: pibrentasvir, SOF: sofosbuvir, RBV: ribavirin, NS5Ai: NS5A inhibitor, PI: NS3/4A protease inhibitor

Optimisation of HCV treatment

Adherence to therapy

Adherence to therapy is one of the most important factors associated with the success of antiviral treatment. The definition of adherence used in the PEG-IFN era was the “80/80 rule”, that is, patients who receive more than 80% of the medication and are treated for more than 80% of the planned duration of treatment are considered adherent. One of the first studies investigating the effect of adherence in PEG-IFN+RBV treatment demonstrated that patients who fulfilled the 80/80 rule had a 63% SVR compared to 52% of those with less than 80% adherence (McHutchison et al., 2002). For the IFN free DAA therapies, adherence to the DAA may be even more important because irregular intake bears the risk of rapid emergence of drug resistance. It will important to collect more real-world data in difficult-to-treat patient cohorts if the SVR is >90% under “normal” non-standardised study conditions. For some patient populations it may be important to treat patients under DOT (directly observed therapy) condition to guarantee adherence (Schütz 2018). Another important and new issue is drug-drug interactions (DDI) that can diminish the effectiveness of the DAAs or induce toxicity of concomitant medications, which may lead to discontinuation of all drugs. Knowledge about DDI is therefore important for the optimal management of patients receiving DAA (Honer Zu Siederdisen 2016).

Simplified HCV Treatment for adults

(Treatment-Naive without cirrhosis or with compensated cirrhosis)

Recent studies with pangenotypic DAA have shown high efficacy and safety. In theory, genotyping and quantitative HCV RNA level are not mandatory to start pangenotypic DAA treatment with either glecaprevir/pibrentasvir (GLE/PIB) or sofosbuvir/velpatasvir (SOF/VEL). Thus, a simplified HCV treatment could be applied to treatment-naïve patients without cirrhosis or with compensated cirrhosis. The idea for a more simplified algorithm is to lower the threshold for the access to treatment. Eligible are treatment-naïve adult patients without cirrhosis or with compensated cirrhosis. HIV- and HBV coinfections, known or suspected hepatocellular carcinoma, a prior liver transplantation as well as a current pregnancy should be excluded from this simplified approach. Patients with cirrhosis should not be decompensated (CHILD-Pugh score < 7) and no physical conditions of decompensation (ascites, hepatic encephalopathy) should be present. If the mentioned conditions are met, a therapy with either GLE/PIB for the duration of 8 weeks or SOF/VEL for the duration

of 12 weeks is possible. The only exception is the therapy of patients with compensated cirrhosis, genotype 3 and a NS5A resistance-associated substitution (RAS) Y93H. In this case SOF/VEL is only recommended in combination with ribavirin. Drug-drug interactions should be included in the decision. On-treatment monitoring may not necessarily be needed if the compliance of the patients is good. For queries or if there is an indication for a decreasing liver function, a specialist should be available. Treatment response should be evaluated 12 weeks after the end of therapy. (<https://www.hcvguidelines.org/treatment-naive/simplified-treatment-compensated-cirrhosis>). However, the SMART-C study (Dore 2019), which evaluated 8-week GLE/PIB in treatment naïve non-cirrhosis patients, showed that a simplified monitoring schedule that included no lab tests or clinic visits during treatment was not equally successful compared to the standard monitoring schedule. For patients' groups with a suspected low adherence (e.g. PWIDs), directly observed therapies may be important to optimize treatment success (McDermott 2018).

Management of side effects and complications

Severe side effects may reduce adherence to therapy and may result in dose modifications that result in a less-than-optimal response. This was the main problem in the IFN era with IFN-induced bone marrow suppression, flu-like symptoms, neuropsychiatric disorders, and autoimmune syndromes. The main problem of RBV is hemolytic anaemia (Manns 2006). First generation PIs BOC and TLV were associated with additional side effects such as rash or dysgeusia and additionally an increase of anaemia that resulted in frequent treatment discontinuations. Thus, many patients could not be treated before the availability of IFN free DAA combinations (Maasoumy 2013b).

In contrast, IFN free DAA therapies are in general very well tolerated (Younossi 2016). If RBV can be omitted, DAA treatment can even improve patient-reported outcomes (PROs) (Höner Zu Siederdisen 2018). With the better tolerability and safety profile of DAAs, eligibility for HCV treatment expanded broadly, including patients with decompensated cirrhosis (Höner Zu Siederdisen 2015). However, studies in patients with decompensated cirrhosis have reported higher rates of serious adverse events and also mortality, which has to be considered (Maan 2016) (see section cirrhosis). In addition, patients with advanced cirrhosis remain at high risk to develop HCC despite HCV eradication (El-Serag 2016). Thus, long-term surveillance of HCV cured patients with cirrhosis is mandatory (EASL 2018).

Drug-drug interactions

With the introduction of DAAs a completely new challenge had to be faced: drug-drug interactions (DDI). First generation PIs underwent extensive hepatic metabolism via the CYP3A pathway (Maasoumy 2013a, Burger 2012). Consequently, up to 49% of hepatitis C patients were at risk for DDI if treated with TLV or BOC due to their co-medication (Maasoumy 2013a).

The next generation PIs SMV and PTV/r as well as the NS5A inhibitors DCV, OBV and LDV have fewer relevant DDIs, but are also metabolised by CYP3A, although to a lesser degree (Kiser 2013). However, DDIs are not limited to the CYP3A pathway. Interactions may also occur with the p-glycoprotein (P-gp) transport or the organic anion transporting polypeptide 1B1 (OATP1B1, OATP1B3 and OATP2B1) as well as other pathways such as CYP2C19, CYP2C9, CYP2D6, UGT1A1 (Kiser 2013).

Currently, 2 or 3 DAAs are used in therapy, each of them with the potential to cause DDIs. In one publication that has assessed the risk for significant interaction with the concomitant medication and OBV/PTV/r + DSV, LDV/SOF, DCV/SOF, SMV + SOF or TLV or BOC a significant amount of interactions occurred (Höner Zu Siederdisen 2016). Potentially significant interactions could be expected in 66% of the patients taking OBV/PTV/r + DSV, in 31% of SOF + SMV patients, 37% of SOF + DCV patients, and 40% of SOF/LDV patients. PPI, thyroid hormones and dihydropyridine derivatives were most frequently involved in possible DDIs. Importantly, the risk for DDIs was higher in patients with advanced cirrhosis due to polypharmacy affecting between 39% and 92% of patients treated with a combination of 2 or more DAAs.

DDIs needs to be considered also while using the newer DAA regimens such as SOF/VEL/VOX, GRZ/EBR and GLE/PIB. For optimal therapeutic management, it is essential to specifically ask patients about concomitant medications and assess if those drugs might interact with the DAAs. In some cases, closer monitoring or slight dose modifications may be sufficient while in other cases some drugs should be strictly avoided especially, if alternatives are available that do not cause interactions. Furthermore, the patient has to be informed that self-medication may also be a problem since interactions are not limited to approved drugs. Even herbals and foods have to be considered. Examples are St. John's Wort, which is a potent inducer of CYP3A and P-gp or naringin, a flavinoid of grapefruit, which is an inhibitor of CYP3A. Drug interactions are usually considered significant if the area under the plasma concentration time curve (AUC) is altered by more than 30%. It is also important to note, that potentially life-threatening interactions are not known yet and only detected after careful observation after market approval. This was the case for the combination of amiodarone

and SOF which led to severe bradycardia (Fontaine 2015). If the patient has no pacemaker, it is recommended to wait at least 3 months before starting a SOF containing regimen after withdrawal of amiodarone.

As the effect of DDI may vary depending on which drugs are used, no strict recommendation or rule can be given regarding the concomitant use of various medications. Therefore it is strongly advised to consider the recommendations in the product label. Supportive online tools or apps for mobile devices are available. One example is the very comprehensive drug interaction resource provided by the University of Liverpool (<http://www.hep-druginteractions.org>). The website provides clinically useful and evidence-based information which is updated when new drug interactions are analysed and published.

The following recommendations are based on the product label and the current EASL guidelines (EASL 2018).

SOF/LDV is affected by and may affect drugs transported or metabolised by intestinal P-gp, breast cancer resistance protein (BCRP) and hepatic organic anion transporting polypeptide (OATP). Interactions may be possible with the following drugs: digoxin, dabigatran, amlodipine, buprenorphine, carvedilol, cyclosporine and rosuvastatin. Patients with concomitant statin therapy should be monitored for statin side effects.

The solubility of LDV is depended on the gastric pH. Thus, PPIs may lead to decreased LDV concentrations with subsequently reduced SVR rates. Concomitant use should be generally avoided. If not possible, intake should be 4 hours apart and the equivalent PPI dosage should not surpass 20 mg omeprazole or pantoprazole. Co-administration with amiodarone should be strictly avoided as mentioned above. *For antiretrovirals see Chapter 15.*

GRZ/ERB are weak inhibitors of CYP3A and P-gp, therefore coadministration with other drugs metabolised by a similar pathway should be avoided or monitored cautiously (e.g. tacrolimus, statins, dabigatran, ticagrelor, quetiapine). Coadministration with cyclosporine is not recommended.

SOF/VEL interact with CYP2B6, CYP2C8, CYP3A4, P-gp, BCRP and OATP1B1. Concomitant intake of potent P-gp or CYP inducers should be strictly avoided (e.g., rifampicin, rifabutin, carbamazepine, phenobarbital, phenytoin, St John's wort). The solubility is also depended on the gastric pH, therefore, the considerations regarding PPI intake for SOF/LDV do also apply for SOF/VEL. Co-administration with amiodarone should be strictly avoided.

GLE/PIB interact with OATP1B1, OATP1B3, P-gp, BCRP and CYP3A. Co-administration with dabigatran, aliskiren, lovastatin, atorvastatin or simvastatin for example is not recommended. Rosuvastatin may need a dose reduction. Contrary, strong inducers of P-gp and CYP3A may reduce GLE/PIB concentrations (e.g. rifampicin, carbamazepine, St. John's

wort, phenytoin, oxcarbazepine or eslicarbazepine). Co-administration of GLE/PIB with ethinylestradiol-containing contraception has led to ALT elevations, therefore co-administration is contraindicated. Instead, progesterone-containing contraception is allowed.

SOF/VEL/VOX has the same interaction potential as SOF/VEL with additional interactions caused by the PI voxilaprevir, which is mainly metabolised by CYP3A4. Strong inhibitors of CYP3A (e.g. azole antifungals, antiretrovirals with boosted protease inhibitors) should not be co-administered. This also applies to most statins except pravastatin. It should be evaluated, if the statin-therapy can be stopped during HCV treatment. Co-administration with dabigatran, edoxaban, cyclosporine, aliskiren and amiodarone is not recommended.

Treatment of hepatitis C in special populations

Patients with acute hepatitis C

The goal of acute hepatitis C treatment is the prevention of persistent HCV infection. Spontaneous clearance of acute hepatitis C occurs in 10–50% (Maasoumy and Wedemeyer 2012). Early treatment with interferon based therapy was more effective, than treating patients with chronic hepatitis C (Jaeckel 2001, Wiegand 2006, Deterding 2013).

This strategy seems obsolete as DAA regimens have a very high efficacy in patients with chronic hepatitis C. Treating patients with acute hepatitis C is today mainly motivated by breaking the transmission chain in people with high risk behaviors.

Several studies have now provided data that short-term treatment of 6–8 weeks with DAA combination therapy (i.e. SOF/LDV) is highly effective in patients with acute (Deterding 2017) or recent (Martinello 2018) HCV infection. However, so far the data are limited to define distinct treatment regimen and treatment duration for patients with acute HCV infection. HIV infected patients with acute or recent HCV infection may need a different approach than monoinfected patients, if ultrashort therapies are applied. For example, 6 weeks SOF/LDV has been investigated in 20 GT1 patients with acute HCV mono-infection (Deterding 2017) and in 26 HIV GT1 and GT4 patients with acute hepatitis C (Rockstroh 2017). All HCV monoinfected patients achieved SVR (Deterding 2017) while three HIV patients relapsed after the end of therapy, two additional with SVR4 were lost to follow up and one patient had a reinfection resulting in only 77% SVR (Rockstroh 2017).

Symptomatic patients in particular with jaundice have a good chance of clearing HCV spontaneously (Gerlach 2003, Hofer 2003), occurring usually in the first 12 weeks after the onset of symptoms. Given the high SVR in

patients with chronic hepatitis C with new DAA therapies, the decision to monitor the natural course may be easier. Thus, monitoring HCV RNA levels at 4 and 12 weeks following diagnosis of acute infection provides an opportunity to assess the likelihood of spontaneous clearance without compromising outcome.

However, treatment of acute or early HCV infection may be important in risk groups to prevent transmission and new infections. The unrestricted DAA availability in the Netherlands and the increase uptake of treatment of acute and early HCV infection was followed by a 51% decrease in acute HCV infections among HIV positive MSM (Boerekamps 2018).

At this stage, a pangenotypic treatment regimen given for 8 weeks may be the best approach for patients with acute hepatitis C (EASL 2018). However, treatment of acute hepatitis C has to be considered an off-label use.

Patients with normal aminotransferase levels

Approximately 30% of patients with chronic hepatitis C maintain persistently normal alanine aminotransferase (ALT) levels despite having detectable HCV RNA in serum. These patients have generally mild liver disease and show a slow progression to cirrhosis. However, up to one third of patients with normal ALT can present with significant liver fibrosis necessitating an effective treatment (Bacon 2002). In current guidelines, ALT elevation is not a prerequisite to start antiviral therapy and the assessment of liver fibrosis stage should be made regardless of ALT (EASL 2018).

Patients with compensated versus decompensated liver cirrhosis

Successful therapy of patients with advanced fibrosis and liver cirrhosis is associated with decreased incidence of HCC, decompensation and liver-related mortality (Morgan, 2010, Veldt 2007, Ioannou 2017). In addition, in patients awaiting liver transplantation, successful therapy prevents graft reinfection (Forns 2003). Thus, patients should be considered for immediate therapy if no contraindications are present. Efficacy data for patients with compensated liver cirrhosis are well defined in several hundred patients. Based on the findings of several Phase 3 trials for the evaluation of IFN-free regimens, patients with compensated liver cirrhosis are expected to have SVR rates $\geq 95\%$ (EASL 2018). However, SVR rates are lower in patients with decompensated cirrhosis and not all DAA combinations can be administered due to contraindications. NS3/4A protease inhibitors are not recommended or even contraindicated in patients with decompensated cirrhosis because

of substantially higher drug exposure with the dose used in compensated liver disease (EASL 2018). Thus, GZR/EBR, GLE/PIB and SOF/VEL/VOX are not recommended in decompensated liver cirrhosis (EASL 2018) (<https://www.hcvguidelines.org/unique-populations/decompensated-cirrhosis>).

Nevertheless, several studies and real-world data have demonstrated that IFN-free PI free DAA therapy is reasonably safe even in patients with advanced liver disease, but these patients still have an increased risk for hospitalisation during treatment, mostly due to complications from liver disease (Höner Zu Siederdisen 2015, Manns 2016, Poordad 2016, Curry 2015b).

The SOLAR-2 study evaluated the use of SOF/LDV + RBV in 329 patients with decompensated cirrhosis for 12 and 24 weeks including patients after liver transplantation (Manns 2016). SVR12 rates were ranging between 87% and 96% for patients with Child-Pugh-Turcotte score (CPT) B patients and 72-85% for CPT C patients in GT 1 (Table 23). Importantly, although the overall number of severe adverse events ranged between 17% and 30% in the SOLAR-2 and ALLY-I (SOF + DCV study) trial, the number of treatment associated severe adverse events was rather low with about 2-5% in the SOLAR-2 trial, suggesting a good safety of DAAs even in decompensated patients, but a high risk for complications due to the underlying liver disease (Manns 2016, Poordad 2016). However, the rate of treatment discontinuations can be higher in RBV treated patients, thus, the use of RBV is still a concern in these patients and the initial dose should be low (i.e. 600mg) (<https://www.hcvguidelines.org/unique-populations/decompensated-cirrhosis>).

The combination of SOF/VEL was studied in patients with CPT B (not CPT C) in the ASTRAL-4 study in GT1, GT2, GT3, GT4 and GT6 (Curry 2015b). Only numerically small differences could be seen between 12 and 24 weeks of SOF/VEL for GT1, 2, 4 and 6, suggesting that 12 weeks of therapy was enough. International guidelines recommend the addition of RBV (low initial dose [600mg] of ribavirin, increase as tolerated) or extension to 24 weeks in RBV ineligible patients (EASL 2018) (<https://www.hcvguidelines.org/unique-populations/decompensated-cirrhosis>). For GT3 the combination of SOF/VEL + RBV for 12 weeks showed the highest response rates with 85% whereas both combinations without RBV showed an SVR12 of only 50%, thus RBV seems important in these difficult-to-treat patients (Table 18).

An important question is, if patients with advanced liver cirrhosis benefit from IFN-free therapies. Early data suggest that patients treated with IFN-free therapies show an improvement of liver function (Deterding 2015). Several study with CPT B and C patients demonstrated that virologic response to DAA therapy for 12-24 weeks was associated with improvements in bilirubin, albumin, MELD and CPT scores (Manns 2016). Recent studies have documented that up to one third of DAA treated HCV patients with decompensated cirrhosis can be delisted as result of clinical improvement, which appears to be remain stable in most patients (Pascasio 2017, Belli 2016).

However, the benefit of treatment in decompensated cirrhosis is still not completely clear to date and further follow-up data are needed to see whether successful treatment in these patient population leads to decreased mortality and prevention of liver transplantation in the long-term. For example, patients with high MELD scores are unlikely to benefit from treatment and in one study no patients with a MELD >20 could be delisted (Pascasio 2017). Several studies evaluated prognostic factors that are associated with improvement of liver function after DAA therapy in decompensated cirrhosis. Albumin < 28 g/l was associated with a poor treatment response and age >65 years and/or an albumin < 35 g/l was associated with an increased rate of adverse events and a lower chance for improvement of liver function in the UK EAP, possibly suggesting a point of no return in these patients (Charlton 2015, Foster 2016). A retrospective analysis of data from 4 clinical trials with SOF-based therapies in patients with decompensated cirrhosis (502 of CPT class B and 120 of CPT class C). Based on the results, the authors developed a scoring system based on 5 baseline factors (body mass index, encephalopathy, ascites, and serum levels of alanine aminotransferase and albumin) which was associated significantly with patient outcomes and was called the “BE3A score” (El-Sherif 2018). It is important that patients with CTP B or C improved to CPT A. However, some patients may achieve a significant decrease of the MELD score but the patient has still a poor prognosis and low quality of life, which has been called the “MELD purgatory” after successful DAA therapy (Tapper 2017).

EASL recommends that patients awaiting liver transplantation should be treated with DAA if the MELD-score is <18-20. Patients with MELD scores ≥18-20 should be transplanted first and treated after liver transplantation (EASL 2018). In certain situations, treatment may be considered before transplantation. However, creatinine and therefore renal function is a main driver of the MELD score, which can be a problem because SOF-based therapies are not recommended, if GFR is <30 mL/min and PIs as well as IFN are contraindicated in decompensated cirrhosis.

Table 23. Pivotal phase 2 and 3 trials with SOF/LDV and SOF/VEL in patients with decompensated cirrhosis. Studies are not head-to-head and it is difficult to compare SVR between different studies because the populations had significant differences in genetic and socioeconomic backgrounds.

Study	Treatment	Child B	Child C
SOLAR-2 (GT1) (Manns et al., 2016)	a) 400/90 mg SOF/LDV + RBV 12 weeks	87% (20/23)	85% (17/20)
	b) 400/90 mg SOF/LDV + RBV 24 weeks	96% (22/23)	72% (13/18)
ASTRAL-4 (GT1,2,3,4,6) (Curry et al., 2015)	a) 400/100 mg SOF/VEL 12 weeks	83% (75/68)	
	b) 400/100 mg SOF/VEL + RBV 12 weeks	94% (82/87)	
	c) 400/100 mg SOF/VEL 24 weeks	86% (77/90)	

GT: genotype, SOF: sofosbuvir, VEL: velpastavir, VOX: voxilaprevir

Patients with hepatocellular carcinoma (HCC)

The ideal timing of DAA therapy in patient with HCC is debated. There are rationales to treat patients with HCC who have an indication for liver transplantation after transplantation. Some studies have shown lower SVR in patients with active HCC compared with patients without HCC or patients with HCC after transplantation (Beste 2017, Prenner 2017) (Table 24). Several studies have also suggested that rates of HCC recurrence in patients with a history of HCC (i.e. after resection or ablative therapies) can potentially be increased after DAA therapy (Reig, 2016, Conti 2016, El Kassas, 2018), while other studies suggested the opposite (Petta 2017, Huang 2018). The timing of DAA therapy in patients with HCC may be crucial. If patients receive potentially curative treatment for HCC it may be best to wait with DAA therapy for 3-6 months after successful HCC therapy. Importantly, SVR rates seem not to be impaired in patients with successfully treated HCC (Persico 2018a).

Overall, the treatment of patients with HCC is an individualised approach and patients should be managed in specialised centres.

Table 24. Studies with DAA in patients with hepatocellular carcinoma (HCC). Studies are not head-to-head and it is difficult to compare SVR between different studies because the populations had significant differences in genetic and socioeconomic backgrounds.

Study	Cohort	SVR HCC	SVR No HCC
(Beste et al., 2017)	DAA therapy: 16,863 non-HCC, 482 HCC, 142 HCC but treatment after LT	74% After LT 94%	91%
(Prenner et al., 2017)	DAA therapy: 284 non-HCC, 137 HCC	79%	88%

LT: liver transplantation, HCC: hepatocellular carcinoma

Patients after liver transplantation

HCV reinfection occurs in almost all untreated patients after liver transplantation. While the course of hepatitis C in liver transplant recipients was believed to be rather benign in the late '80s and early '90s (Böker 1997), More recently HCV infection has been shown to lead to a more rapid progression of liver fibrosis posttransplant (Berenguer 2005, Neumann 2004) with cirrhosis within the first 5-10 years in 20-30% of patients. Because HCV infection takes a more rapid course posttransplant than in immunocompetent individuals, treatment needs are obvious.

Antiviral therapy may be started before transplant to prevent reinfection

of the graft according to the consideration discussed above. If this approach is successful, reinfection can be prevented (Forns 2003, Curry 2015a). The approval of the new IFN-free regimens increased the safety and feasibility of therapy before and after liver transplantation. If available, treatment after liver transplantation should be initiated with IFN-free DAA regimens.

The efficacy of SOF/LDV + RBV has been examined in GT1 and GT4 infection after liver transplantation (Charlton 2015). Patients with prior treatment experience as well as patients with decompensated liver cirrhosis were included. Treatment duration was 12 or 24 weeks for SOF/LDV + RBV. SVR12 data were available in 111 patients without cirrhosis, 51 patients with Child A cirrhosis, 52 patients with Child B cirrhosis, 9 patients with Child C cirrhosis and 6 patients with fibrosing cholestatic hepatitis. In patients with compensated cirrhosis the SVR rates were similar to non-immunocompromised patients. In contrast, in Child C patients, the SVR rate declined to 60% for 12 weeks and 75% for 24 weeks of treatment (Table 25). Treatment-emergent death occurred in four patients due to progressive multifocal leukoencephalitis, thoracic aorta aneurysm dissection, internal bleeding and complications of cirrhosis. Some patients required erythropoietin treatment or blood transfusions due to RBV. Additional data are available from the SOLAR-2 study. In 168 patients with varying degrees of fibrosis including patients with compensated liver cirrhosis, the SVR rate for 12 or 24 weeks of SOF/LDV + RBV treatment was 95% and 98% (Table 25). In patients with decompensated liver disease SVR rates posttransplantation were 95% (19/20) for 12 weeks of treatment and 100% (16/16) for 24 weeks of treatment in CPT B patients. Only 6 patients with CPT C posttransplant were included and showed response rates of 50% (1/2) and 75% SVR (3/4) for 12 and 24 weeks of treatment, respectively (Manns 2016).

SOF/VEL given for 12 weeks has been evaluated in 79 patients with GT1-4 after liver transplantation. The SVR was 96% (Agarwal 2018) (Table 25).

As renal insufficiency is more frequent in transplanted patients, data with SOF free PI based therapies (GZR/EBR and GLE/PIB) are also important. GZR/EBR has been used in some patients after liver transplantation with comparable efficacy than in immunocompetent patients (Miuma 2018). Importantly, cyclosporine cannot be co-administered with GZR/EBR.

12 weeks GLE/PIB has been studied in 80 patients after liver transplantation. Most patients had mild fibrosis. SVR was 98% and only one patient had a virological failure (Reau 2018) (Table 25).

Overall, treatment in patients with compensated liver disease after transplantation is safe and effective with the new DAA and response rates are similar to patients without concomitant immunosuppressive regimens (Liao 2017).

Table 25. Pivotal phase 2 and 3 trials with DAA therapy in patients after liver transplantation.

Studies are not head-to-head and SVR between studies are difficult to compare because there were significant differences in genetic and socioeconomic backgrounds. Dosage of the medications may vary depending on the immunosuppressive co-medication.

Study	Treatment	SVR
SOLAR-1 (Charlton et al., 2015) n=214	a) 400/90 mg SOF/LDV + 600-1200 mg RBV 12 weeks	<F3: 96% CPT A: 96% CPT B: 85% CPT C: 60%
	b) 400/90 mg SOF/LDV + 600-1200 mg RBV 24 weeks	<F3: 98% CPT A: 96% CPT B: 88% CPT C: 75%
SOLAR-2 (Manns et al., 2016) n = 168	400/90 mg SOF/LDV + 600-1200 mg RBV 12 weeks	GT1: 95% GT2: 100%
	400/90 mg SOF/LDV + 600-1200 mg RBV 24 weeks (FO - compensated cirrhosis)	GT3: 97% GT4: 100%
(Agarwal et al., 2018) 79 patients, 47% GT1 44% GT3, 18% cirrhosis	400/100 mg SOF/VEL 12 weeks	95% 98%
(Reau et al., 2018) N=100, n=20 kidney-Tx, n=80 liver-Tx, naive GT1-6, exp. GT1,2,4-6, 80% FO-1	300/120 mg GLE/PIB 12 weeks	98%

RBV: ribavirin, **SOF:** sofosbuvir, **LDV:** ledipasvir, **VEL:** velpatasvir, **GLE:** glecaprevir, **PIB:** pibrentasvir, **CPT:** Child-Pugh-Turcotte score

Patients with chronic kidney disease

Chronic hepatitis C is prevalent in patients with chronic kidney disease (CKD), including those with severe renal impairment (eGFR <30 mL/min/1.73 m²) and those who require hemodialysis or peritoneal dialysis. Treatment needs for HCV patients with CKD are obvious, especially if patients are considered for kidney transplantation. The outcome of HCV post-kidney transplantation is worse than for HCV negative patients after renal transplantation. In the past, patients after kidney transplantation could not be treated because IFN-based therapies were contraindicated posttransplantation since they may induce rejection. This has changed with the advent of DAA therapies (Reau 2018, Colombo 2017). However, SOF and its metabolites are mainly eliminated via renal clearance. Until recently, SOF was not recommended in patients with eGFR <30. Nevertheless, there are some reports about the use of SOF in patients with severe renal insufficiency or hemodialysis showing high efficacy and safety with the full dose of SOF (Manoj 2018). However, patients with CKD treated with

SOF based therapies in the TARGET registry had higher rates of anaemia, worsening renal dysfunction and serious adverse events regardless of use of RBV (Saxena 2016). Nevertheless, SOF based therapies can be used in patients with CKD when no other relevant treatment options are available, e.g. in patients with decompensated cirrhosis where protease inhibitors are not recommended. Recently, the FDA has also updated the label and now state that that no dosage adjustment is recommended in patients with any degree of renal impairment including patients on dialysis based on pharmacokinetic data obtained from studies involving HCV-infected patients with renal impairment including dialysis patients.

Nevertheless, SOF free therapies including NS3/4A protease inhibitors and NS5A inhibitors should be preferred in patients with severe renal impairment (eGFR<30), if possible.

The C-SURFER study investigated 12 weeks GZR/EBR in patients with CKD stage 4-5 including 76% with hemodialysis and compared this to a placebo controlled deferred treatment group (Roth 2015). 12 weeks GZR/EBR showed 99% SVR in the per protocol analysis (Table 26). The treatment regimen was well tolerated with a low rate of adverse events.

12 weeks GL/PIB was investigated in the EXPEDITION-4 phase III trial in 104 patients with stage 4 or 5 CKD. Overall, 98% of patients achieved SVR but none of the patients had a virologic relapse. (Gane 2017a) (Table 26).

Table 26. Pivotal phase 2 and 3 trials with DAA therapy in patients with CKD including haemodialysis. Studies are not head-to-head and SVR between studies are difficult to compare because there were significant differences in genetic and socioeconomic backgrounds.

Study	Treatment	SVR
C-SURFER (Roth 2015) n=224 76% haemodialysis	a) 50/100 mg GZR/EBR 12 weeks (n=111) plus 11 pharmacokinetic study b) placebo (n=113)	94% ITT, 99% mITT GT1a: 100% GT1b: 99%
(Gane et al., 2017a) N=104 CKD 5 87%, GT1 52%, GT3 11%, cirrhosis 19%	300/120 mg GLE/PIB 12 weeks	98%, 100% mITT

RBV: ribavirin, EBR: elbasvir, GZR: grazoprevir, ITT: intention-to-treat, mITT: modified intention-to-treat excluding non-virological failures

Drug use and patients on stable maintenance substitution

Treatment of people who inject drugs (PWID) is an individual approach and should only be performed in an experienced multidisciplinary setting including hepatologists, psychiatrists and addiction specialists. Drug interactions with DAAs need to be considered.

In principle, treatment with DAA is possible and studies show excellent adherence in selected OST (opiate substitution) patients. One study with GZR/EBR showed that OST patients maintain abuse of concomitant drugs such as cocaine, amphetamines, benzodiazepines, but SVR rates were not impaired and adherence was excellent (Dore 2016). Even in patients with more recent active drug use (past 6 months), treatment is possible and effective. The SIMPLIFY study showed that 97 (94%) of 103 PWIDs achieved SVR after 12 weeks of SOF/VEL (Grebely 2018). Drug use before and during treatment did not affect SVR. However, reinfection appeared to be an issue even within the 24 weeks post treatment period (Dore 2016).

Patients with co-infections

Due to the similar routes of transmission, patients with chronic hepatitis C are frequently co-infected with hepatitis B virus, hepatitis D virus or human immunodeficiency virus. These important patient groups are discussed in *Chapters 10, 15 and 16*. Importantly, HBV is usually suppressed in HBV/HCV co-infected patients (Wiegand 2015) and after successful DAA treatment of HCV, HBV reactivation can occur (Mücke 2018). Meanwhile, efficacy and adverse event rates with DAAs among patients with HCV/HIV coinfection are not different from those observed with HCV mono-infection. Meanwhile treatment recommendations are similar for HCV mono-infected and HCV/HIV co-infected patients. However, drug-drug interactions have to be considered (EASL 2018) (<https://www.hcvguidelines.org/unique-populations/hiv-hcv>).

Patients with hemophilia

Due to contaminated clotting factor concentrates, many patients with hemophilia were infected with HCV and/or HIV. Review of available data suggest that treatment success of HCV-infected hemophiliacs is similar to that achieved in the general HCV-infected population (Franchini 2008).

Patients with extrahepatic manifestations

More than 50% of HCV-infected patients suffer from extrahepatic manifestations ranging from fatigue to severe symptoms of mixed cryoglobulinaemia (Cacoub 1999) (*see Chapter 13*). The primary goal of treatment is HCV eradication, which is associated with improvement of clinical symptoms, especially in patients with mixed cryoglobulinaemia

(Negro 2015, Cacoub 2018a). Insulin resistance can be improved, especially in HCV GT1 patients with SVR (Thompson 2012, Cacoub 2018a). Most of the data that elimination of HCV can reduce extrahepatic manifestations are derived from studies with IFN-based treatment.

Meanwhile IFN-free therapies are an option for patients with extrahepatic manifestations and first data are available. Data from a prospective international multi-centre cohort study of 148 patients with symptomatic HCV-associated cryoglobulinaemia vasculitis show high virological and clinical response after DAA therapy (SOF + DCV, n=53; SOF + RBV, n=51; SOF/LDV, n=23; or SOF + SMV, n=18). SVR was documented for 97.2% of patients and a complete clinical response was reported in 73%, a partial response in 23%, and no response in 4.8%. After a median follow-up time of 15.3 months, vasculitis manifestations cleared or significantly improved: purpura 97%, renal involvement 92%, arthralgia 86%, neuropathy 77.1%, and cryoglobulinaemia 52.2% (Cacoub 2018b). Case series have reported regression of non-Hodgkin's lymphoma following SVR with DAA therapies regimen with or without additional chemotherapy (Persico 2018b, Lim 2015, Arcaini 2016).

However, there may also be a point-of-no-return for extrahepatic manifestations. A retrospective analysis of HCV patients with asymptomatic and symptomatic extrahepatic manifestations who were treated with DAA ± PEG-IFN showed high SVR but among 7 patients with severe vasculitis (mostly renal impairment) only 1 had a complete clinical response, with 3 showing a partial response and 2 showing no improvement. Three out four patients with life-threatening vasculitis received rituximab (Emery 2017).

Thus, in patients with severe symptoms of mixed cryoglobulinaemia, treatment with rituximab may be considered (EASL 2018).

Direct-acting antiviral therapy of HCV negative recipients after receiving a HCV positive solid organ

Due to the success of DAA therapy, there is the consideration to use HCV positive solid organs for transplantation and prevent HCV reinfection by prophylactic or preemptive DAA therapy. Several studies have shown that HCV infection can be prevented by an early initiation (at the time of transplantation or within the first two weeks) of DAA therapy in HCV negative patients who received HCV positive solid organs (table 27). Patients receiving anti-HCV positive but HCV RNA negative solid organs may not require DAA therapy. These patients can be monitored and in the rare case of HCV infection, treatment of acute HCV infection should be initiated and is usually effective (see above).

In most cases the HCV genotype is unknown at the time of transplantation

so that a pangenotypic DAA regime such as GLE/PIB or SOF/VEL should be preferred. The concomitant medication after transplantation and known drug-drug interactions should be considered.

Table 27. Selection of trials with DAA therapy in HCV negative patients who received HCV positive solid organs.

Study	Donor status	Start of therapy	DAA	SVR
Bethea et al. 2019 Heart transplantation N=55 Heart N=22 combined heart-kidney N=3	20 NAT+ 5 NAT- HCV antibody pos. (only treated if HCV RNA positive)	Donor NAT+ Pre-emptive therapy, starting before transport to the operating room followed by an 8-week course	GLE/PIB	100%
Kapila et al. 2019 Liver transplantation N=24	24 NAT+	Post-Transplant (Median 123 days post Tx)	LED/SOF N=12 LED/SOF/RBV N=8 SOF/VEL N=2 DAC/SOF/RBV N=1 GLE/PIB N=1	95.8% One patient developed HCV recurrence after achieving SVR12
Aslam et al. 2019 N=21 heart N=18 combined heart-kidney N=3	19 NAT+ 2 NAT-		GLE/PIB 14/19 SOF/VEL 2/19 ELB/GRA 2/19 LED/SOF 1/19	100%
Kwong et al. 2019 Liver transplantation N=10	10 NAT+	Post-Transplant (Median 43 days post Tx)	SOF/DCV/RBV 24 weeks N=1 SOF/LDV/RBV 24 weeks N=1 SOF/VEL 12 weeks N=3 SOF/VEL/RBV 12 weeks N=2 SOF/LDV 12 weeks N=2 SOF/VEL 24 weeks N=1	100%
Molnar et al. 2019 Kidney transplantation N=53	53 NAT+	Post-Transplant (Median 76 days post Tx)	GLE/PIB 47/53 SOF/VEL 5/53 SOF/LED 1/53	100%
Durand et al. 2018 Kidney transplantation N=10	10 NAT+	GZR/EBR immediately before transplantation	GT1 continued GZR/EBR GT2 or 3 continued SOF/GZR/EBR	100%
Friebus-Kardash et al. 2019 Kidney transplantation N=7	7 NAT+	Post-Transplant (Median 7 days post Tx)	SOF/LED N=4 SOF/VEL N=3	100%

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13. Extrahepatic manifestations of chronic HCV

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Introduction

Patients with chronic hepatitis C virus (HCV) infection are at risk of a variety of extrahepatic manifestations (EHMs) (Table 1). It is estimated that about 90% of patients with chronic HCV infection will develop one or more EHMs during the course of the disease (Tang 2016, Negro 2015). EHMs may often be the first and only clinical sign of chronic hepatitis C infection. Evidence of HCV infection should always be ruled out in cases of non-specific chronic fatigue and/or rheumatic, hematological, endocrine or dermatological disorders. The pathogenesis of EHM is still not fully understood although most studies suggest that the presence of mixed cryoglobulinaemia, particularly HCV lymphotropism, molecular mimicry and non-cryoglobulinemic autoimmune phenomena constitute the major pathogenic factors (Ferri 2007). Nevertheless, the pathogenesis and epidemiology of many EHMs require further investigation (Figure 1). Our aim is to give a brief insight into the epidemiology, pathogenesis, clinical relevance and therapeutic management of HCV-associated EHM (Zignego 2007a).

Mixed cryoglobulinaemia

Cryoglobulinaemia refers to the presence of abnormal immunoglobulins in the serum, which have the unusual property of precipitating at temperatures below 37°C and redissolving at higher temperatures. The phenomenon of cryoprecipitation was first described in 1933 (Wintrobe 1933). Cryoglobulins (CGs) are nowadays classified into three types (Table 2) based on their clonality. Type II CG and type III CG, consisting of monoclonal and/or polyclonal immunoglobulins, are prevalent in patients with chronic HCV infection, while type I CGs, consisting exclusively of monoclonal components, are mostly found in patients with lymphoproliferative disorders (multiple myeloma, B cell lymphoma, Waldenström macroglobulinaemia). Type II or type III mixed cryoglobulinaemia is found in 19%-50% of patients with chronic HCV but leads to clinical manifestations through vascular precipitation of immunocomplexes in only 30% of them (Lunel 1994, Wong

1996). Asymptomatic mixed cryoglobulinaemia (MC) during the course of chronic HCV infection may evolve into symptomatic disease. Patients with symptomatic mixed cryoglobulinaemia (MCS) exhibit higher cryoglobulin concentrations (cryocrit >3%) and lower concentrations of complement factors C3 and C4 (Weiner 1998). Thus CG-triggered complement activation may constitute a key incidence in cryoglobulinaemia-derived pathogenesis. Factors that seem to favour the development of MC are female sex, age, alcohol intake (>50g/d), advanced liver fibrosis and steatosis (Lunel 1994, Wong 1996, Saadoun 2006).

Table 1. Extrahepatic manifestations of chronic hepatitis C infection

Organ/System involved	Manifestation
Endocrine disorders	<ul style="list-style-type: none"> Autoimmune thyroidopathies (in particular, Hashimoto thyroiditis) Insulin resistance/diabetes mellitus* Growth hormone (GH) insufficiency Vitamin D deficiency Osteopenia and Osteoporosis
Rheumatic disorders	<ul style="list-style-type: none"> Mixed cryoglobulinaemia* Cryoglobulinemic vasculitis* Peripheral neuropathy* Membrano-proliferative glomerulonephritis (GN)* Membranous GN* Rheumatoid arthralgias/oligopolyarthritis Rheumatoid factor positivity* Sicca syndrome
Hematologic disorders	<ul style="list-style-type: none"> Lymphoproliferative disorders/Non-Hodgkin Lymphomas* Immune thrombocytopenic purpura (ITP) Monoclonal gammopathies* Autoimmune hemolytic anaemia
Dermatologic disorders	<ul style="list-style-type: none"> Palpable purpura Porphyria cutanea tarda (PCT) Lichen planus Pruritus
Cardiovascular disorders	<ul style="list-style-type: none"> Cardiomyopathy/Myocarditis Carotid atherosclerosis Increased risk for peripheral arterial disease (PAD), cardiovascular (CV) mortality, and ischemic stroke
Central nervous system disorders	<ul style="list-style-type: none"> Chronic fatigue*, subclinical cognitive impairment, psychomotoric deceleration, symptoms of depression* Neurocognitive disorders
Miscellaneous	<ul style="list-style-type: none"> Myopathy Idiopathic pulmonary fibrosis Increased risk for non-liver solid cancers (rectum, pancreas, lung and bronchus, kidney) (Allison 2015)

*Associations based on strong epidemiological prevalence and/or clear pathogenetic mechanisms

Table 2. Types of cryoglobulinaemia

Type	Clonality
Type I	Monoclonal immunoglobulins (IgG or IgM)
Type II	Polyclonal immunoglobulins (mainly IgG) and monoclonal IgM with rheumatoid factor activity (RF)
Type III	Polyclonal IgG and IgM

Principal HCV-EHDs classified according to the strength of the association			
1 Strong association	2 Significant association	3 Possible association	4 Anecdotal association
Mixed cryoglobulinaemia syndrome (cryogl. vasculitis) B-cell NHL	Monocl. gammopathies PCT, Sjögren/sicca s. lichen planus glomerulonephritis autoimmune thyroiditis papillary thyroid cancer diabetes m. type 2 cardiovascular inv.	PAN, IBM, Polyarthritis, Sarcoidosis, Pruritus Osteosclerosis fibromyalgia Peripheral neuropathy Lung alveolitis	Large-vessel vasculitis ANCA-vasculitis Other SAD (PM/DM, SLE, APS, Behcet's s., etc.) Chronic urticaria Psoriasis Mooren corneal ulcer

Figure 1. Schematic representation of EHM categories according to the strength of association (Ferri 2016). 1: Strong association with HCV as main etiological agent, 2: Association demonstrated in a significant proportion of patients compared to the general population. 3: Suggested role of HCV infection demonstrated in cohort studies. 4: Anecdotal observations suggested a possible role of HCV. PCT: porphyria cutanea tarda, PAN: periarteriitis nodosa, IBM: inclusion body myositis, SLE: systemic lupus erythematoses, PM/DM: polymyositis/dermatomyositis, APS: anti-phospholipid syndrome.

Diagnosis

Detection of CG is carried out by keeping patient serum at 4°C for up to 7 days. When cryoprecipitate is visible, CG can be purified and characterised using immunofixation electrophoresis. In case of evidence of mixed cryoglobulinaemia in HCV positive patients, cryoglobulinemic syndrome needs to be looked for. Vigilant monitoring is required, as asymptomatic mixed cryoglobulinaemia patients may develop MC-related disorders in the course of the disease. The diagnosis of the MC syndrome is based on serologic, pathologic and clinical criteria (Table 3).

Table 3. Diagnostic criteria of cryoglobulinemic syndrome

Serologic	Histopathologic	Clinical
<ul style="list-style-type: none"> • C4 reduction • Positive rheumatoid factor (RF) • CGs, type II or III • HCV antibodies 	<ul style="list-style-type: none"> • Leucocytoclastic vasculitis • Monoclonal B-cell infiltrates 	<ul style="list-style-type: none"> • Purpura • Fatigue • Arthralgia • Membranoproliferative GN • Peripheral neuropathy

In the presence of mixed CG, low C4 counts, leucocytoclastic vasculitis and purpura, a definite symptomatic MC can be diagnosed. Rheumatoid factor (RF) determination constitutes a reliable surrogate marker for detection of CG. Finally, presence of CG may impair HCV RNA determination as viral RNA can accumulate in precipitated cryocrit (Colantoni 1997).

Clinical presentation

HCV-related MC proceeds mostly asymptotically and has no significant influence on the course of chronic liver inflammation. On the other hand, symptomatic mixed cryoglobulinaemia is associated with higher mortality (Ferri 2004).

Systemic vasculitis

HCV-related vasculitis relies on a deposition of immunocomplexes containing CGs, complement and large amounts of HCV antigens in the small- and medium-sized blood vessels. HCV accumulates in the CG immunoglobulins. Pathohistological findings reveal a leucocytoclastic vasculitis (Agnello 1997). The most common symptoms of mixed cryoglobulinemic vasculitis are weakness, arthralgia and purpura (the Meltzer and Franklin triad). Mixed cryoglobulinemic vasculitis may also lead to Raynaud's Syndrome and Sicca Syndrome, glomerulonephritis and peripheral neuropathy.

Renal impairment

The predominant renal impairment associated with mixed cryoglobulinaemia is the membranous proliferative glomerulonephritis (MPGN), characterised in most cases by proteinuria, mild hematuria and mild renal insufficiency. The presence of kidney impairment is considered

to be a negative prognostic factor in the course of the disease (Ferri 2004). In 15% of patients, MC-related nephropathy may progress to terminal chronic renal failure requiring dialysis (Tarantino 1995).

Peripheral neuropathy

Peripheral neuropathy, on the basis of endoneural microangiopathy, constitutes a further typical complication of mixed cryoglobulinaemia. MC-related neuropathy, presenting clinically as mononeuropathy or polyneuropathy, is mostly sensory and is characterised by numbness, burning skin, a crawling sensation, and pruritus, predominantly in the hands and feet (Tembl 1999, Lidove 2001). Epidemiological data from Italy suggests that peripheral neuropathy is the second most common symptom after the Meltzer and Franklin triad in patients with symptomatic HCV-associated mixed cryoglobulinaemia (Ferri 2004).

Cirrhosis

The causal association between CG and progression of liver fibrosis suggested by numerous authors was not confirmed in a published 10-year prospective study. The 10-year rates of progression to cirrhosis were similar in cryoglobulinemic and non-cryoglobulinemic HCV-infected patients (Vigano 2007). From this, it is unlikely that mixed cryoglobulinaemia constitutes an independent risk factor for the progression of liver fibrosis.

Malignant lymphoproliferative disorders/NHL

The association between infectious agents and potentially reversible "antigen driven" lymphoproliferative disorders, such as *Helicobacter pylori*-related gastric marginal zone B cell lymphoma has been known for many decades. There seems to be a causative association between HCV and Non-Hodgkin Lymphoma (NHL) (Mele 2003, Duberg 2005, Giordano 2007). HCV infection leads *per se* to a two-fold higher risk of developing NHL (Mele 2003, Duberg 2005). A meta-analysis of seven studies with over 10,000 patients found a higher incidence of B-cell NHL associated with HCV infection (De Sanjose 2008). The most prevalent HCV-associated lymphoproliferative disorders according to the REAL/WHO classification are: follicular lymphoma, B cell chronic lymphocytic leukemia/small lymphocyte lymphoma, diffuse large B cell lymphoma and marginal zone lymphoma, including the mucosa-associated lymphoid tissue lymphoma. Overall, marginal zone lymphoma appears to be the most frequently encountered low grade B cell lymphoma in HCV patients. The role of HCV in

the genesis of lymphoma can be either explained by the direct lymphoma-inducing effects of HCV during viral replication in normal B cells or by being a stochastic process as a result of HCV-induced proliferation of B cells (Agnello 2004, Figure 2). More recent data from a large population-based study comparing HCV-infected patients with the general population showed a more than doubled age-adjusted mortality rate for NHL among HCV-infected patients. In addition, there was a trend towards higher grades and stages of NHL in the HCV group compared to the control population (Allison 2015).

HCV-associated lymphoproliferative disorders (LPDs) are observed over the course of MC. 8-10% of mixed cryoglobulinaemia type II evolve into B cell NHL after long-lasting infection. However, a remarkably high prevalence of B cell NHL was also found in HCV patients without mixed cryoglobulinaemia (Silvestri 1997). Genetic predisposition and other factors seem to have a major impact on the development of LPDs in HCV positive patients (Matsuo 2004).

Aetiology and pathogenesis of LPDs in patients with HCV infection

In the development of LPDs direct and indirect pathogenic HCV-associated factors (Figure 2) are seen. Sustained B cell activation and proliferation, noticed during chronic HCV infection, is an indirect pathogenic mechanism.

Direct pathogenic mechanisms are based on lymphotropic properties of HCV, hence on HCV's entry into the B cells. HCV RNA sequences were first detected in mononuclear peripheral blood cells (Zignego 1992). Especially CD19⁺ cells seem to be permissive for certain HCV quasispecies (Roque Afonso 1999). Active replication of the HCV genome in B cells is associated with activation of anti-apoptotic gene BCL-2 and inhibition of p53 or c-Myc-induced apoptosis (Sakamuro 1995, Ray 1996). In this light, direct involvement of HCV in the immortalisation of B cells can be imagined (Zignego 2000, Machida 2004).

More recent data show that the lymphotropism of HCV with its association to B cells is mediated by the complement system involving the complement receptor 2 (CD21) and CD19 as well as CD81 complex (Wang 2016). A complex dysregulated cytokine network involving Th1 immune response and proinflammatory cytokines has been shown to be present in HCV-EHMs. IFN γ as well as CXCL9, CXCL10 and CXCL11 chemokines are responsible mediators of liver inflammation and parenchyma damage during the course of the infection. CXCL10 levels have been shown to decrease after successful DAA therapy of HCV infection (Fallahi 2017).

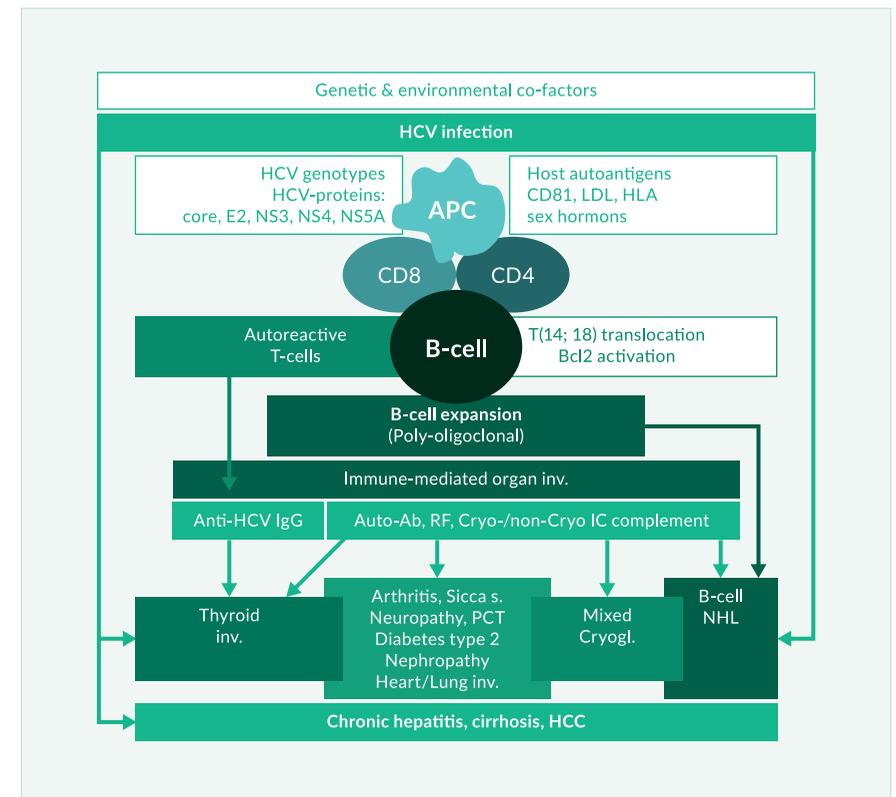


Figure 2. Aetiology and pathomechanisms involved in chronic HCV infection and the development of malignant lymphoproliferative disorders. Multiple pathogenic processes are involved such as chronic HCV infection, environmental triggers or genetic predisposition. HCV-induced lymphoproliferation and autoantibody production can be distinguished from oncogenic alterations leading to B cell neoplasias and other malignancies (B-NHL, HCC, papillary thyroid cancer). Significant molecularpathogenetic steps are binding of HCV protein E2 to CD81 following t(14;18) translocation as well as proto-oncogen bcl-2 activation, cross reaction between HCV antigens and host autoantigens and direct B cell infection by HCV followed by neoplastic transformation. HCV-induced B cell proliferation with production of various autoantibodies may be the pathological substrate of organ- and non-organ-specific autoimmune disorders, such as MC syndrome or cryoglobulinemic vasculitis (RF: rheumatoid factor, NHL: non-Hodgkin's lymphoma, HCC: hepatocellular carcinoma, PCT: porphyria cutanea tarda, modified after Ferri C et al., 2017).

Treatment of lymphoproliferative disorders

Because of the close correlation between the level of viral suppression and improvement of HCV-associated extrahepatic symptoms, the most effective antiviral strategy should be considered when dealing with HCV-related extrahepatic diseases. New interferon-free combinations of direct acting antiviral drugs (DAA) are the standard of care for HCV infection types 1-6. Therefore these regimens can also be regarded as treatment of choice in HCV-infected patients with extrahepatic manifestations.

However, the clinical experience of DAA use in patients with EHM remains limited because only less than 100 of such cases were reported in the last two years. Moreover, there are only few systematic studies on the effect of SVR caused by DAA therapy on EHMs (Younossi 2016). Compared to interferon-based therapies the newer DAAs have a very small number of true contraindications. However drug-drug interactions due to CYP3A or P-glycoprotein metabolism need to be taken into account and concomitant medications need to be assessed and adjusted accordingly. For further information, see the other HCV chapters.

In the treatment era of DAA regimens, several prospective studies have reported on the use of DAA regimens in HCV-induced EHMs since 2015. Most out of these had cryoglobulinemic vasculitis (Ramos-Casals 2017; see also Table 4). Furthermore, recent data show the benefit of viral clearance induced by DAA therapy on liver- and non-liver-related mortality compared to matched untreated controls (Negro 2019). There is first data revealing a successful treatment of a genotype 3 HCV patient with decompensated cirrhosis and renal failure secondary to MCS. 12 week-treatment with sofosbuvir, ledipasvir and ribavirin led to SVR and improvement of liver and renal function in this patient, yet further studies with larger cohorts are required to confirm these results (Flemming 2016). In another large retrospective cohort of DAA-treated patients, a successful treatment with SVR was associated with significant risk reduction of developing MC, glomerulonephritis and lichen planus, but no significant effect on the risk of diabetes and NHL (El-Serag 2019).

Mixed cryoglobulinaemia

While asymptomatic mixed cryoglobulinaemia (MC) *per se* does not constitute an indication for treatment, symptomatic mixed cryoglobulinaemia (MCS) should always be treated. Because asymptomatic cryoglobulinaemia may evolve into symptomatic CG in the course of disease, vigilant monitoring is required and introduction of antiviral therapy in terms of prophylaxis should be considered.

Because a causal correlation between HCV infection and mixed cryoglobulinemia has been established, the therapeutic approach of symptomatic mixed cryoglobulinemia should primarily concentrate on the eradication of the virus. In the therapeutic era of interferon, IFN α has been shown to be a promising therapeutic tool in HCV-induced MC due to its antiviral, and antiproliferative properties on IgM-RF-producing B cells and stimulation of macrophage-mediated clearance of immunocomplexes, suggesting that IFN α may lead to clinical amelioration even in virologic non-responders. Clinical improvement of MC is reported in 50 to 70%

of patients receiving antiviral therapy with IFN α plus RBV and mostly correlates with a significant reduction of HCV RNA concentrations (Calleja 1999). However, cryoglobulinemic vasculitis following successful antiviral treatment persists in a small collective (Levine 2005). Data from a large prospective study in chronically HCV-infected patients with MC who have been treated with Peg-IFN α plus ribavirin confirmed the close relationship between virologic response and clinical-immunological response. Indeed, all patients with sustained virologic response also experienced a sustained clinical response, either complete or partial. In the majority of sustained virologic response patients all MCS symptoms persistently disappeared (36 patients, 57%); in only two (3%) did definite MCS persist. All virologic non-responders were also clinical non-responders, in spite of a transient improvement in some cases (Gragani 2015). The amount of the clinical benefit of HCV therapy seems to be inversely correlated with the time from diagnosis to initiation of antiviral therapy, which favors an early start of treatment (Mahale 2018). A therapeutic limitation seems to be the possible persistence of B-cell clones in a dormant state long after HCV eradication has been established, as there is a risk of reactivation leading to recurrent cryoglobulinemia syndrome (Visentini 2019). In case of treatment failure of antiviral therapy and/or fulminant manifestations, contraindications or severe side effects, alternative therapeutic strategies such as cytostatic immunosuppressive therapy and/or plasmapheresis have been considered in the interferon era (Craxi 2008) (Figure 3, Table 4). Rituximab has been shown to be an effective and safe treatment option for MC even in advanced liver disease. Moreover, B cell depletion can lead to improvement of cirrhotic syndrome by mechanisms that remain to be elucidated (Petrarca 2010).

Systemic vasculitis

In cases of severe systemic vasculitis, initial therapy with rituximab, a monoclonal chimeric antibody against CD20 B cell-specific antigen, is suggested. Its efficacy and safety have been demonstrated in patients with symptomatic MC resistant to IFN α therapy, even though HCV RNA increased approximately twice the baseline levels in responders (Sansonna 2003). In the interferon era, a combined application of rituximab with PEG-IFN α plus ribavirin was considered a rational approach for cases with severe mixed cryoglobulinemia-related vasculitis resistant to antiviral therapy alone (Saadoun 2008). However, the future role of rituximab and other immunosuppressive regimens remain to be seen in the light of interferon-free antiviral treatment options, in which nearly all patients can be quite effectively treated and cured from their HCV infection. Still, clinical experience in treatment of EHMs with DAA therapy is rather limited. First

evidence of the efficacy and safety of DAA based treatment with sofosbuvir-based regimens in patients with HCV-induced MC was recently published by Sise et al. demonstrating a SVR12 rate of 83%. Interestingly, treatment response was associated with an improvement in eGFR and a reduction in proteinuria (Sise 2015). In severe mixed cryoglobulinemia-related vasculitis or acute manifestations refractory to both, antiviral and rituximab-based approaches, cycles of plasma exchange plus corticosteroids and eventually cyclophosphamide are indicated. Further studies showed that low dose interleukin-2 can lead to clinical improvement of vasculitis and has immunologic effects such as recovery of regulatory T cells (Saadoun 2011).

Regarding the IFN-free DAA regimens, there is a cohort of 24 HCV patients with CV who were treated with sofosbuvir+RBV. A clinical response was observed in most patients (87,5%) and 74% of patients achieved SVR24 (17/23) (Saadoun 2015b). Treatment with sofosbuvir+RBV (n=18) as well as combination of sofosbuvir+RBV plus simeprevir or ledipasvir or daclatasvir was analysed in a recent trial with 28 CV patients from whom 12 patients had cirrhosis. Here SVR24 was 100%, although there are no data on immunological response (Gagnani 2016). There are also some data on DAA regimens in patients with EHM without the use of RBV. In a retrospective study of 8 CV patients who were treated with sofosbuvir and simeprevir, there was an SVR12 rate of 87,5% (7/8). A complete clinical response for CV was only seen in half of the patients (4/8) (Sise 2016). More recently, data of n=30 and n=16 HCV patients with CV were published using many different DAA regimens for HCV treatment, including 3D, sofosbuvir, simeprevir, daclatasvir, grazoprevir and elbasvir. The CGs became negative in 12 of 30 patients and the SVR24 rates were constantly high (29/30 and 16/16 patients) (Bonacci 2016, Gagnani 2016). Another long-term follow-up analysis investigated 148 patients with HCV-associated CV who were treated with different DAA regimens (sofosbuvir+daclatasvir, sofosbuvir+RBV, sofosbuvir+ledipasvir or sofosbuvir+simeprevir) for 12 or 24 weeks. More than 95 % of patients achieved a full or partial response of CV symptoms following DAA therapy (Cacoub 2019).

Therefore, studies with DAA therapy have shown discordant rates regarding SVR and CV response rates (Sise 2016). Taken together, an interferon-free DAA regimen represents the current standard of care for HCV-infected patients with EHM. For selecting the most appropriate DAA regimen certain host and viral factors but also co-medications have to be taken into considerations as outlined in current HCV treatment guidelines (Ramos-Casals 2017; EASL guideline 2018).

Peripheral neuropathy

Cryoglobulinemia-induced peripheral neuropathy has been a significant issue in the past, because the effectiveness of interferon-based antiviral therapy on peripheral neuropathy has been debated intensely. Data on efficacy of DAA treatment on HCV-related neuropathy show promising results with total resolution of polyneuropathy in 21 of 25 patients under sofosbuvir-based regimens (Hegazy 2016). Therefore, peripheral neuropathy should not be considered as a contraindication for antiviral therapy of the chronic hepatitis C. However, another study found a small proportion of DAA-treated patients with presence of severe cryoglobulinemic vasculitis and peripheral neuropathy being associated with non-response to therapy (Cacoub 2019).

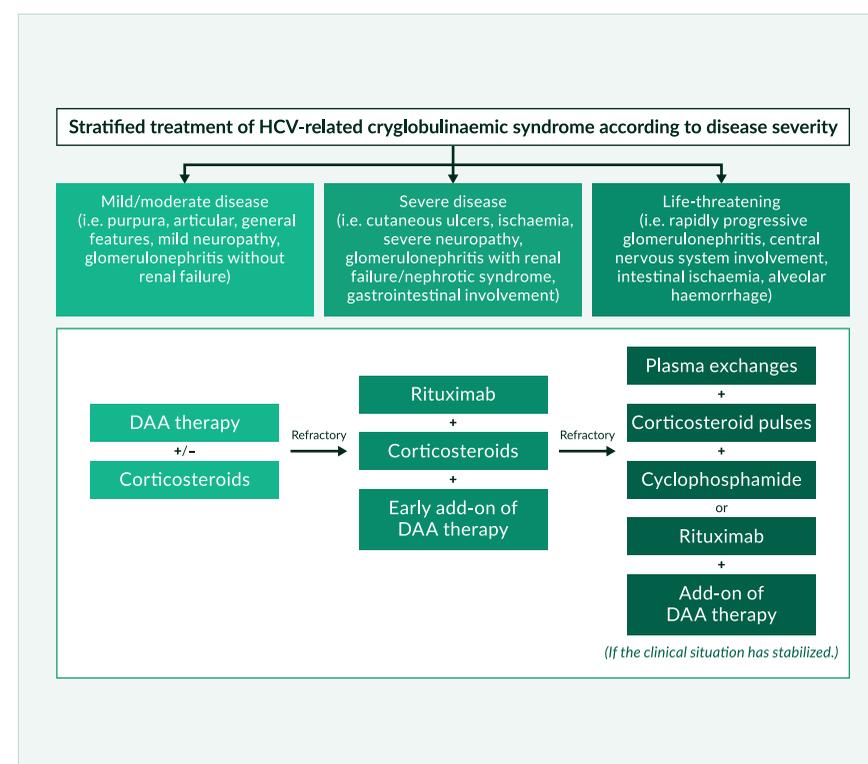


Figure 3. Therapeutic algorithm for symptomatic HCV-related mixed cryoglobulinaemia (Ramos-Casals 2012). Antiviral therapy, i.e., combination therapy with direct acting antivirals (+/- ribavirin), is regarded as first-line therapy in cases of mild/moderate manifestations. In case of contraindications, patients should be treated primarily with corticosteroids. Long-term therapy with corticosteroids may result in elevation of viral load and progression of hepatic disease. In light of this, rituximab represents an attractive alternative, because in this case, drug-induced viral load escalation is minor. In patients with severe manifestations, treatment should focus on both DAA therapy and immunosuppression (\pm plasmapheresis). Due to its excellent immunosuppressive properties and relatively mild side effect profile, use of rituximab should be favoured. Therapy-refractory cases require individual treatment according to the particular center's experience.

As eradication of *Helicobacter pylori* may lead to complete remission of MALT lymphoma, antiviral therapy can lead to regression of low-grade NHL in patients with HCV-related malignant lymphoproliferative disorders. Combination therapy with direct acting antivirals (+/- ribavirin) should be regarded in such cases as first-line therapy (Giannelli 2003, Vallisa 2005). Remission of the hematologic disorders is closely associated with virologic response or rather achievement of sustained virologic response.

Table 4. Overview of selected studies evaluating different antiviral but also rituximab-based treatment strategies of cryoglobulinaemia-related disorders in patients with chronic HCV infection

Author	Patients	Treatment	Result
Sansonno	N=20 MC vasculitis and peripheral neuropathy resistant to IFN α monotherapy	Rituximab 375 mg/m ² /4x/wk	16 patients with complete clinical response; 12 sustained response throughout follow-up. Viraemia increases in responders
Saadoun	N=16 MC vasculitis in relapsers or non-responders to IFN α /PEG-IFN α + RBV	Rituximab 375 mg/m ² /4x/wk; PEG-IFN α 1.5 ug/kg/wk + RBV (600–1200 mg/d) for 12 months	10/16 report complete clinical response; CGs and HCV RNA undetectable in responders
Roccatello	N=6 MC systematic manifestations predominantly renal (5/6)	Rituximab 375 mg/m ² /4x/wk + rituximab 375 mg/m ² 1 month and 2 months later	Decrease of cryocrit and proteinuria at months 2, 6, 12
Sise	N=12 HCV-related MC with systemic vasculitis; renal manifestation (N=7)	Sofosbuvir + ribavirin (12 or 24 wks) or sofosbuvir + simeprevir (12 wks)	Overall SVR12 83%; 86% SVR12 in patients with kidney involvement (6/7); decrease of CG levels in 89%
Gragnani	N=44 HCV-related MC with active cryoglobulinemic vasculitis	Sofosbuvir monotherapy or + simeprevir or + daclatasvir or + ledipasvir (+/- ribavirin)	SVR12 and SVR24: 100%, MC response at SVR24: 36% full complete response, 41% complete response, 23% partial response
Bonacci	N=30 MC vasculitis	Ombitasvir + paritaprevir + ritonavir + dasabuvir or Ledipasvir + sofosbuvir or simeprevir + daclatasvir or grazoprevir + elbasvir and other regimens	SVR24: 97% (29/30); complete clinical response 22/30, CGs negative 12/30
Saadoun	N=41 MC vasculitis	Sofosbuvir + daclatasvir (12 or 24 wks)	SVR12: 100% (41/41); 90,2% complete clinical response (37/41); no CGs detectable at week 36 in 50%

Author	Patients	Treatment	Result
Shahin	N=24 Arthropathia and vasculitis	Sofosbuvir + ribavirin (N=17) or sofosbuvir + simeprevir (n=7)	SVR24: 100% (24/24); Tender Joint Count and pain visual analogue scale improved significantly; vasculitis clinically improved in all patients
Cacoub	N=148 HCV-related CV	Sofosbuvir + daclatasvir or sofosbuvir + RBV or sofosbuvir + ledipasvir or sofosbuvir + simeprevir	SVR: 97,2 %; complete clinical response 72,6 % (106/148); partial clinical response 22,6 % (33/148); no clinical response 4,8 % (7/148)

Treatment of HCV-infected patients with high-grade NHL should be based on cytostatic chemotherapy according to current guidelines from hematologic societies. HCV infection does not constitute a contraindication for cytostatic chemotherapy. Unlike HBV infection, antiviral prophylaxis before chemotherapy introduction is not obligatory. Chemotherapy may lead to a substantial increase in viremia. Consecutive exacerbation of the infection, making discontinuation of chemotherapy mandatory, is however unlikely to occur. However, treatment-related liver toxicity is more frequent in HCV-positive NHL and is often associated with severe hepatic manifestations (Besson 2006, Arcaini 2009). Current data suggest that antiviral treatment may serve as maintenance therapy for achieving sustained remission of NHL after chemotherapy completion (Giannelli 2003). Recent data also show the concomitance of DAA therapy and chemotherapy to be safe and effective in influencing remission of high-grade NHL in HCV patients with a higher disease-free survival in treated HCV patients with aggressive NHL (Persico 2018).

Further hematological manifestations

HCV-associated thrombocytopenia

Thrombocytopenic conditions (platelet counts below 150 x 10³/uL) are often observed in patients with chronic hepatitis C and result mainly from advanced liver fibrosis and manifest cirrhosis with portal hypertension and consecutive splenomegaly (Wang 2004). Lack of hepatic-derived thrombopoietin can inter alia be recognised as an important causal factor (Afdhal 2008). As HCV RNA can be abundant in platelets (Takehara 1994) and megakaryocytes of thrombocytopenic patients, direct cytopathic involvement of HCV can be hypothesised (Bordin 1995, De Almeida 2004). Furthermore, it has been suggested that exposure to HCV may be a

causative factor for the production of platelet-associated immunoglobulins, inducing thrombocytopenia through a similar immunological mechanism to that operating in immune thrombocytopenic purpura (ITP) (Aref 2009). There is a high HCV prevalence in patients with ITP (García-Suaréz 2000), and these patients exhibit diverse characteristics to HCV negative patients with ITP, which supports the hypothesis of direct viral involvement in the development of thrombocytopenia (Rajan 2005).

There is no consensus regarding the optimum treatment of HCV-related ITP. Along with classical therapeutic approaches such as corticosteroids, intravenous immunoglobulins and splenectomy, antiviral therapy constitutes another option. A substantial increase of platelets after application of antiviral therapy is registered in a significant percentage of patients with HCV-related ITP (Iga 2005), although evidence from further studies is required to confirm this hypothesis. However, caution is recommended in thrombocytopenic patients treated with PEG-IFN α plus ribavirin, as significant aggravation of HCV-related ITP may occur on this regimen (Fattovich 1996). On the other hand, long-term use of steroids or immunosuppressive drugs is limited by an increased risk of fibrosis progression or a substantial elevation of virus, respectively.

An orally active thrombopoietin receptor agonist, eltrombopag, may be used in thrombocytopenic HCV patients. Its efficacy has been documented in patients with HCV-related ITP (Bussel 2007) as well as in HCV positive patients suffering from thrombocytopenia due to cirrhosis (McHutchison 2007), although, in another study treating patients with eltrombopag in combination with PEG-IFN α and ribavirin, portal vein thrombosis was observed in a number of patients as an unexpected complication (Afdhal 2011). FDA recently approved a new indication for eltrombopag for patients with thrombocytopenia with chronic hepatitis C to allow the initiation and maintenance of interferon-based therapy. However, in countries with access to interferon-free regimens this indication may become obsolete as direct acting antivirals do not aggravate thrombocytopenia.

In case of refractory disease or aggravation during the course of antiviral therapy, rituximab should be considered (Weitz 2005).

HCV-related autoimmune hemolytic anaemia

Interpretation of autoimmune hemolytic anaemia (AHA) as a possible EHM is based mainly on a few well-documented case reports (Chao 2001, Fernández 2006, Srinivasan 2001). AHA has been frequently observed in HCV patients treated with IFN α with and without ribavirin and consequently recognised as a possible side effect of antiviral treatment (De la Serna-Higuera 1999, Nomura 2004). Recently, a large-scale epidemiological

study confirmed a high incidence of AHA in HCV patients undergoing antiviral treatment. However, the incidence rate of AHA in treatment-naïve HCV patients was statistically insignificant (Chiao 2009). Therefore, for the time being, there is little evidence for regarding AHA as a possible EHM of chronic HCV infection.

HCV-related glomerulonephritis

Data from national cohort studies show that HCV-infected patients have a higher prevalence of chronic kidney disease (CKD) and especially diabetes, hyperlipidemia and cirrhosis showed to increase the risk for CKD in HCV-infected individuals (Chen 2014). Moreover, presence of HCV has been shown to be associated with deterioration of kidney function. A large cohort study with over 100.000 HCV infected US veterans and over 900.000 non-HCV controls found an almost two-fold increased risk of developing end-stage renal disease in HCV-infected individuals compared to non-HCV-infected controls (Molnar 2015). According to the recently revised KDIGO (Kidney Disease: Improving Global Outcomes) guidelines, all patients with chronic kidney disease should be tested for the presence of HCV infection and all HCV patients should be assessed for kidney damage (Kidney International Supplements 2018).

Glomerulonephritis (GN) constitutes a rare extrahepatic complication of chronic HCV. Predominant manifestations are cryoglobulinemic or non-cryoglobulinemic membranous proliferative GN and mesangioproliferative GN. Far less common is membranous nephropathy (Arase 1998). Other forms of GN do not correlate significantly with HCV infection (Daghestani 1999). Microhematuria and proteinuria are among the most frequent medical findings in patients with membranous proliferative GN. Approximately 50% of patients exhibit a mild renal insufficiency. 20-25% may present an acute nephritic syndrome (hematuria, hypertension and proteinuria), as in 25% of patients nephrotic syndrome represents the initial manifestation. In contrast, >80% of patients with HCV-related membranous nephropathy suffer primarily a nephrotic syndrome (Doutrelepon 1993, Rollino 1991). The mesangioproliferative form proceeds mostly asymptotically, with typical findings such as hematuria and proteinuria often missing (McGuire 2006).

The pathomechanism of renal impairment is yet not fully understood. It can be hypothesised that glomerular injury is primarily caused by a deposition of circulating immunocomplexes containing anti-HCV antibodies, HCV antigens and complement factors. Formation and deposition of such immunocomplexes occurs also in the absence of CGs. HCV proteins in glomerular and tubulointerstitial structures are immunohistologically detectable in approximately 70% of patients with chronic HCV (Sansonnò

1997). Further possible pathomechanisms of glomerular injury encompass formation of glomerular autoantibodies, glomerular impairment due to chronic hepatic injury, or IgM overproduction with consecutive glomerular IgM deposition as a result of HCV-triggered cryoglobulinaemia type II. GN prevalence in HCV patients is estimated at 1.4% and is comparably high due to its prevalence among blood donors (Paydas 1996).

HCV-induced GN has mostly a benign prognosis (Daghestani 1999). 10-15% of patients with nephritic syndrome experience spontaneous complete or partial remission. Frequently persisting mild proteinuria exhibits no tendency to progression. It is estimated that only approximately 15% of the patients with HCV-related GN develop terminal renal failure requiring dialysis (Tarantino 1995). Nevertheless, presence of kidney impairment is considered to be a negative prognostic factor for long-term survival (Ferri 2004).

Patients with HCV-related GN should be primarily treated with direct acting antivirals. In cases of mild renal impairment, sustained viral response normally leads to amelioration of proteinuria or even full remission of GN. With high baseline viremia and advanced renal insufficiency, antiviral therapy is subject to certain limitations (Sabry 2002). Despite amelioration of proteinuria achieved after antiviral therapy, significant improvement of renal function is often lacking (Alric 2004). Ribavirin dosage must be cautiously adjusted to glomerular filtration rate (GFR), in order to mainly prevent ribavirin accumulation with consecutive hemolytic anemia (Fabrizi 2008). RBV-induced hemolytic anemia was efficiently treated by administration of erythropoietin and erythrocyte concentrates (van Leusen 2008). As determination of RBV blood levels is not an established laboratory procedure, implementation of such a therapeutic approach in clinical routine remains arduous. Renal impairment was observed as an adverse event associated with the use of telaprevir and boceprevir (Mauss 2014). In patients with severe renal insufficiency (eGFR <30 ml/min), data for the use of simeprevir, ledipasvir, sofosbuvir and other direct acting antivirals are emerging. Although the use of sofosbuvir is currently not recommended in patients with eGFR <30 ml/min, safety and efficacy of full dose sofosbuvir regimen was recently shown in this population (Hundemer 2015). The 3 D regimen, comprising of paritaprevir/ritonavir/ombitasvir + dasabuvir as well as the NS5A inhibitor daclatasvir have been safely administered in patients with severe renal insufficiency (GFR <30 ml/min) due to the predominant biliary elimination of these drugs (Fabrizi 2015). The regimen of grazoprevir plus elbasvir has been evaluated in a large cohort of 235 HCV patients with serious renal involvement. These drugs have a renal elimination rate less than 1%. Therefore, the 3 D regimen as well as grazoprevir plus elbasvir are currently the only approved combination therapies in end-stage renal disease (GFR <15 ml/min) (Cacoub 2016). However, therapy with glecaprevir/

pibrentasvir is also possible and should be favoured and, if available, replace the 3 D regimen. Data from a recent cohort of 101 HCV patients with chronic kidney disease stage 3b, 4 or 5 treated with glecaprevir/pibrentasvir showed an SVR rate of 97% (98/101) with no virologic failure and no safety signals, which support the use of this regimen in HCV patients with advanced and end-stage renal disease (Lawitz 2019). Currently, grazoprevir plus elbasvir is the recommended therapeutic regimen in HCV type 1 patients with severe or terminal renal insufficiency (AFEF 2016).

Fulminant manifestations with impending acute renal failure can be treated with corticosteroids, cyclosporine, and other immunosuppressive drugs such as cyclophosphamide and eventually plasmapheresis (Garini 2007, Margin 1994). In case of simultaneous bone marrow B cell infiltration and/or resistance to conventional therapy, application of rituximab is indicated (Roccatello 2004). Rituximab may be used as an alternative first line therapy in severe renal manifestations (Roccatello 2008). Antiviral and immunosuppressive therapy should always be supplemented with ACE inhibitors or AT1 receptor antagonists (Kamar 2006).

Endocrine manifestations

Thyroid disease is found more commonly in patients with chronic HCV infection than in the general population. About 13% of HCV-infected patients have hypothyroidism and up to 25% have thyroid antibodies. Thyroid disease is found to be one of the most common endocrine disorders in chronic HCV infection (Antonelli 2004). The hypothesis of chronic immune system stimulation in patients with MC who also presented autoimmune thyroiditis is underlined by the elevated levels of CXCL9, CXCL10 and CXCL11 chemokines as well as higher IL-6 levels which can be found in these patients (Ferri 2017). *In vitro* experiments showed that Hepatitis C virus is even capable of infection of human thyroid cells due to membrane expression of the HCV receptor CD81 (Blackard 2012). There is also evidence that IFN α may induce thyroid disease or unmask preexisting silent thyroidopathies (Graves disease, Hashimoto thyroiditis) (Prummel 2003). Furthermore, the presence of thyroid autoantibodies increases the risk of developing an overt thyroiditis during interferon-based therapy.

The association between chronic HCV infection and development of insulin resistance and diabetes mellitus has been discussed in the past (Knobler 2000, Mason 1999, Hui 2003, Mehta 2003). A meta-analysis of retrospective and prospective studies confirms a higher risk for the development of diabetes mellitus type II in patients with chronic HCV infection (OR=1.68, 95% CI 1.15-2.20) (White 2008). Similar results were reported in a recent prospective study from Taiwan with more than 21000

participants (hazard ratio 1.53, 95% CI 1.29–1.81) (Lin 2016). Viral induction of insulin resistance seems to be HCV-specific, as prevalence of diabetes mellitus in HBV-infected patients is significantly lower (White 2008, Imazeki 2008). The pathomechanism of HCV-induced insulin resistance is yet not fully understood. It has been suggested that the appearance of insulin resistance could correlate with certain genotypes of HCV. By altering host lipid metabolism to favour its own replication, HCV infection leads to hepatic steatosis especially in HCV type 3 infections. Moreover, the occurrence and severity of steatosis correlates with viral load and response to interferon-based therapy in HCV type 3 patients (Rubbia-Brandt 2001). Furthermore, HCV-dependent upregulation of cytokine suppressor SOC-3 may be responsible for the induction of cell desensitisation towards insulin. Peroxisome proliferator-activated receptor- γ coactivator 1 α is induced after HCV infection, thereby upregulating gluconeogenesis and providing a potential target for treatment (Shlomai 2012). Insulin resistance in turn represents an independent risk factor for progression of liver fibrosis and lower SVR in patients with chronic HCV infection (Moucari 2008, Kawaguchi 2004). As the development of insulin resistance or type 2 diabetes mellitus may contribute to the progression of liver disease during HCV infection, some international guidelines recommend that antiviral treatment should be promptly initiated in HCV infected patients with insulin resistance or diabetes (Cacoub 2018).

A causal association is backed up by studies demonstrating that antiviral therapy resulting in SVR correlates with improved diabetic metabolic status and partial resolution of insulin resistance (Kawaguchi 2007, Zhang 2012).

There is growing evidence that a majority of HCV-infected patients also suffer from vitamin D deficiency. Recent clinical data show higher vitamin D levels as an independent predictive factor of SVR following antiviral therapy (Cholongitas 2012). Because of its anti-inflammatory and anti-fibrotic effects, vitamin D supplementation might therefore protect against progression of liver disease (Rahman 2013). HCV-infected patients are at a significantly higher risk of developing osteoporosis and osteoporosis-associated bone fractures. Chronic HCV infection leads to a reduction in bone density due to imbalance in calcium and vitamin D homeostasis and a decreased synthesis of insulin-like growth factor-1 (IGF-1) (Marek 2015). Additionally, it has been shown that bone density decreases with progression of liver fibrosis due to HCV (Lin 2012). In another study with relatively young HCV patients (aged 40–60 years) without advanced fibrosis, 42% had reduced bone density and 12% osteoporosis (Lai 2015). Furthermore, a large cohort study from Taiwan with over 10,000 HCV patients and 41,000 controls reported a 1.33-fold increased incidence of osteoporosis in the HCV group versus controls (Chen 2015). Another study from Denmark compared the overall incidence of bone fractures from over 12,000 HCV patients and 60,000 matched controls. HCV

patients had a 2.15-fold increased incidence of bone fractures. There was no significant difference in fracture incidence between patients with active versus successfully treated chronic hepatitis C (Hansen 2014).

Finally, a link between HCV, growth hormone (GH) insufficiency and low insulin-like growth factor (IGF1) has been hypothesised. Reduced GH secretion could be the result of a direct inhibitory effect of HCV infection at the level of the pituitary or hypothalamus (Plöckinger 2007).

Cardiovascular manifestations

There is increasing evidence that chronic HCV infection may also increase the risk for cardiovascular disease. Many direct and indirect factors which contribute to the development of atherosclerosis and cardiovascular disease have been identified, such as insulin resistance, oxidative stress, lipopolysaccharides and proinflammatory cytokines (see Figure 4, Adinolfi 2018). A nearly 1.65-fold increase in cardiovascular disease-related mortality was reported in a large meta-analysis of observational studies. The risk for cerebrovascular or cardiovascular disease in HCV infection was even 1.71-fold higher when additional risk factors like diabetes and arterial hypertension were present (Petta 2016). Moreover, a cohort study from Taiwan found chronic hepatitis C to be an independent predictor of stroke (Negro 2014). Another data from a recent American study with a cohort of 1434 HCV-positive participants showed a significantly higher incidence of coronary heart disease events in patients with detectable HCV RNA than in those who were HCV RNA negative (Pothineni 2014). A 1.43-fold increased risk of developing peripheral arterial disease (PAD) in chronically HCV infected Taiwanese patients as compared to uninfected controls might be suggestive that PAD can be regarded as an extrahepatic manifestation of chronic HCV infection (Hsu YH 2015). Potential pathomechanisms might include metabolic factors such as insulin resistance with hyperglycemia, endothelial dysfunction and inflammation leading to damage of vessels and instability of plaques but also other systemic processes associated with the chronic inflammatory state and potentially leading to atherosclerosis have to be considered (Negro 2015). In addition, chronic HCV infection is independently associated with peripheral arterial stiffness. Compared to controls, HCV patients had increased arterial stiffness with lower compliance indices in a cohort of 221 participants (Chou 2017).

However SVR in HCV infection results in an increase in total and LDL cholesterol, which seems contradictory to the data reported above (Mauss 2017). The interaction of terminating a possibly proatherogenic chronic infection which results in an increase of proatherogenic lipids requires further research to draw a clinical relevant conclusion.

First data on DAA therapy effects in patients with advanced liver fibrosis and compensated cirrhosis revealed a significant improvement in carotid atherosclerosis measured by intima media thickness and carotid thickening, although data about major cardiovascular outcomes under DAA therapy are still absent (Petta 2018). A recent nationwide cohort study from Taiwan demonstrated a significant reduction in the incidence of end-stage renal disease, acute coronary syndrome and ischemic stroke in HCV treated patients compared to untreated controls. Treated patients also showed a significant improvement in non-liver death-related survival (see Figure 5, Hsu YC 2015). The current available data show that the eradication of HCV by DAA therapy is associated with an improvement of atherosclerosis and metabolic conditions which may lead to the development of cardiovascular disease at least in the short term and at early disease stages, yet further studies are needed to clarify these results (Adinolfi 2018).

Occasionally, chronic HCV infection has been seen in association with other cardiac pathologies such as chronic myocarditis and dilated/hypertrophic cardiomyopathy. Pathogenesis seems to rely on genetic predisposition and is assumed to be immunologically triggered (Matsumori 2000).

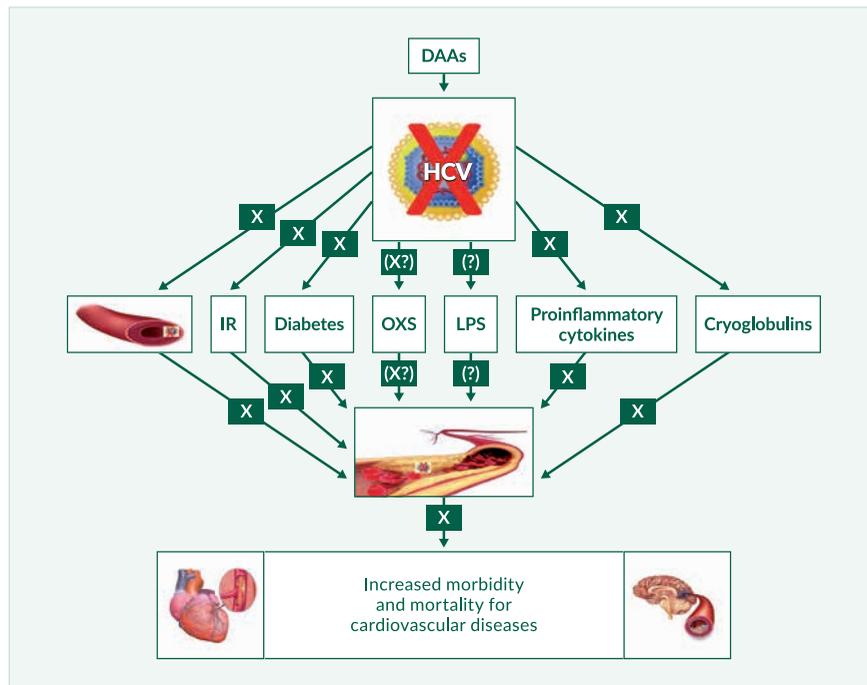


Figure 4. Direct and indirect factors associated with the development of atherosclerosis and cardiovascular disease in HCV patients and the possible effect of viral clearance by DAA therapy. (IR: insulin resistance, OXS: oxidative stress, LPS: lipopolysaccharide, according to Adinolfi LE et al. 2018).

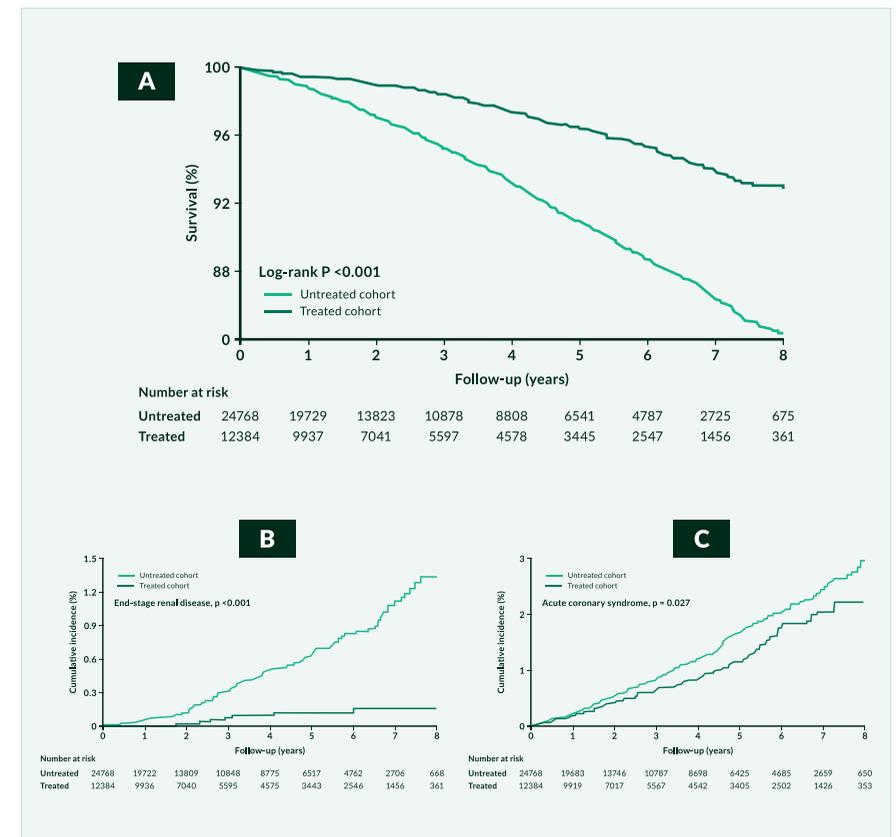


Figure 5. A) Overall mortality survival curves for non-liver related causes in treated and untreated patients with chronic HCV infection B) and C) Cumulative incidence of extrahepatic outcomes between treated and untreated HCV cohorts for end-stage renal disease and acute coronary syndrome (according to Hsu YC et al. 2015).

Central nervous manifestations

Numerous central nervous manifestations have been described in association with HCV infection. Cryoglobulinemic or non-cryoglobulinemic vasculitis of cerebral blood vessels may be responsible for the relatively high prevalence of both ischemic and hemorrhagic strokes in young HCV positive patients (Cacoub 1998). Transverse myelopathies leading to symmetrical paraparesis and sensory deficiency have been observed (Aktipi 2007).

Furthermore, chronic HCV infection is associated with significant impairment of quality of life. 35-68% of HCV patients suffer from chronic fatigue, subclinical cognitive impairment and psychomotor deceleration. Symptoms of depression are evident in 2-30% of HCV patients examined (Perry 2008, Forton 2003, Carta 2007). Psychometric as well as functional magnetic resonance spectroscopy studies suggest altered neurotransmission in HCV-infected patients (Weissenborn 2006, Forton

2001). In addition, significant tryptophan deficiency is detectable in patients with chronic HCV infection. Deficiency of tryptophan-derived serotonin is likely to favour an occurrence of depressive disorders. There is evidence to suggest that antiviral therapy can lead to elevation of tryptophan blood levels and thus contribute to amelioration of depressive symptoms in HCV patients (Zignego 2007c).

While the aetiology of cognitive dysfunction in HCV patients is not completely understood, it is hypothesised that the virus has a direct neurotoxic effect by entering the CNS via the PBMCs. This may be accompanied by an indirect neurotoxic effect via cerebral and/or systemic inflammation, for example increased pro-inflammatory cytokines over many years of infection. These cytokines may cross the blood-brain barrier and contribute to cognitive disorders (Senzolo 2011). More recent studies indicate that brain microvascular endothelial cells serve as a preferential site of HCV tropism and replication and that alteration of the blood-brain barrier could lead to activation of microglia and entry of inflammatory cytokines (Fletcher 2012). Supporting new data shows evidence for the affection of mostly memory tasks in HCV-infected children with significant correlations between endogenous cytokines like IL-6 and IFN α and cognitive dysfunction (Abu Faddan 2014).

Significant improvements of mental health and central nervous manifestations (i.e. fatigue and health related quality of life) have been consistently demonstrated during interferon-free DAA-based regimens (Younossi 2014a, Younossi 2014b). A recent study evaluated a significant improvement in health related quality of life due to DAA therapy in patients with cryoglobulinemic vasculitis (Saadoun 2015b). According to recent guidelines (EASL 2018), the presence of such EHM (i.e. debilitating fatigue) should be regarded as a priority treatment indication with DAA, even in the absence of significant liver damage (Negro 2015).

Dermatologic manifestations

A multitude of cutaneous disorders has been sporadically associated with chronic HCV infection (Hadziyannis 1998). Epidemiologic studies have confirmed the existence of a strong correlation between the sporadic form of porphyria cutanea tarda (PCT) and HCV, though the presence of HCV in PCT patients seems to be subject to strong regional factors. Indeed, HCV prevalence in PCT patients is above 50% in Italy, while only 8% in Germany (Fargion 1992, Stölzel 1995). Because therapy with PEG-IFN and ribavirin may exacerbate the cutaneous manifestations in PCT patients, these individuals might strongly benefit from therapy with DAAs (Negro 2015).

Evidence of a close association between HCV and lichen planus was

provided by studies performed in Japan and southern Europe (Nagao 1995, Carrozzo 1996), yet these observations do not apply to all geographic regions (Ingafou 1998). HLA-DR6 has been recognised as a major predisposing factor for development of lichen planus in HCV positive patients. One hypothesis suggests that geographical fluctuation of HLA-DR6 is responsible for the diverse prevalence among HCV patients (Gandolfo 2002). A recent study from Japan showed seven HCV patients with oral lichen planus who were treated with daclatasvir and asunaprevir for 24 weeks. In addition to the SVR24 rate of 100%, the cutaneous manifestations disappeared in four and improved in the remaining three subjects, underlining the close association between replicative HCV infection and oral lichen planus (Nagao 2016).

Recent data from a large cohort of HCV-infected patients showed a higher incidence and mortality for several types of non-liver cancers in individuals with HCV compared to the general population such as pancreas, lung, kidney and rectum malignancies. However it remains elusive if higher smoking rates in the observed HCV cohort could have been a possible confounding factor (Allison 2015). New data from a french population showed only hematological malignancies and oral cancer to be at higher risk in HCV patients compared to the general population. Especially HCV cirrhosis was associated with a higher age-adjusted incidence of non-liver cancers, even after achieving SVR (Allaire 2018).

Idiopathic pulmonary fibrosis (IPF) may potentially be an EHM, as prevalence of anti-HCV in patients with this disease is notably high (Ueda 1992). Interestingly, alveolar lavage in therapy-naïve HCV patients yielded frequent findings consistent with a chronic alveolitis. Alveolar lavage in the same patients after completion of antiviral therapy showed a remission of inflammatory activity (Yamaguchi 1997). Involvement of CGs in the genesis of IPF is also probable (Ferri 1997).

Several ocular abnormalities such as sicca syndrome due to reduced lacrimation as well as a peripheral corneal ulceration which is called Mooren's ulcer have been reported in association with HCV infection, although the pathogenesis of such abnormalities is not completely understood (Wilson 1993, Tang 2016).

Miscellaneous manifestations

Recent data from a large cohort of HCV-infected patients showed a higher incidence and mortality for several types of non-liver cancers in individuals with HCV compared to the general population such as pancreas, lung, kidney and rectum malignancies. However it remains elusive if higher smoking rates in the observed HCV cohort could have been a possible confounding factor (Allison 2015). New data from a french population

showed only hematological malignancies and oral cancer to be at higher risk in HCV patients compared to the general population. Especially HCV cirrhosis was associated with a higher age-adjusted incidence of non-liver cancers, even after achieving SVR (Allaire 2018). A higher prevalence of extrahepatic malignancies than expected was also found in a cohort of 431 HCV patients who were treated with DAAs in Israel. The incidence of lymphoma was about 100 times higher than in the general population. Also, the incidence of pancreatic, endocervix, breast and squamous cell lung carcinoma increased 29-, 1.6-, 136- and 2,5-fold, respectively. One possible cause might be down-regulating of immune response after successful DAA therapy with higher vulnerability to the development of neoplasia, yet larger multicentered studies are required to confirm these results (Khouri 2019).

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Several ocular abnormalities such as sicca syndrome due to reduced lacrimation as well as a peripheral corneal ulceration which is called Mooren's ulcer have been reported in association with HCV infection, although the pathogenesis of such abnormalities is not completely understood (Wilson 1993, Tang 2016).

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14. Management of HBV/HIV coinfection

Stefan Mauss and Jürgen Kurt Rockstroh

Introduction

The prevalence and transmission routes of HBV coinfection in the HIV+ population vary substantially by geographic region (Alter 2006, Konopnicki 2005). In the United States and Europe the majority of HIV positive gay men have evidence of past HBV infection, and 5–10% show persistence of HBs-antigen, with or without replicative hepatitis B as defined by the presence of HBV DNA (Konopnicki 2005). Overall, rates of HBV/HIV coinfection are slightly lower among intravenous drug users compared to gay men and much lower among people infected through heterosexual contact (Núñez 2005).

In endemic regions of Africa and Asia, the majority of HBV infections are transmitted vertically at birth or before the age of 5 through close contact within households, medical procedures and traditional scarification (Modi 2007). The prevalence among youth in most Asian countries has substantially decreased since the introduction of vaccination on nationwide scales (Shepard 2006). In Europe, vaccination of children and members of risk groups is promoted and reimbursed by health care systems in most countries.

The natural history of hepatitis B is altered by simultaneous infection with HIV. Immune control of HBV is negatively affected leading to a reduction of HBs-antigen seroconversion. If HBV persists, the HBV DNA levels are generally higher in HIV positive patients not on antiretroviral therapy (Bodsworth 1989, Bodsworth 1991, Hadler 1991). In addition, with progression of cellular immune deficiency, reactivation of HBV replication despite previous HBs-antigen seroconversion may occur (Soriano 2005). However, after immune recovery due to antiretroviral therapy, HBe-antigen and HBs-antigen seroconversion occur in a higher proportion of patients compared to HBV monoinfected patients treated for chronic hepatitis B (Schmutz 2006, Piroth 2010, Kosi 2012).

In untreated HIV infection, faster progression to liver cirrhosis is reported for HBV/HIV-coinfected patients (Puoti 2006). Moreover, hepatocellular carcinoma may develop at an earlier age and is more aggressive in this population (Puoti 2004, Brau 2007).

Being HBV-coinfected results in increased mortality for HIV positive

individuals, even after the introduction of effective antiretroviral therapy (ART), as demonstrated by an analysis of the EuroSIDA Study, which shows a 3.6-fold higher risk of liver-related deaths among HBsAg positive patients compared to HBsAg negative individuals (Konopnicki 2005, Nikolopoulos 2009) (Figure 1). In the Multicentre AIDS Cohort Study (MACS), an 8-fold increased risk of liver-related mortality was seen among HBV/HIV-coinfected compared to HIV-monoinfected individuals, particularly among subjects with low nadir CD4+ cell counts (Thio 2002). Even at present, despite the widespread use of tenofovir, HBV/HIV coinfection is still associated with an increased morbidity (Crowell 2014), and liver-related deaths in HBV/HIV-infected patients still do occur (Rosenthal 2014).

The beneficial impact of treatment of HBV in HBV/HIV coinfection was first demonstrated by data from a large cohort showing a reduction in mortality with lamivudine treatment compared to untreated patients (Puoti 2007). This result is even more remarkable because lamivudine is the least effective HBV polymerase inhibitor due to the rapid development of drug resistance. In general, because of its limited long-term efficacy, lamivudine monotherapy cannot be considered as appropriate therapy for either mono HBV infection or HBV/HIV coinfection (Matthews 2011).

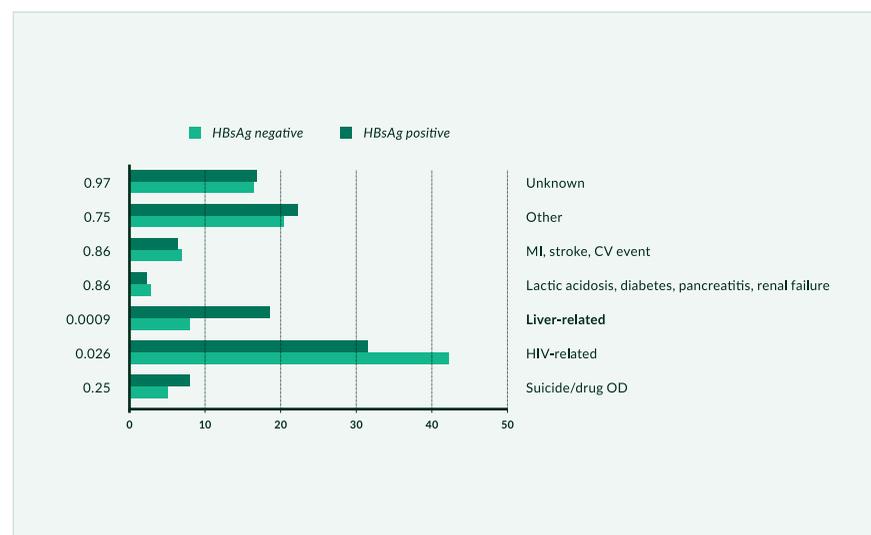


Figure 1. Association of HBV/HIV coinfection and mortality (Konopnicki 2005). More than one cause of death allowed per patient; p-values from chi-squared tests.

In addition, two large cohort studies (EuroSIDA and MACS) plus data from HBV monoinfection studies showing a reduction in morbidity and mortality established the need to treat chronic hepatitis B in HBV/HIV-coinfected patients.

Treatment of chronic hepatitis B in HBV/HIV-coinfected patients on antiretroviral therapy

In general, starting hepatitis B therapy depends on the degree of liver fibrosis and the HBV DNA level. But as ART is now recommended for all HIV patients independent of CD4-count to reduce HIV-associated morbidity and mortality and to prevent HIV transmission, all HBV/HIV-coinfected patients are considered eligible for ART by current guidelines (e. g. EACS 2016). The previous complicated recommendations for how to treat chronic hepatitis B in patients without ART are now obsolete. As antiretroviral drugs that are also active against HBV can usually be used, interferon-based treatment of HBV is now rarely indicated. Data in the literature for HIV-coinfected patients on interferon therapy for HBV infection are limited and not very encouraging (Núñez 2003). In addition, intensified treatment studies combining pegylated interferon with adefovir or intensifying TDF therapy with pegylated interferon for one year showed no increase in HBV seroconversion rates (Ingiliz 2008, Boyd 2016).

In general, tenofovir is the standard of care for HBV in HIV-coinfected patients, because of its strong HBV polymerase activity and antiretroviral efficacy. Tenofovir has been a long-acting and effective therapy in the vast majority of treated HBV/HIV-coinfected patients (van Bömmel 2004, Matthews 2009, Martin-Carbonero 2011, Thibaut 2011). Its antiviral efficacy is not impaired in HBV/HIV-coinfected compared to HBV-monoinfected patients (Plaza 2013). No conclusive pattern of resistance mutations has been identified in studies or cohorts (Snow-Lampart 2011). These data are still valid at the end of 2016. In theory, resistance may occur in patients on long-term therapy, as with any other antivirals.

For patients with HBV DNA <2000 IU/mL and no relevant liver fibrosis, no specific ART regimen is recommended. However due to the favourable resistance profile a regimen including tenofovir is the first choice. When choosing an HBV polymerase inhibitor, complete suppression of HBV DNA is important to avoid the development of HBV drug resistance.

When HBV DNA is above 2000 IU/mL in HBV treatment naïve patients a combination of tenofovir plus lamivudine/emtricitabine to treat both infections is usually recommended. Even for patients who harbor lamivudine-, telbivudine- or adefovir-resistant HBV due to previous therapies this strategy stands. The recommendation to continue lamivudine/emtricitabine is based on delayed resistance to adefovir seen when doing so (Lampertico 2007), but the same effect has not been in combination with tenofovir (Berg 2010, Patterson 2011).

Initiating ART including tenofovir resulted in higher rates of HBe antigen loss and seroconversion as expected from HBV-monoinfected

patients (Schmutz 2006, Piroth 2010, Kosi 2012). This may be due to the additional effect of immune reconstitution in HBV/HIV coinfecting patients complicating the immunological control of HBV replication.

For patients with advanced liver fibrosis or liver cirrhosis a maximally active continuous HBV polymerase inhibitor therapy is important to avoid further fibrosis progression and hepatic decompensation and to reduce the risk of developing hepatocellular carcinoma. Tenofovir plus lamivudine/emtricitabine is the treatment of choice. If the results are not fully suppressive, adding entecavir should be considered (Ratcliffe 2011). A reduction in the incidence of hepatocellular carcinoma has been shown for patients on HBV polymerase inhibitors compared to untreated patients, strengthening the antiproliferative effects of suppressive antiviral therapy (Hosaka 2012).

Liver ultrasound is needed at least every six months, for early detection of hepatocellular carcinoma. In patients with advanced cirrhosis, esophagogastrosopy should be performed as screening for oesophageal varices. For patients with hepatic decompensation and full treatment options for HBV and who have stable HIV infection, liver transplantation should be considered as posttransplant life expectancy seems to be the same as for HBV-monoinfected patients (Coffin 2007, Tateo 2009). Patients with hepatocellular carcinoma may also be considered liver transplant candidates, although according to preliminary observations from small cohorts, the outcome may be worse than for HBV-monoinfected patients (Vibert 2008).

In prospective controlled studies, tenofovir was clearly superior to adefovir for the treatment of HBe antigen positive and HBe antigen negative patients (Marcellin 2008).

The acquisition of adefovir resistance mutations and multiple lamivudine resistance mutations may impair the activity of tenofovir (Fung 2005, Lada 2012, van Bömmel 2010), although even in these situations tenofovir retains sufficient activity against HBV (Berg 2010, Patterson 2011, Petersen 2012).

In lamivudine-resistant HBV the antiviral efficacy of entecavir in HIV-coinfecting patients is reduced, as it is in HBV monoinfection (Shermann 2008). Because of this and the property of tenofovir as a fully active antiretroviral, tenofovir-DF is the preferred choice in treatment-naïve HBV/HIV coinfecting patients who will use ART. The use of entecavir, telbivudine or adefovir as an add-on to tenofovir or other drugs in the case of not fully suppressive antiviral HBV therapy has not yet been studied in HBV/HIV coinfection. This decision should be made on a case-by-case basis.

Based on the history of ART, combination HBV therapy of tenofovir plus lamivudine/emtricitabine was expected to be superior to tenofovir monotherapy, in particular in patients with highly replicative HBV infection. However, this hypothesis has not as yet been supported by

studies (Schmutz 2006, Mathews 2008, Mathews 2009, Price 2013). There are data showing better viral suppression for entecavir and tenofovir-DF compared to entecavir monotherapy in highly replicative patients with HBV-monoinfection, but no such a study is available for a comparison with tenofovir monotherapy (Lok 2012).

In the case of HIV resistance to tenofovir, it is usually important to continue using tenofovir for HBV activity when switching to other ART. Discontinuation of the HBV polymerase inhibitor without maintaining the antiviral pressure on HBV can lead to necroinflammatory flares that can result in acute liver decompensation, particularly in patients with liver cirrhosis.

In 2015, tenofovir alafenamide (TAF) was approved as antiretroviral therapy in Europe and the US. TAF is a new formulation of tenofovir with lower plasma exposure of the active drug tenofovir compared to tenofovir diproxitil fumarate (TDF). TAF has not shown superior antiviral activity against HIV or HBV compared to TDF, but may offer advantages concerning long term toxicities involving bone and kidney over TDF (Agarwal 2015, Sax 2015). TAF can substitute TDF as HBV therapy in HBV/HIV-coinfecting patients (Gallant 2016). In November 2016, TAF was approved for HBV treatment in the US, followed by the approval in Europe in January 2017.

The potentially nephrotoxic effect of TDF is a concern. Although nephrotoxicity is rarely observed in HIV negative patients treated with TDF monotherapy (Heathcote 2011, Mauss 2011), renal impairment has been more frequently reported in HIV positive patients using TDF as a component in ART and may be associated in particular with the combined use of TDF and ritonavir-boosted HIV protease inhibitors (Mauss 2005, Fux 2007, Goicoechea 2008, Mocroft 2010). In addition, the recently approved cytochrome P450 3A inhibitor cobicistat can also increase creatinine levels. Regular monitoring of renal function in HBV/HIV-coinfecting patients including estimated glomerular filtration rate (eGFR) and assessment of proteinuria is necessary. In the case of a reduced eGFR, TDF should be substituted by TAF or should be dosed at a reduced frequency according to the label. In the case of significant proteinuria, TDF should also be replaced by TAF. Alternatively in specific situations in the case of tenofovir associated nephrotoxicity tenofovir can also be replaced by entecavir.

Conclusion

The number of available HBV polymerase inhibitors for chronic hepatitis B has increased substantially over the last few years. In general, the choice is confined to two mostly non-cross-resistant classes, the nucleotide and nucleoside compounds.

For HBV/HIV coinfecting patients, ART is indicated to treat both infections simultaneously. The HBV treatment of choice is tenofovir. Due to rapid development of resistance when HBV is not fully suppressed HBV monotherapy with either lamivudine or emtricitabine should not generally be considered. A combination of tenofovir plus lamivudine or emtricitabine as a primary combination therapy has theoretical advantages over tenofovir alone, but studies supporting this concept have not been published to date. However as tenofovir is combined with emtricitabine or lamivudine in most antiretroviral regimen today this seems to be a more theoretical argument and not reflected by reality.

In general, treatment of HBV as a viral disease follows the same rules as HIV therapy, aiming at full suppression of the replication of the virus to avoid the development of resistance. Successful viral suppression of hepatitis B results in inhibition of necroinflammatory activity, reversion of fibrosis, and most importantly a decrease in the incidence of hepatic decompensation and hepatocellular carcinoma.

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15. Management of HCV/HIV coinfection

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Epidemiology of HCV/HIV coinfection

HIV and HCV share transmission pathways which explain the high rate of coinfection with both viruses. It is estimated that at 2-15% of the 37.9 million people living with HIV globally had HCV coinfection (WHO 2020, Platt 2016). While both viruses are transmitted with high efficacy via blood-to-blood contact, HCV is less easily transmitted sexually. Thus, the prevalence of HCV coinfection within different countries, regions and populations is generally closely related to the prevalence of blood-borne HIV transmission – mainly among people who inject drugs (PWID).

However, this has changed for a subpopulation of HIV coinfecting gay men, as there is an ongoing epidemic of sexually transmitted HCV that is closely related to the use of some recreational drugs. A high incidence of HCV among HIV positive men who have sex with men (MSM) is reported from several major European cities including London, Paris, Amsterdam and Berlin as well as from the US, Canada, Australia and Taiwan. This documents that HCV may well be sexually transmitted through traumatic sex practices or at least transmitted in the context of sexual intercourse (e.g., intravenous administration of recreational drugs called “chemsex”) and should therefore also be taken into account as a sexually transmitted disease in this context resulting in regular sexual health screenings including HCV (Gotz 2005, Danta 2007, Vogel 2009, Vogel 2010, Matthews 2011, Schmidt 2011, Boesecke 2015).

In the past before the wide spread use of interferon free HCV therapy in HIV cohorts in Europe, Australia and the US, in average one out of four patients was coinfecting with HCV (Rockstroh 2004, Peters 2014). Higher prevalence rates of HCV/HIV coinfection, i.e. up to 70% have been reported for countries with the main transmission risk of intravenous drug use in Eastern European countries like Belarus and the Ukraine, and in Middle Eastern countries such as Iran (SeyedAlinaghi 2011). On the other hand, in Central European countries such as Belgium, Austria or Germany, where HIV is predominantly sexually transmitted, HCV coinfection rates were lower, i.e. between 10 and 15% (Rockstroh 2005, CDC 2011, Peters 2014). Similarly lower rates were reported for Australia (Jin 2009) and the UK (Turner 2009). Data from the US indicated that 25% to 35% of patients with

HIV were coinfecting with HCV (Singal 2009, CDC 2011), reflecting the contribution of at-risk populations such as prison inmates and a higher proportion of intravenous drug use to the overall numbers. 65-70% of HIV-infected prisoners in the US are coinfecting with HCV, in contrast to 18 to 25% of the overall US HIV positive population (Weinbaum 2005, CDC 2014). In Asia, coinfection rates of up to 85% have been reported among Chinese plasma donors whereas in countries with predominantly heterosexual HIV transmission like Thailand, coinfection rates are around 10% (Qian 2006). In Sub-Saharan Africa, where the primary route of transmission of HIV is sexual, HCV coinfection rates have so far been reported to be low.

However, since 2015, the prevalence of HCV/HIV coinfection has substantially decreased in HIV patients in continuous care, due to the availability of highly effective direct acting antiviral (DAA) HCV treatment in most high-income countries (Berenguer 2018, Boerekamps 2018, Sacks-Davis 2018, Braun 2018).

Vertical transmission of HCV is a concern. HCV is detected after birth in 4 to 8% of infants born to HCV positive mothers (Bevilacqua 2009). HCV/HIV coinfection increases the risk for transmission of both viruses and high levels of HCV viraemia in the mother increases the risk of perinatal HCV transmission (Zanetti 1995). However, the risk of HCV transmission is reduced to less than 1% in mothers with HCV/HIV coinfection receiving antiretroviral therapy (ART) and undergoing caesarean section.

In summary, the prevalence of HCV within the HIV positive population is far higher than in the HIV negative population. This highlights the importance of preventing further spread of HCV as one of the major comorbidities in HIV positive people. The average estimated risks of transmission are included in Table 1. Although sharing common routes of infection, both viruses are transmitted with varying efficacy depending upon the mode of transmission.

Table 1. Average estimated risks of transmission for HIV, HCV and HCV/HIV simultaneously

Mode of transmission	HIV	HCV	HCV / HIV coinfection
Perinatal	7-50%	1-7%	1-20%
Sexual contact*	1-3%	<1%	<4%
Needle stick injury	0.3%	<1%	Unknown

* For sexual contact the risk refers to cumulative exposure

Diagnosis of HCV in HIV coinfection

Detection of HCV antibodies via ELISA testing shows HCV exposure. However, the presence of HCV RNA is needed to prove active HCV infection. In acute hepatitis C, HCV RNA is detectable before the presence of HCV antibodies. In addition, in rare cases of HCV/HIV coinfection, the loss of HCV antibodies is observed in very advanced immune deficiency and does not necessarily indicate viral clearance (Cribier 1999). Therefore, a single negative HCV antibody ELISA does not necessarily exclude exposure to HCV in HIV positive patients, especially in severe immune deficiency. Additionally, a rise of liver transaminases (particularly ALT) may be more sensitive for detecting acute hepatitis C in HIV positive patients than repeated testing for the presence of HCV antibodies (Thomson 2009). In cases of suspected early acute hepatitis C testing for HCV RNA is therefore justified to establish the diagnosis.

Higher concentrations of HCV RNA are found in HIV positive individuals than in HIV negative patients with HCV mono-infection (Perez-Olmeda 2002). Interestingly, data from a cross-trial comparison showed that HIV positive patients were less likely to present with elevated serum ALT and clinical signs or symptoms of hepatitis than HIV negative patients (Vogel 2009). In observations patients with haemophilia, mean HCV RNA concentrations increased by 1 log₁₀ over the first two years after HIV seroconversion (Eyster 1994). Levels of HCV viraemia increase eight times faster in HIV positive compared to HIV negative individuals. The highest concentrations for HCV viraemia have been reported in people who subsequently developed liver failure.

Spontaneous clearance of HCV RNA seems to be less frequent in the setting of HCV/HIV coinfection (Thomson 2011). However, interestingly spontaneous clearance of HCV RNA has been observed in some patients with chronic HCV/HIV coinfection that experience significant immune reconstitution following initiation of ART, particularly in patients with the favourable IL28B CC genotype (Fialaire 1999, Thomson 2009, Stenkyist 2014).

The distribution of HCV genotypes in HIV positive patients reflects the route of transmission. Genotype 1b accounts for two-thirds of post-transfusion HCV infections and is the predominant genotype in people with haemophilia. In contrast, genotypes 1a and 3a are more common in people who inject drugs (PWID) (Pol 1994, Soriano 2008) and specific clusters of viruses are traced in gay men engaged in chemsex with HCV genotype 1a and 4 being the most frequent (Caro-Pérez 2017, Van de Laar 2009).

Natural course of HCV in HIV coinfection

Various studies have demonstrated that underlying HIV weakens the immune response to HCV, thereby reducing the chance of spontaneous HCV clearance. Data from the European cohorts of sexually transmitted acute HCV in HIV positive gay men suggest that with underlying HIV spontaneous resolution of HCV occurs in 10-20% of new HCV infections (Vogel 2010, Thomson 2010, Boesecke 2018). Genome-wide association studies identified single nucleotide polymorphisms (SNP) near the IL28B gene encoding for interferon lambda that comprise a crucial part of the innate immune defense against HCV in HCV monoinfection (Thomas 2009). Individuals with HCV monoinfection with the CC genotype were three times more likely to clear HCV RNA and to better respond to interferon-based HCV therapy compared with individuals with CT and TT genotypes (Rauch 2010, Grebely 2010, Nattermann 2011, Rallón 2011). Similar observations were made in individuals with HCV/HIV coinfection (Clausen 2010). Interestingly, these SNPs could explain differences in spontaneous clearance rates between different ethnicities as the frequency of the protective allele varies across ethnic groups. The prevalence of the CC genotype being lower in those of African origin compared to Asian patients, with Europeans being in-between (Thomas 2009).

Numerous large cohort studies have demonstrated that once chronic HCV is established, HIV leads to a faster progression of liver fibrosis due to the lack of critical CD4+ T cell responses against HCV (Danta 2008). In the American multicentre Haemophilic Cohort Study liver failure occurred in 9% of multi-transfused HCV/HIV coinfecting adult haemophiliacs without an AIDS-defining opportunistic infection or malignancy (Eyster 1993). In the same period, no cases of liver failure were observed in HCV positive haemophiliacs who were HIV negative. Subsequent studies confirmed the unfavourable course of HCV in haemophiliacs with HIV coinfection, particularly in the setting of progressive immunodeficiency and lower CD4 counts (Rockstroh 1996, Puoti 2000).

In addition, the time interval between HCV infection and development of cirrhosis is shorter in coinfection. Within the first 10 to 15 years of HCV infection, 15 to 25% of patients with HIV coinfection patients developed cirrhosis compared with only 2 to 6% of HIV negative patients (Soto 1997). Importantly, in men with haemophilia, mortality due to advanced liver disease occurs ten years earlier with HCV/HIV coinfection than with HCV monoinfection (Darby 1997). The incidence of hepatocellular carcinoma also seems to be higher in coinfecting patients (Giordano 2004).

Effect of HCV on HIV

While the impact of HIV on accelerating HCV-associated liver disease is clear, the impact of HCV on the course of HIV disease seems less pronounced. The Swiss Cohort first revealed a blunted CD4+ T cell response associated with a faster progression to AIDS after initiation of ART in patients with HCV/HIV coinfection (Greub 2000). However, an updated analysis with additional four-years of follow-up from the same cohort study could not confirm this initial observation. There were no significant differences with regard to CD4+ T cell count recovery between HIV positive patients with and without HCV coinfection (Kaufmann 2003). Subsequent studies found that no difference in CD4+ T cell count recovery was observed after adjusting for use of ART (Sulkowski 2002). Updated information from an analysis of the EuroSIDA cohort, after taking into account ongoing chronic (persistent HCV replication) and resolved (positive HCV antibodies but negative HCV RNA) HCV infection, confirm that no difference in CD4+ T cell count recovery is observed in patients with chronic HCV and detectable HCV RNA in comparison to patients with HIV monoinfection (Rockstroh 2005). In addition, data from the same cohort revealed that CD4+ T cell recovery in HIV positive patients with maximal suppression of HIV replication is not influenced by HCV serostatus in general or HCV genotype or level of HCV RNA in particular (Peters 2009).

Effect of ART on HCV

In patients with HCV/HIV coinfection starting ART, a transient increase in HCV RNA levels may occur at week 4, but thereafter, no significant changes in concentrations of HCV RNA happen over the first six months of treatment (Rockstroh 1998). However, a 1 log₁₀ decrease of HCV RNA has been reported in individuals with HCV/HIV coinfection individuals receiving more than 12 months of ART who have significant immune reconstitution (Rockstroh 2007). Moreover, case reports of HCV eradication has been reported in patients receiving ART following CD4 count recovery (Jones 2011). Other investigators, however, have not observed this decrease in HCV RNA (Grint 2013).

There is evidence that ART-induced immune reconstitution might reverse the unfavourable accelerated liver fibrosis progression in patients with severe HIV-associated immune deficiency (Verma 2006, Vogel 2009). Taking into account that liver disease progresses especially in those whose CD4+ T cell count drops below 200 cells/μL it is appealing to think that CD4 increases under ART may impact the further course of liver disease. In an early study of 162 individuals with HCV/HIV coinfection who underwent

liver biopsy, the use of protease inhibitors as part of their ART was associated with significantly lower rates of progression of liver fibrosis that could not be explained by other cofactors (Benhamou 2000). These findings were then confirmed by several cohort analyses which showed that individuals with HCV/HIV coinfection on ART had significantly lower liver-related mortality than patients receiving either suboptimal ART (only one or two nucleoside reverse transcriptase inhibitors) or no ART (Qurishi 2003).

In line with these observations, the amount of immune reconstitution achieved on ART was reported to affect the subsequent risk for developing hepatic decompensation in individuals with HCV/HIV coinfection (Pineda 2007). Those patients who experienced the highest CD4+ T cell count gain on ART were the least likely to develop further complications of liver disease. Given that national and international HIV guidelines now recommend ART regardless of CD4 cell count the previous recommendations for earlier ART in HCV/HIV coinfection are obsolete in settings where universal ART is available (EACS 2016). Short-term and long-term virologic success rates of ART in HCV/HIV coinfection, however, may be limited by an increased risk of hepatotoxicity (Sulkowski 2000). Various studies have shown that the presence of HCV is independently associated with an increased risk of rises in serum aminotransferases, highlighting the need for close monitoring or better elimination of HCV (Vispo 2013).

Treatment of HCV in HIV coinfection

After the broad availability of interferon free DAA regimen with high efficacy, good tolerance and short treatment duration elimination of HCV in HIV coinfecting individuals has become easy and the prevalence of HCV/HIV coinfecting patients has declined substantially in countries with broad access to DAA regimen. From a medical point of view there are no reasons left not to treat HCV, however cost of therapy may be a challenge still to overcome.

Several studies have been able to demonstrate that successful treatment of coinfection dramatically reduces subsequent complications of preexisting liver disease in HIV positive patients (Erqou 2013, Mira 2013). This implies that once viral clearance is achieved the prognosis of liver disease dramatically improves (even in the presence of already developed liver cirrhosis) and once HCV is eradicated, further liver complications in patients with low-grade liver fibrosis are very unlikely. Therefore, regardless of stage of liver fibrosis, HCV treatment should be considered for all people living with HCV/HIV coinfection.

The degree of liver fibrosis is important for choosing the optimal therapy, as treatment duration may be prolonged or ribavirin (RBV) added in patients with liver cirrhosis. In patients with advanced liver cirrhosis

(Child-Pugh B and C) the use of HCV protease inhibitors such as glecaprevir, voxilaprevir, grazoprevir or paritaprevir is not advisable due to marked increases in drug levels (*see also Chapter 12*).

In addition, patients with liver cirrhosis have to be regularly followed up after viral elimination as the risk of developing hepatocellular carcinoma or hepatic decompensation persists. Monitoring should include six monthly ultrasound examinations of the liver or alternative imaging procedures, when ultrasound is not available or the quality of the examination is low (EACS 2019).

Liver biopsy is not mandatory for assessing the degree of liver fibrosis when non-invasive methods such as serotests for fibrosis (e. g. Fibrotest) or transient elastography or acoustic radio force impulse (ARFI) are available (Rockstroh 2009, Resino 2011). When liver biopsy or non-invasive tests for assessing hepatic fibrosis demonstrate lower grades of liver fibrosis (F0-F1) regardless of HCV genotype, treatment can be deferred, if there are economic constraints. In this case, fibrosis progression should be frequently assessed (*also see Chapter 17*).

The goal of HCV treatment is to achieve persistently undetectable HCV RNA levels and to reverse liver fibrosis by terminating the necroinflammatory activity in the liver. Viral elimination is generally referred to as a sustained virologic response (SVR). It is defined as undetectable HCV RNA 12 weeks (SVR12) or 24 weeks (SVR24) after completion of HCV therapy.

Treatment in countries with access to interferon-free DAA combinations

For patients with HCV/HIV coinfection in countries with access to interferon-free DAAs, HCV treatment has changed dramatically. These simplified DAA-based and interferon-free HCV therapy regimens are characterised by high efficacy, good tolerance and short treatment durations. The use of ribavirin is reserved for specific situations, e.g. treating patients with liver cirrhosis or retherapy in the presence of resistance mutations. Treatment with DAA regimen has demonstrated comparable efficacy in HCV/HIV coinfection compared to HCV mono-infection. However some subpopulations, e.g. decompensated liver cirrhosis, were not extensively studied in patients with coinfection. Because of this, treatment recommendations are based on trial results from HCV mono-infection.

In clinical practice, the main remaining difference compared to HCV mono-infection is the higher number of possible drug-drug interactions which may lead to an adjustment of ART or other co-medications.

Generally HCV protease inhibitors such as glecaprevir, voxilaprevir, grazoprevir or paritaprevir are not recommended in patients with decompensated liver cirrhosis due to marked increases in drug levels (summary of product characteristics EMEA).

As cost of non-generic DAA regimens are substantial and reimbursement differs on a local level the guidance below may be useful in a context of economic constraints and cost efficiency. Because of this approach, treatment is still structured by HCV genotype. In countries with broad access to pangenotypic regimen a much simpler decision tree exists.

Selection of DAA combinations is based upon HCV GT, stage of liver fibrosis, pre-treatment history and resistance-associated substitutions (RAS) if tested (see Table 2). Use of older, first generation HCV PIs (boceprevir and telaprevir) or older second generation PIs such as simeprevir is no longer recommended because of increased toxicities and lower efficacy. Due to drug-drug interactions in particular with HIV and HCV PIs, careful checking for interactions is urgently recommended prior to starting HCV therapy.

As a general guidance concerning drug-drug interactions any strong inducers of the cytochrome P 450 3A enzyme family or inducers of p-glycoprotein should be avoided. Antiretroviral drugs such as efavirenz, nevirapine, lopinavir/ritonavir and elvitegravir/cobicistat are generally not recommended with DAA regimens. Treatment with rifampicin, rifabutin, carbamazepine and phenytoin should also be avoided. Increases of tenofovir levels during treatment with sofosbuvir/ledipasvir are not considered clinically relevant (Kaur 2015). For specific information on drug-drug interactions consultation of the website <http://www.hiv-druginteractions.org> is recommended.

Table 2. HCV treatment options in people with HCV/HIV coinfection (except for persons pre-treated with Protease or NS5A inhibitors; adapted from EACS 2019)

HCV GT	Treatment regimen	Treatment duration & RBV usage		
		Non-cirrhotic	Compensated cirrhosis	Decompensated cirrhosis CTP class B/C
1 & 4	EBR/GZR	12 weeks ⁽ⁱ⁾		Not recommended
	GLE/PIB	8 weeks	12 weeks	Not recommended
	SOF/VEL	12 weeks		12 weeks with RBV
	SOF/LDV +/- RBV	8-12 weeks without RBV ⁽ⁱⁱ⁾	12 weeks with RBV ⁽ⁱⁱⁱ⁾	
2	GLE/PIB	8 weeks	12 weeks	Not recommended
	SOF/VEL	12 weeks		12 weeks with RBV
3	GLE/PIB	8 weeks ^(iv)	12 weeks ^(iv)	Not recommended
	SOF/VEL +/- RBV	12 weeks ^(v)	12 weeks with RBV ^(vi) or 24 weeks without RBV	
	SOF/VEL/VOX	-	12 weeks	Not recommended
5 & 6	GLE/PIB	8 weeks	12 weeks	Not recommended
	SOF/LDV +/- RBV	12 weeks +/- RBV ^(vii)	12 weeks with RBV ⁽ⁱⁱⁱ⁾	
	SOF/VEL	12 weeks		12 weeks with RBV

EBR = elbasvir **GLE** = glecaprevir **GZR** = grazoprevir
LDV = ledipasvir **PIB** = pibrentasvir **RBV** = ribavirin
SOF = sofosbuvir **VEL** = velpatasvir **VOX** = voxilaprevir
RAS = Resistance Associated Substitutions

- i. Extension of treatment to 16 weeks and addition of RBV in PLWH with GT1a with baseline HCV-RNA > 800.000 IU/mL and/or NS5A RASs causing at least 5-fold reduction in activity of EBR to minimise the risk of treatment failure and in HCV GT4 experienced PLWH with HCV-RNA > 800.000 IU/mL. 8 weeks can be considered in GT 1b treatment-naïve with F0-F2
- ii. 8 weeks treatment without RBV only in treatment-naïve PLWH with F < 3 and baseline HCV-RNA < 6 million IU/mL
- iii. In persons intolerant to RBV, treatment may be prolonged to 24 weeks. RBV can be omitted in treatment-naïve or -experienced PLWH with compensated cirrhosis without baseline NS5A RAS
- iv. Treatment duration in HCV GT3 who failed previous treatment with IFN and RBV +/- SOF or SOF and RBV should be 16 weeks
- v. Addition of RBV in treatment experienced PLWH with baseline NS5A RASs, if RAS testing available; if these persons are intolerant to RBV, treatment may be prolonged to 24 weeks without RBV
- vi. If RAS testing is available and demonstrates absence of NS5A RAS Y93H, RBV can be omitted in treatment naïve PLWH with compensated cirrhosis
- vii. In treatment experienced (exposure to IFN/RBV/SOF) PLWH add RBV treatment for 12 weeks or prolong treatment to 24 weeks without RBV

Treatment in countries without access to interferon-free DAA combinations

Because treatment with the new DAAs, if patent protected, is very expensive, access to these drugs is not available in some healthcare systems. Access to generic drugs may be an alternative solution in almost all of these areas.

In case only interferon and ribavirin are available the standard dosage for PEG-IFN α -2a is 180 μ g SC once weekly and for PEG-IFN α -2b 1.5 μ g/kg body weight SC once weekly plus RBV 1000 mg (<75 kg body weight) and 1200 mg (\geq 75 kg body weight). Duration of therapy is individualised between 24 to 48 weeks taking into account factors for HCV treatment response such as genotype, baseline viral load and virologic response.

If an early virologic response (decline of at least 2 log₁₀ reduction in HCV RNA at week 12 from baseline) is not achieved when treating HCV with PEG-IFN and RBV, treatment should be stopped.

During PEG-IFN+RBV therapy, didanosine (ddI) is contraindicated in persons with cirrhosis and should be avoided in persons with less severe liver disease. Stavudine (d4T) and zidovudine (ZDV) should also be avoided.

For details please consult previous recommendations (EACS 2015).

Treatment of HCV for relapse or non-response

For patients with coinfection in countries with access to DAAs, interferon-free DAA-based HCV treatment should be the first choice for retreatment patients with chronic HCV. The rules are essentially the same as for HCV mono-infection (*see Chapter 12*). However, due to possible drug-drug interactions, the concomitant ART should be assessed before initiating HCV therapy (*see EACS guidelines 2019 or visit www.hep-druginteractions.org*). Before retreatment patients with virologic failure, adherence should be assessed and reinfection excluded. Resistance testing, if available, should be performed before re-treatment of persons who failed after a PI-and/or NS5A inhibitor-containing agent. The triple combination of SOF/VEL/VOX for 12 weeks is the treatment of choice for re-treatment, especially if resistance testing is not available. In persons with complex mutations patterns SOF+GLE/PIB + RBV for 12-16 weeks can also be considered. In case of unavailability of SOF/VEL/VOX or SOF + GLE/PIB other regimens with at least two active DAAs could be combined with the preferential use of one drug with high genetic barrier to resistance and with extended treatment durations and potentially addition of RBV. In patients with decompensated cirrhosis SOF/VEL + RBV for 24 weeks is the only available option for re-treatment in case of contraindication to liver transplantation.

The main risk factor for virologic failure in adherent patients is liver

cirrhosis. Because of this, these patients will accumulate in the group of patients requiring retreatment. In particular, patients with hepatic decompensation are challenging as treatment is associated with hepatic decompensation, infectious complications and has a mortality rate of up to 10% in clinical studies. In some patients, liver transplantation followed by DAA therapy may be an alternative strategy (*see Chapter 20*).

In countries with no access to interferon free DAA regimen, patients with a history of interferon based HCV therapy who were either non-responders or who relapsed while on previous HCV therapy need to be reassessed with regard to the next HCV treatment optimising the dose and duration of PEG-IFN and RBV as well as potentially adding simeprevir, daclatasvir or sofosbuvir as a third drug. Interferon-based therapy is contraindicated in patients with hepatic decompensation.

Treatment of acute HCV in HIV

In the past, interferon-based regimen were more efficacious, when used in the acute phase of HCV infection, but, given the SVR rate of >90% with most DAA regimen in chronic HCV, this advantage is no longer important.

IFN-containing HCV regimens are no longer recommended. After diagnosis of recently acquired HCV infection, HCV RNA should be re-measured 4 weeks later. Treatment is recommended in PLWH without a decrease of 2 log of HCV RNA at 4 weeks compared with initial HCV RNA, due to the very low probability of spontaneous clearance, and in persons with persistent serum HCV RNA 12 weeks after diagnosis of recently acquired HCV, see Algorithm for Management of Recently acquired HCV in Persons with HIV Co-infection. HCV treatment immediately after diagnosis may be considered in PLWH with ongoing risk behavior to reduce onward transmission. However, counseling strategies to change the risk behavior are also an essential part of the prevention measures. IFN-free treatment with DAAs is recommended as in naïve non-cirrhotic (except for those with pre-existing cirrhosis). For more detailed information on the management of recently acquired HCV infection we refer to the European AIDS Treatment Network (NEAT) consensus conference guideline, www.neat-id.org. In case of interferon-based therapy as the only available option, duration of treatment should be based on rapid virologic response (RVR) regardless of genotype. Early discontinuation of interferon based therapy is justified in persons experiencing significant side effects of PEG-IFN and/or RBV. Also patients who do not achieve a \geq 2 log₁₀ decrease in HCV RNA level at week 12 should discontinue therapy (NEAT 2011).

Management of liver cirrhosis and liver transplantation in people with HCV/HIV coinfection

In general, compared to HCV mono-infection, individuals with HCV/HIV coinfection develop more rapid HCV-related hepatic injuries such as liver fibrosis and cirrhosis. Additionally, HCV/HIV coinfection is associated with an increased rate of hepatocellular carcinoma (HCC). Typically, HCC occurs in coinfection at an earlier age and the course is more aggressive, with a shorter survival compared to HCV mono-infection (Klein 2016). An ultrasound of the liver should be performed every six months for HCC surveillance in patients with F3/F4 fibrosis, according to the recommendations of EACS (EACS 2019).

As upper gastrointestinal bleeding is another important complication the presence of oesophageal varices using upper-gastrointestinal endoscopy should be monitored in patients with liver cirrhosis every year.

Liver transplantation should be considered in patients with decompensated liver cirrhosis. To fulfil the selection criteria for a liver transplant in individuals with HCV/HIV coinfection, the CD4⁺ T cell count has to be at least 100 cells/ μ L. Additionally, the patient has to have either undetectable HIV viraemia (<50 copies/mL) or at least rational treatment options to control HIV infection successfully after liver transplantation. Further contraindications for transplantation are opportunistic diseases, ongoing alcohol or drug use, large multilobar HCC or HCC metastasis in other organs, a second malignant disease, advanced cardiopulmonary disease or older age with an elevated perioperative mortality risk.

The possibility to eradicate HCV in virtually all patients posttransplant due to the high efficacy of DAA regimen will positively affect transplant survival. On the other hand, the need for liver transplantation due to chronic HCV will be substantially reduced over the years to come in countries with large scale access to DAAs.

For more details, refer to Chapter 20 on liver transplantation in HCV/HIV coinfection.

Conclusion

Uncontrolled HIV infection accelerates the progression of hepatitis C, resulting in higher liver disease-related mortality and morbidity in HCV/HIV coinfection compared to either HCV or HIV mono-infection. In countries with access to DAAs, interferon-free DAA-based treatment is strongly recommended in all patients. Treating with DAA based regimen

has eliminated the lower efficacy of HCV therapy as known from interferon based therapies. Drug-drug interactions between ART and the DAAs inhibitors require careful selection of both HIV and HCV drugs before initiating therapy. After elimination of HCV monitoring for HCC and hepatic decompensation has to be implemented for patients with advanced liver fibrosis.

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16. HBV/HCV coinfection

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Epidemiology of HBV/HCV coinfection

Infection with either hepatitis B (HBV) or hepatitis C (HCV) virus is one of the major causes of chronic liver disease globally (Konstantinou 2015). Due to shared routes of transmission, coinfection with HBV and HCV is not uncommon among individuals in areas of high HBV prevalence and among individuals at high risk of parenterally transmitted infections, such as people who inject drug (PWID) (Pallas 1999), those with an increased number of lifetime sexual partners (Bini 2010), patients on haemodialysis (Reddy 2005), patients undergoing organ transplantation (Aroldi 2005) and HIV positive individuals (Zhou 2007, Jansen 2015). Due to a lack of large-scale population-based studies the exact number of people coinfecting with HBV/HCV is unknown. Dual infection ranges from 9% to 30%, depending on the geographic region (Zarski 1998, Liaw 1995, Tyson 2013). These numbers may underestimate the true number of people with HBV/HCV coinfection, as there is a well-known entity of occult HBV infection (patients with negative hepatitis B surface antigen [HBsAg] but detectable serum HBV DNA) in patients with chronic HCV (Cacciola 1999, Torbenson 2002, Raimondo 2005, Wiegand 2015).

Screening for HBV/HCV coinfection

People with a first episode of acute hepatitis should be screened for all viral causes including HBV and HCV (see Chapter 8 on diagnostic tests in acute and chronic hepatitis B and Chapter 11 for hepatitis C). Some patients may be inoculated with both viruses simultaneously and will present with acute hepatitis due to both viruses. In addition, HBV superinfection in patients with chronic HCV, and HCV superinfection in patients with chronic HBV have both been reported (Liaw 2000, Liaw 2002, Liaw 2004). Therefore, episodes of acute hepatitis in patients with known chronic HBV or HCV infection, especially those with ongoing risk behavior for hepatitis infections such as injecting drug use or multiple sex partners, should undergo screening for superinfection. In addition, in patients with chronic HCV, ruling out occult HBV infection beyond HBsAg testing, e.g., by polymerase chain reaction (PCR), should be done when clinically indicated (Squadrito 2013).

Viral interactions between HBV and HCV

Patients with both HBV and HCV may show a large spectrum of virologic profiles and different viral dominance patterns have been documented. In most cases, HCV is dominant and suppresses HBV replication (Liaw 2001), resulting in lower HBV DNA levels and decreased activity of HBV DNA polymerase (Chu 1998). Moreover, HCV was demonstrated to inhibit HBsAg production by mechanisms mediated by host immune responses. HBsAg levels were found even lower compared with HBV-monoinfected patients undergoing treatment with nucleos(t)ide analogues but comparable to low replicative HBsAg carriers (Wiegand 2015). Superinfection with HCV in patients with chronic HBV might even induce seroconversion of HBsAg (Liaw 1994, Liaw 1991). Most recent clinical findings postulate that HCV coinfection itself is not associated with seroconversion but a higher ALT level >80U/L is the major determinant of HBsAg loss in patients with HBV/HCV coinfection (Yang 2016).

Several authors have reported that HBV can reciprocally inhibit HCV replication (Sato 1994). HBV DNA replication has been shown to correlate with decreased HCV RNA levels in coinfecting patients (Zarski 1998). Coinfection with HBV was sometimes associated with a higher spontaneous HCV clearance (Islam 2016).

Furthermore, patients with coinfection have lower levels of both HBV DNA and HCV RNA than corresponding monoinfected controls, indicating that simultaneous suppression of one virus by the other might occur (Jardi 2001). Thus, either HBV or HCV can play the dominant role, HBV and HCV can inhibit each other simultaneously and they can alternate their dominance (Liaw 1995). Both viruses have the ability to induce seroconversion of the other. The chronology of infection may have a role in determining the dominant virus.

Interestingly, recent *in vitro* studies revealed that there is most probably no direct interference between HBV and HCV replication, making interindividual differences in innate and/or adaptive host immune responses responsible for viral interference observed in coinfecting patients (Bellecave 2009, Eyre 2009). A modulation of human dendritic cells induced by the combined effects of HBV and HCV core proteins, leads to an inefficient antigen presentation to CD4+ T cells and thus suppresses the induction of cellular immune response (Agrawal 2014, Yoshio 2016). These findings show a possible mechanism by which HBV and HCV synergistically induce immune tolerance that may be fundamental in establishing chronic, persistent infection.

Clinical scenarios of HBV and HCV infection

Different scenarios of infection have been described with HBV/HCV coinfection including acute hepatitis with HBV and HCV (Alberti 1995), occult HBV coinfection of chronic HCV (Sagnelli 2001), and superinfection by either virus in patients with pre-existing chronic hepatitis due to the other virus (Figure 1). Frequently the sequence of infection cannot be defined.

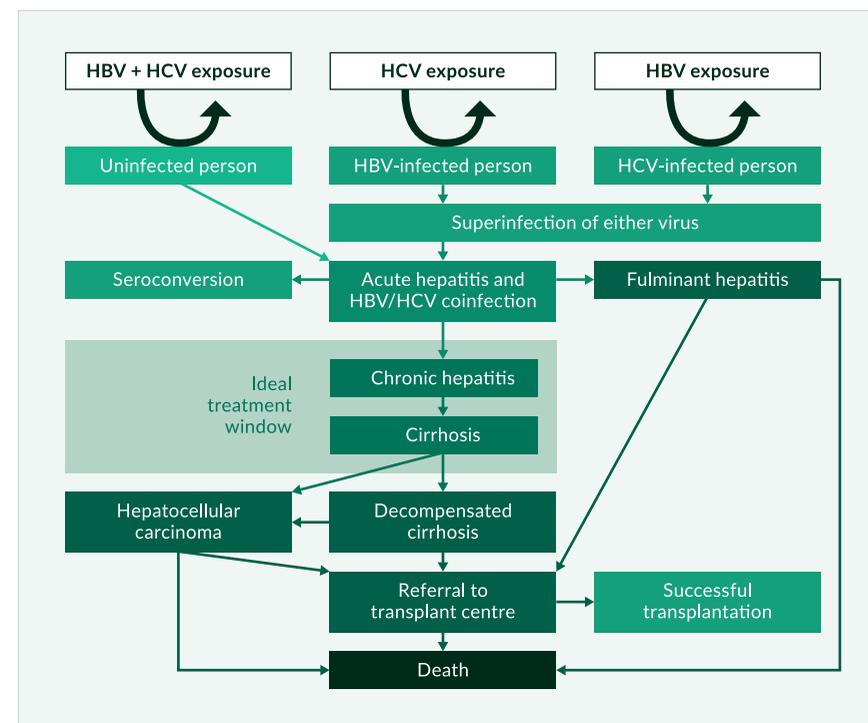


Figure 1. Clinical scenarios of HBV/HCV coinfection (modified after Crockett 2005)

Acute hepatitis by simultaneous infection of HBV and HCV

Simultaneous coinfection with HBV and HCV is rarely seen, but the interaction of HBV and HCV appears to be similar to chronic infection. In acute infection with HBV and HCV, patients showed delayed HBsAg appearance and a shorter hepatitis B surface antigenaemia compared to those with acute HBV alone (Mimms 1993). Biphasic alanine aminotransferase (ALT) elevation was found in some patients, although rates of viral clearance were similar to those in patients with HBV or HCV monoinfection (Alberti 1995). Simultaneous infection often has a self-limiting, benign course with complete recovery from one or both infections (Chen 2007, Chu 1995).

HCV superinfection

HCV superinfection is frequent in endemic areas of HBV infection, such as Asia, South America and sub-Saharan Africa (Liaw 2002, Liaw 2004), which can result in the suppression of HBV replication and termination of HBsAg carriage. However, long-term follow-up analyses have described a higher rate of liver cirrhosis and hepatocellular carcinoma (Liaw 2004, Yang 2016). Fulminant hepatic failure was significantly higher among patients with underlying HBV infection than those without (23% vs. 3%) (Chu 1999, Wu 1994, Chu 1994).

HBV superinfection

HBV superinfection is less common in people living with HCV and limited data is available. In a case control study, HCV RNA was undetectable in all observed patients during acute HBV infection, indicating that superinfection of HBV leads to long lasting suppression of HCV (at one year 71% remained negative, at two years 42%) and in up to 25% of cases even can lead to permanent clearance of chronic HCV infection, especially in patients with severe acute HBV infection (Sagnelli 2009, Liaw 2000, Wietzke 1999). Patients with superinfection and those with HBV mono-infection showed similar initial HBV viral load and a similar trend of becoming negative for HBV DNA. HBV superinfection is associated with acute deterioration of liver function and showed a severe course during acute illness more frequently (34.5% in superinfection versus 6.9% in HBV mono-infection). The risk of fulminant hepatitis is increased (Sagnelli 2009, Sagnelli 2002).

Occult HBV infection in patients with HCV infection

Occult HBV infection, defined as detectable HBV DNA in liver or serum and undetectable HBsAg (Ozaslan 2009, Torbenson 2002), has been identified in up to 50% of patients with chronic HCV (Matsuoka 2008). Importantly, a relation to HCV treatment outcomes has been described (Zignego 1997, Fukuda 2001, Sagnelli 2001). HCV infection with occult HBV infection has been associated with higher ALT levels, greater histological activity index and liver disease more often progressing to liver cirrhosis (Fukuda 1999, Cacciola 1999, Sagnelli 2001). Occult HBV infection seems to significantly shorten life expectancy compared to HCV mono-infection (Squadrito 2014, Coppola 2016).

Chronic hepatitis in HBV/HCV coinfection

Patients with detectable serum HBV DNA and HCV RNA are at highest risk of severe liver disease and therefore should be considered for treatment. Large follow-up studies show that patients with HBV viraemia are at higher risk for cirrhosis, HCC and overall death than people with HCV mono-infection (36.8, 6.9, and 41.7 versus 17.4, 3.6, and 31.4 per 1000 person years, respectively) (Kruse 2014, Bini 2014). Active HCV infection (HCV RNA+) in the setting of inactive HBsAg (HBsAg+/HBV DNA-) is associated with a clinical course similar to that of HCV mono-infection. Another possibility is active HBV infection in patients with inactive or prior HCV infection (HBV DNA+/HCV RNA-/anti-HCV+). This immune profile is less common, and may indicate HBV suppression of HCV. A longitudinal study of virologic monitoring of 103 HBV/HCV-coinfected patients revealed a fluctuation in the virologic pattern (Raimondo 2006). Asian ethnicity is a major independent predictor of HBV dominance, while HCV-dominant disease is more common in non-Asian individuals (Nguyen 2011). Thus, careful longitudinal follow-up of levels of serum HBV DNA and HCV RNA is needed for a correct diagnosis and decision on the most successful treatment strategy. Table 1 shows the immune profiles found in patients with chronic HBV/HCV infection.

Table 1. Immune profiles in patients with chronic HBV/HCV infection

	HBV and HCV active	Occult HBV in chronic active HCV	HCV active in HBsAg carrier
HBsAg	+	-	+
HBV DNA	+	+	-
Anti-HCV	+	+	+
HCV RNA	+	+	+

Cirrhosis

Higher rates of cirrhosis have been shown in people with HBV/HCV coinfection. In comparison to patients with HBV mono-infection, higher rates of cirrhosis (44% vs. 21%) and decompensated liver disease (24% vs. 6%) were demonstrated in people with coinfection (Fong 1991). Compared to HCV mono-infection, a higher rate of cirrhosis (95% vs. 49%) and more decompensated liver disease (Child-Pugh class C 37% vs. 0%) were found in people with HBV/HCV coinfection (Mohamed Ael 1997).

Hepatocellular carcinoma

In many studies, coinfection with HBV and HCV is associated with an increased risk of HCC development, confirmed by three large meta-analyses (Cho 2011, Shi 2005, Donato 1998).

In one longitudinal study, incidence of HCC was 6.4 per 100 person years in people with HCV/HBV coinfection compared to 2.0 and 3.7 in HBV and HCV monoinfection, respectively. The cumulative risk of developing HCC after 10 years was 45% in HBV/HCV coinfection compared to 16% in HBV and 28% in HCV monoinfection (Chiaromonte 1999). Possible associated risk factors for HCC development in coinfection are longer duration of infection, higher HCV RNA levels, and higher levels of fibrosis (Zampino 2015). Patients with HBV/HCV coinfection should undergo a screening routine for HCC with liver ultrasound and α fetoprotein levels in serum at least every six months.

In this context, however, it has to be mentioned that dually infected patients are an extremely heterogeneous population and most of the data available does not take into account the differences in the viruses (genotypes, main HBV genomic mutations, activity status of one or both viruses, etc.) or those regarding patients' characteristics and comorbidities (presence of diabetes, alcohol intake, etc.) (Huang 2011).

Treatment of HBV and HCV coinfection

Despite the individual clinical importance, solid evidence and well-established treatment guidelines for HBV/HCV coinfection are currently lacking. Generally, treatment guidelines for monoinfection should be applied to coinfection after carefully characterising the replicative status of HBV, HCV and hepatitis delta virus infection. Due to the variety of virologic profiles in HBV/HCV coinfection it is important to assess the dominant virus prior to initiating therapy. In people with coinfection, treatment should be initiated when inclusion criteria for standard treatment guidelines of HBV and HCV monoinfection are met (*see Chapter 9 on HBV treatment and Chapter 12 on HCV treatment*). Treating HBV/HCV coinfection leads to a risk reduction of HCC and improved survival (Liu 2014, Konstantinou 2015). As with monoinfection, treatment of people with coinfection should be started before liver decompensation occurs.

Due to loss of viral suppression from the successfully treated dominant virus, acceleration of liver disease has been reported (Yalcin 2003) and caution must be exercised upon initiation of therapy.

In coinfection with dominance of HCV infection, PEG-IFN plus ribavirin is still used because of its proven activity against both viruses (Table

2). Data from a meta-analysis show that the SVR achieved in HBV/HCV coinfection are comparable to those in HCV monoinfection (OR = 1.03, 95% CI: 0.37–2.82 and OR = 0.87, 95% CI: 0.62–1.21, respectively) (Liu 2012, Kim 2011, Liu 2009). HCV SVR is maintained in 97% in a five-year follow-up (Yu 2013). Furthermore, HBsAg loss occurs in about 30% within five years after treatment start and there is evidence of an increased possibility of HBeAg seroconversion during or post-treatment with PEG-IFN and ribavirin (Liu 2016, Yu 2013, Liu 2009, Viganò 2009, Yu 2009).

Table 2. PEG-IFN plus ribavirin treatment trials in people with HBV/HCV coinfection

Patients (n)	HCV SVR (%)	HBV DNA negative (%)	HBsAg loss (%)	HBV reactivation # (%)	Reference
19	70*, 78**	33	0	31	Potthoff 2008
161	72*, 83**	56	11	35	Liu 2009
17	6	na	na	na	Senturk 2008
50	40*, 75**	100	0	24	Yu 2009
22	41	86	36	na	Viganò 2009
18	60*, 88**	12	na	na	Kim 2011

*HCV GT 1, **HCV GT 2/3, na=not applicable, # HBV DNA negative pre-treatment

HBV replication may become detectable in up to 60% of patients with undetectable pre-treatment HBV DNA levels, either during the course of treatment (38%) or during the treatment follow-up (60%). Reactivation was only transient in 45% (Liu 2014, Yu 2013, Potthoff 2009, Liu 2009). HBV DNA reactivation was found to be independently associated with younger age, HCV SVR and baseline HBV DNA ≥ 2000 IU/mL (Hung 2012). Thus, close monitoring of both viruses is recommended during and after combination therapy. In case of HBV reactivation or if HBV replication is detectable at a significant level, concurrent HBV nucleos(t)ide analogue (NA) therapy is indicated.

Direct acting antivirals (DAA) still need to be further evaluated in people with HBV/HCV coinfection. IFN-free DAA-based regimes will not be able to clear HBsAg and simultaneous or on-demand nucleos(t)ide analogues will be needed if clinically indicated. In the setting of the IFN-free DAA-based therapies, the possibility of HBV reactivation during HCV treatment is raised due to viral interferences. Post-marketing cases of HBV reactivation under different combinations of DAAs have been reported (De Monte 2016, Hayashi 2016, Takayama 2016, Collins 2015). On the other hand, a recent analysis of 103 previously HBV infected patients showed no evidence of HBV reactivation under DAA treatment (Sulkowski 2016). Nevertheless, positive HBsAg status before DAA treatment is a strong risk factor for developing

hepatitis due to HBV activation during treatment (HR 15.0) (Wang 2017). Due to this potential risk of early HBV reactivation during IFN-free HCV therapies, it is necessary to closely monitor and preemptively treat HBV coinfection, regardless its stage (chronic, occult, resolved), whatever HCV genotype or class of DAA used. Furthermore, in patients receiving tenofovir as concomitant anti-HBV treatment, the eGFR and tubular function should be monitored during treatment with simeprevir or sofosbuvir/ledipasvir as tenofovir exposure is significantly increased.

In patients with dominant HBV, IFN +/- HBV polymerase inhibitors are an upcoming option. Data exists from a small cohort of people with HBV/HCV coinfection treated with lamivudine in combination with standard interferon for 12 months followed by lamivudine for an additional 6 months (Marrone 2004). In this study, clearance of HBeAg was found in 3/8, two patients showed HBeAg seroconversion, and HBV DNA clearance was observed in 3/8 at the end of therapy. HBV DNA became detectable again in two patients at the end of follow-up. HCV clearance was achieved in 50%. In another study, tolerability and efficacy of anti-HBV nucleos(t)ide analogues (lamivudine plus adefovir [n=10], entecavir [n=7], telbivudine [n=4], tenofovir disoproxil fumarate [n=3]) were investigated in a cohort of 24 cirrhotic patients with HBV/HCV coinfection (Coppola 2013). Clearance of HBV DNA was found in 96% of patients after 18 months, while HCV reactivation was low (12.5%). However, while the virologic response was favourable in all patients and treatment was well tolerated, progression of liver cirrhosis was seen in up to one-third. Patients who were HCV RNA positive at baseline deteriorated more frequently. Thus, a favourable clinical impact in HBV/HCV cirrhotic patients was seen only in patients who were HCV RNA negative at baseline.

Based on these observations, NA such as tenofovir, adefovir, entecavir and telbivudine showing a higher genetic barrier in combination with PEG-IFN are a possible treatment option. In cirrhotic patients with HBV/HCV coinfection with detectable HCV RNA, exclusive treatment with NA has a high risk of clinical deterioration. However, further studies are needed to estimate the treatment value of these newer drugs in different clinical scenarios.

Interestingly, fibrosis progression rate after orthotopic liver transplantation in patients with HBV/HCV coinfection is lower compared with HCV mono-infection (Taniguchi 2000, Féray 1999). The one- and five-year patient and graft survival rates were 80% and 70%, respectively. The five-year fibrosis progression rate was 0.17 +/- 0.08 units (Manzia 2010).

Conclusion

Coinfection with HBV and HCV is not uncommon, especially within areas of high hepatitis B prevalence. HBV/HCV coinfection is a challenge for clinicians due to the complex interactions of HBV and HCV, and the propensity for developing severe liver disease. No treatment standard has been established for patients with HBV/HCV coinfection. Treatment decisions must be made based upon identification of the dominant virus. Combination therapy of PEG-IFN plus ribavirin has been shown to be highly effective in inducing virologic response. Systematic treatment experience with DAAs in the setting of HBV/HCV coinfection is lacking and decisions currently have to be made on an individual basis.

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17. Assessment of hepatic fibrosis and steatosis

Frank Grünhage and Frank Lammert

Introduction

Non-invasive methods for the assessment of liver fibrosis and steatosis are replacing invasive liver biopsy due to patient wariness and the low but ever-present morbidity of biopsies. The use of non-invasive markers is also increasing because clinical questions concerning the presence or absence of steatosis, fibrosis and cirrhosis as well as treatment monitoring and follow-up can be sufficiently answered by these tests and procedures. Today, despite the dogma of the biopsy being the gold standard, the use of non-invasive liver fibrosis detection vastly outnumbers biopsies in chronic liver diseases. Non-invasive tests have problems in discriminating accurately between early stages of fibrosis, i.e., F0-F2. Notwithstanding, non-invasive markers can be used as it primarily relevant to discriminate between early stages and advanced fibrosis in clinical practice.

In addition, non-invasive tests carry the potential of being used as screening tools in population-based studies and can detect fibrosis even in individuals with normal liver function tests. Non-invasive markers should be able to reliably identify liver cirrhosis in order to initiate further diagnostic procedures to exclude portal hypertension and to intensify surveillance strategies. Non-invasive strategies are also warranted for monitoring the disease while on therapy and ideally document the regression of fibrosis during follow-up.

Non-invasiveness for the detection of fibrosis has become reality in clinical practice and has been approved for clinical studies but for hepatologists fibrosis assessment is only one puzzle piece and more information is needed. Other endemic conditions such as fatty liver disease with or without inflammatory changes and or fibrosis increase the need for other non-invasive tests that also provide information on hepatic fat contents and inflammation. Whereas ultrasound-based methods such as the controlled attenuation parameter (CAP) can be used for the rapid and easy assessment of steatosis, specific non-invasive test to classify the mode and extend of inflammation in the liver are still missing.

With most experts agreeing that non-invasive techniques do not replace liver biopsies completely, they have reduced the number of biopsies substantially (Leroy 2007, Pinzani 2005, Sebastiani 2006). Hence, the

clinical question remains: Will the information change my practice or advice? Recently three major guidelines have been published on the use of elastography and other methods for non-invasive assessment of liver fibrosis, which are recommended for further reading:

- <http://www.easl.eu/research/our-contributions/clinical-practice-guidelines/detail/non-invasive-tests-for-evaluation-of-liver-disease-severity-and-prognosis>
- <http://www.efsumb.org/guidelines/guidelinesOI.asp>
- <http://www.wfumb.org/reports/>

This chapter reviews non-invasive (serum markers and liver stiffness measurement) markers of liver fibrosis as well as fatty liver disease and aims to illustrate what is relevant in clinical practice.

Mechanisms of liver fibrosis in chronic viral hepatitis

Liver fibrosis is characterised by the loss of hepatocytes, destruction of hepatic (micro)architecture, proliferation of hepatic (myo)fibroblasts, and excess deposition of extracellular matrix (Friedman 2008). The final stage of liver fibrosis (cirrhosis) may result in insufficient detoxification, portal hypertension, renal and pulmonary failure and hepatocellular carcinoma, and is associated with excess mortality. Liver cirrhosis is the common end-stage of chronic liver diseases such as chronic viral hepatitis, non-alcoholic and alcoholic liver diseases as well as autoimmune and metabolic liver diseases. The mechanisms of fibrogenesis in all aetiologies share certain aspects but differ in detail. Consequently, the non-invasive assessment of liver fibrosis also varies between diseases.

A key feature of hepatic fibrosis is the activation and proliferation of fibroblasts and hepatic stellate cells. Chronic liver injury leads to activation of these cells, which become contractile, produce the extracellular matrix components and secrete inflammatory and profibrotic cytokines and chemokines such as transforming growth factor. The activation of these cells is believed to represent the key event in hepatic fibrogenesis (Friedman 2008). Hepatic stellate cell activation depends on signalling by Kupffer cells, endothelial cells, hepatocytes, and platelets. The deposition of the extracellular matrix is constantly opposed by the degradation of these proteins. In progressive liver fibrosis, this balance is skewed in favour of excess extracellular matrix deposition. Matrix metalloproteinases and their regulators (tissue inhibitors of metalloproteinases, TIMPs) control matrix deposition and degradation.

Liver biopsy – the “gold standard” for staging of liver fibrosis

Liver biopsy may be obtained via different routes (Table 1). The most common is the ultrasound-guided percutaneous biopsy.

Table 1. Pros and cons of methods for liver biopsy

Procedure	Advantages	Disadvantages	References
Percutaneous biopsy	<ul style="list-style-type: none"> • Easy to perform • Out-patients procedure in selected cases 	<ul style="list-style-type: none"> • Low patient acceptance • Complication rate 0.75–14% (90% detected within 24 hrs) • Mortality 0.001–0.003% • Higher complication rates in advanced fibrosis • Not advised in patients with ascites 	Terjung 2003, van der Poorten 2006, Myers 2008, Chi 2017
Transiugular biopsy	<ul style="list-style-type: none"> • Applicable in patients with ascites and coagulation deficiencies 	<ul style="list-style-type: none"> • Expensive • In-patient procedure • Smaller biopsies may hamper fibrosis assessment • Usually interventional radiologist required 	Cholongitas 2006, Wolska-Krawczyk 2013
(Mini-) Laparoscopy	<ul style="list-style-type: none"> • Higher detection rates for cirrhosis • Bleeding can be treated directly 	<ul style="list-style-type: none"> • Expensive • Not available in all centres 	Helmreich-Becker 2003, Denzer 2007

The quality and reliability of fibrosis staging via histopathological assessment of liver biopsy specimens depends largely on the size of the specimen and the number of portal fields. The biopsy should be at least 20–25 mm long and more than 11 portal tracts should be visible (Bedossa 2003, Cholongitas 2006, Rousselet 2005). However, in daily practice these requirements may not be easy to achieve; and even if a large enough biopsy is acquired, the specimen only reflects about 1/50,000 of the whole liver.

Thus, liver biopsies are particularly prone to sampling errors and may – like non-invasive markers – have difficulties in discriminating between adjacent stages of fibrosis (i.e., F1 vs. F2 or F2 vs. F3). Discrepancies of more than one stage are rare (Regev 2002, Siddique 2003, Skripinova 2007). Intra- and inter-observer variability may be unaffected by specimen sizes but can lead to discrepancies in up to 20% of cases, even if one stage difference between estimates is accepted (Gronbaek 2002, Petz 2003). Standardised automated staging using deep learning algorithms may improve diagnostic accuracy (Yu 2018).

There is a wide variability in the use of other staging systems in patients with chronic viral hepatitis. In Germany, current guidelines recommend the Desmet & Scheuer staging system (Supplementary Table 1) (Batts 1995, Desmet 1994, French METAVIR Cooperative Study Group 1994, Ishak 1995, Knodell 1981, Schirmacher 2004).

Supplementary Table 1. Commonly used liver fibrosis staging scores

Staging System	Fibrosis stages	Remarks	
METAVIR	F0, F1, F2, F3, F4	Best evaluated in HCV fibrosis	French METAVIR Cooperative Study Group 1994
Knodell	F0, F1, F3, F4	No intermediate stage	Knodell 1981
Desmet & Scheuer	Analogous to METAVIR	Recommended by the German guidelines for the assessment of liver fibrosis	Desmet 1994, Schirmacher 2004
Batts & Ludwig	Similar to METAVIR		Batts 1995
Ishak	F0, F1, F2, F3, F4, F5, F6		Ishak 1995

Surrogate markers of liver fibrosis

Liver fibrosis develops as a continuous process rather than in a stepwise manner. Thus, so-called surrogate markers, which are also continuous variables, may provide more precise information. Surrogate markers can be subdivided into two groups (Table 2):

Direct markers reflect changes in the content of extracellular matrix proteins (such as collagen) in the liver.

Indirect markers indicate alterations in hepatic function, increase in portal hypertension with subsequent splenic enlargement, and/or grade of hepatic inflammation that may correlate with fibrosis stage.

Direct and indirect markers may be used alone or, more commonly, in combination ("composite scores"). The calculation of such scores can be simple (e.g., APRI, FIB-4, FORNS) or based on complicated formulas (e.g., Fibrotest, Fibromax, Fibrosure).

Advantages of surrogate markers are (EASL 2015):

- Good reproducibility
- High applicability (95%)
- No cost and wide availability (non-patented)
- Well validated
- Can be performed in the outpatient clinic

Disadvantages of surrogate markers include (EASL 2015):

- Non-specific of the liver
- Unable to discriminate between intermediate stages of fibrosis
- Performance not as good as elastography for cirrhosis
- Cost and limited availability (proprietary)
- Limitations (haemolysis, Gilbert syndrome, inflammation, cholestasis, heart failure)

Table 2. Summary of non-proprietary direct and indirect surrogate markers of liver fibrosis (modified from Pinzani 2008)

Index	Variables	Formula for calculation	Interpretation
Direct surrogate marker			
MP3	PIIINP, MMP-1	$0.5901 (\log \text{PIIINP} [\text{ng/mL}]) - 0.1749 (\log \text{MMP-1} [\text{ng/mL}])$	<0.3 ≈ F0-2 >0.4 ≈ F3-4 <0.3 ≈ F0-1 >0.4 ≈ F2-4
Indirect surrogate markers			
Forns	Age, plt, γ GT, cholesterol	$7.811 - 3.131 \times \ln(\text{plt}) + 0.781 \times \ln(\gamma\text{GT}) + 3.467 \times \ln(\text{age}) - 0.014 (\text{cholesterol})$	>6.9 ≈ Scheuer 2-4 <4.2 ≈ Scheuer 0-1
APRI	AST, plt	$([\text{AST}/\text{ULN}]/\text{plt} [\times 10^9/\text{L}]) \times 100$	>1.5 ≈ Ishak 3-6 ≤0.5 ≈ Ishak 0-2
Fibroindex	Plt, AST, γ GT,	$1.738 - 0.064 (\text{plt} [\times 10^4/\text{mm}^3]) + 0.005 (\text{AST} [\text{IU/L}]) + 0.463 \times (\gamma\text{GT} [\text{g/dL}])$	≤1.25 ≈ F0-F1 ≥2.25 ≈ F2-F3
Testa	Plt, spleen diameter	Plt count/spleen diameter	>1750 ≈ Ishak ≤2 ≤1750 ≈ Ishak >2
Fibrosis probability index	AST, cholesterol, past alcohol intake, HOMA, age	$E^x/1 + e^x$, where $x = -10.929 + (1.827 \times \ln[\text{AST}]) + (0.081 \times \text{age}) + (0.768 \times [\text{past alcohol use graded as 0-2}]) + (0.385 \times \text{HOMA})$	<0.2 ≈ F0-F1 ≥0.8 ≈ F2-F4
FIB-4	Plt, AST, ALT, age	$(\text{Ages} \times \text{AST})/(\text{plt count} \times$	<1.45 ≈ Ishak <4-6 >3.25 ≈ Ishak ≥4-6
Bonancini	ALT, AST, INR, plt	Sum (range 0-11) of (plt score) + (ALT/AST score) + (INR score) plt ($\times 10^9/\text{L}$): >340 = 0; 280-339 = 1; 220-279 = 2; 160-219 = 3; 100-159 = 4; 40-99 = 5; <40 = 6 ALT/AST ratio: >1.7 = 0; 1.2-1.7 = 1; 0.6-1.19 = 2; <0.6 = 3 INR: \1.4 = 2	>8 ≈ Knodell 3-4
Pohl	AST, ALT, plt	Positive if: $\text{AST}/\text{ALT} \geq 1$ and platelet count $<150 \times 10^9/\text{L}$	Positive ≈ F3-F4
Age-Platelet	Plt, age	Age score + plt score (0-10 possible score) age: <30 = 0; 30-39 = 1; 40-49 = 2; 50-59 = 3; 60-69 = 4; ≥70 = 5. Plt ($\times 10^9/\text{L}$): ≥225 = 0; 200-224 = 1; 175-199 = 2; 150-174 = 3; 125-149 = 4; ≥125 = 5	≥6 ≈ F2-F4

Index	Variables	Formula for calculation	Interpretation
Combined direct and indirect surrogate markers			
SHASTA	HA, AST, albumin	$-3.84 + 1.70$ (1 if HA 41–85 ng/mL, 0 otherwise) + 3.28 (1 if HA >85 ng/mL, 0 otherwise) + 1.58 (1 if HA <3.5 g/dL, 0 otherwise) + 1.78 (1 if AST >60 IU/L, 0 otherwise)	$>0.8 \approx$ Ishak ≥ 3 $<0.3 \approx$ Ishak ≤ 2
FM	plt, PI, AST, HA, $\alpha 2$ -MC, gender, age	-0.007 plt (G/L) – 0.049 PI (%) + 0.012 AST (IU/L) + 0.005 $\alpha 2$ -MC (mg/dL) + 0.021 HA (μ g/L) – 0.270 urea (mmol/L) + 0.027 age (years) + 3.718	$\geq F2$
Hepascore	HA, $\alpha 2$ -MC, γ GT, age, gender	$y/1 + y$, where $y = \exp [-4.185818 - (0.0249 \times \text{age}) + (0.7464 \times \text{sex}) + (1.0039 \times \alpha 2\text{-MC}) + (0.0302 \times \text{HA}) + (0.0691 \times \text{bilirubin}) - (0.0012 \times \gamma\text{GT})]$	$\geq 0.5 \approx$ F2–F4 $<0.5 \approx$ F0–F1

$\alpha 2$ -MC = $\alpha 2$ -macroglobulin, **HA** = hyaluronic acid, **MMP-1** = matrix metalloproteinase 1, **PIIINP** = aminoterminal peptide of type III procollagen, **plt** = platelets

Primary endpoints of the studies that evaluated surrogate markers vary from discrimination of no fibrosis and cirrhosis to the determination of the stages of fibrosis. With the occurrence of the new antiviral treatment options for HCV patients that allow the treatment even in decompensated patients with advanced cirrhosis, the detection of fibrosis in HCV patients has become a less relevant clinical information. However, in areas with limited treatment access where treatment is prioritised the determination of advanced fibrosis stages may guide the decision of whom to treat first. In addition patients with liver cirrhosis need continuous monitoring even after viral elimination due to an increased risk of the occurrence of hepatocellular carcinoma.

From the whole range of surrogate markers only a few are in broad clinical use. The simple APRI score has been widely studied in HBV and HCV as well as in co-infected patients (Cacoub 2008, Lebensztein 2005, Vallet-Pichard 2008, Wai 2006). A comprehensive meta-analysis of the performance of the APRI test showed that its major strength is the exclusion of significant fibrosis, defined as F2–F4, or cirrhosis with cut-offs of 0.5 and 1.5, respectively. Importantly, the test performance varied with the quantity of advanced fibrosis in the different patient groups (Shaheen 2007 & 2008). Fibrotest has also achieved some clinical significance. However, this test may not be available for all patients. Meta-analyses of the predictive performance of Fibrotest summarise that the reliability for the detection of advanced fibrosis or cirrhosis is adequate for clinical practice, and a cut-off of 0.6 has been suggested (Poynard 2007, Shaheen 2007 & 2008). Of note, the reliability for the detection of earlier fibrosis stages appears to be relatively low (Poynard 2007, Shaheen 2008).

It has to be pointed out that the performance of these markers differs

among liver diseases. For instance, a study evaluating indirect markers in >2,000 patients with chronic liver diseases detected a higher accuracy for detecting significant fibrosis in HCV patients than in NALFD (Sebastian 2011). A comprehensive paper reviewing the diagnostic accuracy of surrogate markers of fibrosis in HCV patients from 172 studies concluded that these tests based on different biomarkers are equally effective in diagnosing cirrhosis (Chou 2013). Combinations of different scores may be more effective in avoiding biopsies.

Non-invasive markers have potential value beyond the prediction of fibrosis. Surrogate markers and in particular elastography techniques have been evaluated for the prediction of liver-related complications and mortality. A number of studies aimed to test composite scores in this context for a variety of liver diseases such as PBC, alcoholic liver disease or HCV as well as mixed cohorts, describing AUROCs of 0.73 – 0.86 for mortality prediction (Mayo 2008, Naveau 2009, Parkes 2010, Vergniol 2011).

In summary, surrogate markers may support the clinical decision making process, but a single surrogate marker or score cannot replace liver biopsy. On the other hand, attempts have been made to combine different surrogate markers and biopsy in clinical decision algorithms that aim to reduce the need for liver biopsy.

Ultrasound-based elastography

Several methods for ultrasound-based elastography of the liver have been developed. These methods can be subdivided into two categories. The readouts of these measurements are either kPa or m/s, or both. Transient elastography has been available and evaluated since 2005, whereas the other technologies have become commercially available thereafter. Hence, transient elastography is the most common elastography method today but the success in non-invasive evaluation of liver fibrosis obviously has stimulated others manufacturers of ultrasound machines to promote their own specific technology. However, although similar in read-outs, not all specific machines have been evaluated in detail.

1. Shear wave speed techniques and readout values*

- Transient elastography (Fibroscan, Echosens)
 - kPa
- Point shear wave speed measurement
 - Virtual touch tissue quantification (ARFI, Siemens)
 - m/s (kPa, calculated)
 - ElastPQ (Philips)
 - kPa

- Shear wave speed imaging (Supersonic)
 - m/s or kPa

2. Strain/displacement techniques

- Strain elastography (colour coded, Hitachi)

Initially elastography was used to assess liver fibrosis stages without the need for biopsy and to exclude cirrhosis. Over time clinicians and researchers broadened the application and tried to answer more questions using elastography:

- Prediction of liver related complications (HCC, portal hypertension, mortality)
- Monitoring progression or regression of liver disease
- Screening for patients with increased liver stiffness in the normal population

Transient elastography

Transient elastography (TE) is a non-invasive technique to assess liver fibrosis (Sandrin 1999). TE allows the assessment of liver fibrosis by calculating the velocity of a low-frequency transient shear wave produced by a mechanical probe that is placed directly on the skin of the patient. The velocity of the wave that penetrates the liver tissue depends on the stiffness of the liver, which in turn correlates with the extent of liver fibrosis. In practice, a probe is placed in an intercostal space at a position that is comparable to the position for standard liver biopsy. Ten successful measurements are usually necessary for the assessment of liver stiffness. This can be done in less than 5 minutes. At present TE machines are exclusively available from Echosens (FibroScan®). Liver stiffness is expressed in kilo Pascal (kPa). The method is easy to learn and quick, results are available immediately, and a technical assistant can perform the procedure. In most studies, TE displays robust intra- and inter-observer variability (Fraquelli 2007) and may be used in children as well as adults (de Ledinghen 2007).

Normal liver stiffness

Evaluation of liver stiffness in subjects without apparent liver disease shows that liver stiffness is influenced by sex and body mass index (BMI). In general, liver stiffness is higher in men than in women (5.8 ± 1.5 vs. 5.2 ± 1.6 kPa) and in obese vs. non-obese (6.5 ± 1.6 vs. 5.3 ± 1.5 kPa). (Roulot 2008). Interestingly, TE may be used as a screening tool for the general population to identify patients with unrecognised liver disease (Ginès 2016, Roulot 2011).

Taken together one might say that liver stiffness values < 7.5 kPa appear to reflect the normal range, i.e. the absence of advanced liver fibrosis (Castera 2008, Ferraioli 2015).

Confounding factors

Common sources of false interpretation of results (usually elevated liver stiffness measurements) have been identified and should be taken into account when setting up TE measurements in clinical routine (Table 3). Acute liver injury such as acute viral or alcoholic hepatitis, or chronic viral hepatitis flares can lead to overestimation of liver fibrosis (Arena 2008, Coco 2007, Sagir 2008). Other interfering conditions include cardiac failure, Valsalva manoeuvre, pulmonary hypertension, amyloidosis, pregnancy, cholestasis, or steatosis, with the latter being more relevant in HCV than in HBV (Arena 2008, Fraquelli 2007). Another relevant artefact is the examination of a patient within 2 hours after a meal, which increases resistance by up to 2 kPa (Mederacke 2009). However, this effect was not observed using CAP for the detection of steatosis (Silva 2019). Special probes have been developed to overcome problems with measurements in children and in obese patients (“S-probe”, “XL-probe”) (Engelmann 2011).

Table 3. Reasons and conditions for unreliable TE measurements

Confounder	Countermeasure	Comment
Obesity (BMI >30 kg/m ²)	Use XL probe	Cut-offs may be slightly lower with XL probe
Age >52 years		
Steatosis		Relevant only in HCV patients
Non-fasting	Re-measure after 3–6 h fasting period	
Cardiac failure	Reassessment after cardiac recompensation	
High necroinflammatory activity (AST/ALT ratio)	Reassessment after cessation of inflammatory flare	
Ascites	Use other non-invasive procedures such as ARFI, SSI or MR elastography, or re-measure after complete paracentesis	
Cholestasis	Decompression	Stiffness reduction in PSC is incomplete after stenting, but changes in stiffness during long-term follow-up are associated with severity of fibrosis and outcomes

Reliability

Common quality criteria applied to certify an acceptable quality of TE measurements are: 10 successful measurements with >60% successful measurements and an interquartile range (IQR)/median (M) ratio <0.30. However, the relevance of these criteria has been questioned, and a three-category classification system of reliability has been suggested: “very reliable” (IQR/M ≤0.10), “reliable” (0.10 < IQR/M ≤ 0.30, or IQR/M >0.30 with median liver stiffness <7.1 kPa), and “poorly reliable” (IQR/M >0.30 with median liver stiffness ≥7.1 kPa). Applying these categories to the clinical endpoint “cirrhosis” leads to the correct classification of 90.4%, 85.8% and 69.5% patients, respectively (Boursier 2012). In a large overview of 12,000 examinations 4% of measurements with the M-probe were not successful, and 17% were rated as unreliable (Castera 2010). Multivariate assessment of factors responsible for failure or unreliability were obesity and limited operator experience. Interestingly, not BMI in general but a lipohypertrophy in the thoracic belt in particular was the limiting factor for the success rate. It is important to note that the applicability of TE is limited to relatively lean patients (BMI <28 kg/m²), patients without ascites, and “cooperative” patients. The special “XL-probe” for obese patients has broadened the applicability of TE and is recommended for patients with a skin-capsule distance of >2.5 cm (but below 3.5 cm) (Myers 2011). Sequential use of M- and XL-probes show that XL-probes tend to produce higher stiffness measurement values in the same patient as compared to the M-probe, thus the former probes may overestimate liver stiffness in these patients.

Unlike liver histology, no published data is available on the variability (“sampling error”) of TE results. TE correlates well with other surrogate markers of liver fibrosis such as APRI and FIB-4 (Vidovic 2010). In patients with chronic liver disease eligible for TE, liver stiffness values correlate with the stage of fibrosis, irrespective of the underlying disease aetiology. TE has been evaluated in patients with chronic viral hepatitis, PBC, PSC, NASH, haemochromatosis, and Wilson disease. Due to high acceptance by patients, it can easily be used to monitor progression or regression of fibrosis in patients under observation or on therapy (Wilson 2006, Wong 2011). TE has been evaluated for the detection of liver fibrosis in patients with acute and chronic viral hepatitis and has also been positively evaluated for HCV/HIV-coinfected patients and in patients with HCV recurrence posttransplantation (Carrion 2006, de Ledinghen 2006, Maida 2007).

Cut-offs for liver fibrosis

Studies comparing TE with liver biopsy demonstrate both high sensitivity and specificity for the detection of advanced fibrosis and cirrhosis. However, TE performance is less reliable for the detection of fibrosis stages ≥F2 as compared to more advanced stages of liver fibrosis (sensitivity 56-67%),

resulting in moderate negative predictive values. Thus, the assessment of liver fibrosis by TE alone may result in the underestimation of liver fibrosis in some patients. Vice versa, if TE predicts significant fibrosis, a biopsy will not be necessary.

An authoritative meta-analysis that evaluated the predictive performance of TE in patients with chronic liver disease suggested that the optimal cut-off value for the diagnosis of significant fibrosis is 7.65 kPa and 13.0 kPa for cirrhosis (Friedrich-Rust 2008). For chronic liver diseases other than HCV the specific cut-off values for cirrhosis are 11.7 kPa in HBV, 10.3 kPa in non-alcoholic fatty liver disease, 17.9 kPa in biliary liver diseases, and 22.7 kPa in alcoholic liver disease if drinking and 12.5 kPa if abstinent (Trapper 2015).

A meta-analysis of the performance of TE in patients with alcoholic liver disease is less enthusiastic about the exactness of TE in this context and suggests to use both TE and liver biopsy sequentially in some cases to establish the correct fibrosis stage in all patients; the authors stress the importance of TE in ruling out cirrhosis or advanced fibrosis rather than defining exact fibrosis stages (Pavlov 2015).

Apparently different diseases have somewhat different cut-offs. However, rather than using fixed cut-offs the application of TE in a more continuous manner and follow-up procedure to assess changes in liver stiffness (Castera 2008). Whereas liver stiffness values >12.5 kPa are highly suggestive for advanced liver fibrosis or cirrhosis, patients with lower values (<7.5 kPa) are unlikely to suffer from advanced disease. Intermediate patients may qualify for liver puncture to clarify fibrosis stage if not answered by other non-invasive procedures.

Since the amount of data for TE and different liver disease etiology is continuously rising, we summarize available cut-offs and potential diagnostic consequences for clinicians in Table 4.

Table 4. Staging of liver fibrosis according to liver stiffness measurements in TE

Disease	Cut-off	Diagnosis	Consequence / comment	References
Chronic hepatitis C virus infection	6.8 - 7.6 kPa	Risk of F2 - F4	Antiviral therapy Liver biopsy	Talwalkar 2007 Nahon 2008
	13.1 kPa	F4	Check for portal hypertension HCC screening	Friedrich-Rust 2008
HIV/HCV co-infection	≥ 7.1 kPa ≥ 12.5 kPa	≥ F2 F4	TE outperforms APRI and FT	Castera 2014
Chronic hepatitis B virus infection	6.6 - 8.8 kPa > 11.7 kPa	≥ F2 F4		Chon 2012 Xu 2015
Chronic hepatitis D virus infection	> 14.0 kPa	Cirrhosis	HDV-infected patients tend to have higher TE values due to higher necroinflammatory activity	Da 2019
Non-alcoholic fatty liver disease	< 7.9 kPa ≥ 9.3 kPa	Rules out F3 fibrosis with 90% sensitivity and specificity High risk of F3 fibrosis with 90% sensitivity and specificity	M-probe with significantly lower cut-offs than XL-probe	Wong 2010
	14.3 kPa (M-probe) 16.8 kPa (XL-probe)	F4	Screen for complications	Oeda 2019
			In subjects with F0-F2 fibrosis, the rates of false-positive LSM results for F3-F4 fibrosis increased according to CAP tertiles (7.2% in lower versus 16.6% in middle versus 18.1% in higher)	Petta 2017
Alcohol-related liver disease	7.0 kPa 9.0 kPa 12.1 kPa 18.6 kPa	≥ F1 ≥ F2 ≥ F3 F4	12.1 kPa in patients with AST < 38 IU/L and bilirubin < 9 μmol/L 25.9 kPa in patients with AST > 75 IU/L and bilirubin > 16 μmol/L	Nguyen-Khac 2018
Primary biliary cholangitis	7.1 kPa 8.8 kPa 10.7 kPa 16.9 kPa	≥ F1 ≥ F2 ≥ F3 F4	Cut-off of 2.1 kPa per year was associated with an 8.4-fold increased risk of liver decompensation, liver transplantation, or death	Corpechot 2012
	<20 kPa and platelets >150,000/mm	No need for screening for esophageal varices		Moctezuma-Velazquez 2019

Disease	Cut-off	Diagnosis	Consequence / comment	References
Primary sclerosing cholangitis	7.4 kPa 8.6 kPa 9.6 kPa 14.4 kPa	≥ F1 ≥ F2 ≥ F3 = F4	Progression rate > 1.3 kPa per year is associated with decreased survival	Corpechot 2014
Autoimmune hepatitis	5.8 kPa 10.4 kPa 16.0 kPa	≥ F2 ≥ F3 Cirrhosis	Treatment response and fibrosis regression may be monitored by TE	Hartl 2016
Haemochromatosis	<6.4 kPa	Rules out significant fibrosis	Limited prospective data	Legros 2015
Wilson disease	≥ 9.9 - 10.1 kPa	Cirrhosis	Adult population	Paternostro 2020 Karlas 2012
			Liver stiffness is higher at diagnosis and decreases with treatment	Stefanescu 2016
Cystic fibrosis (CF)-associated liver disease	≥ 5.2 - 5.95 kPa	Suspicion of CF-associated liver diseases	Combination with APRI may yield higher diagnostic value	Lam 2019
Screening	9.1 kPa 9.5 kPa	≥ F2 in general population ≥ F2 in patients at risk for alcohol-related liver disease	Screening with TE is cost-effective	Serra-Buriell 2019
Portal hypertension	> 25 kPa	High risk for varices	Endoscopy screening for varices	Castera 2011 Robic 2012
HCC	> 25 kPa	> 45-fold increase for risk of developing HCC in viral hepatitis, but increased risk starts at 10.0 kPa	HCC screening	Masuzaki 2009 Fung 2011

Incremental increase of liver stiffness is associated with worse prognosis

Elastography may be used for monitoring stiffness changes over time. Rather than focusing at a given stiffness at a certain time point clinicians may use stiffness measurements for monitoring changes in liver stiffness. Studies highlighted that the consecutive increase of liver stiffness is related with higher mortality and liver-related events such as variceal bleeding or hepatic encephalopathy, especially in patients with liver stiffness >12.5 kPa (Perrez-Latorre 2016, Vergiol 2014).

Monitoring treatment with TE

TE may be used to monitor changes in liver stiffness following either the natural course or changes in stiffness on and after treatment. Whereas in the first scenario prediction of disease progression rates may be useful, the latter reflects the regression of inflammation and/or fibrosis. The longitudinal monitoring of patients with chronic HBV and HCV infections but also Wilson disease has documented reduction in liver stiffness upon treatment response (Andersen 2011, Fung 2011, Hezode 2011).

Correlation of liver stiffness with complications and outcome

In addition to the assessment of liver fibrosis stages, TE might be used to predict the presence of portal hypertension (Rockey 2008). Of note, a cut-off value of >25 kPa has been associated with a >45-fold increased risk of developing HCC in viral hepatitis. However, the risk seems to increase in a linear fashion starting from 10 kPa (Fung 2011, Masuzaki 2009). Furthermore, TE values >21 kPa are associated with portal hypertension as well as the risk of portal hypertension-related complications and indicate that endoscopy is indicated to assess oesophageal varices as well as the need for primary prophylaxis with non-selective β -blockers (Castera 2011, Robic 2011).

Combination of non-invasive tests

A combination of non-invasive tests in the form of surrogate markers, elastography methods or both have the potential of reducing the number of biopsies, lead prioritisation strategies for treatment and surveillance and predict morbidity and mortality. Despite a number of studies in this field we currently do not have a definite algorithm that is widely accepted in clinical practice. However, the WHO highlights the combination of APRI, FIB-4 and TE in order to identify patients at risk and to start treatment in HCV-infected patients (WHO 2014).

The current EASL guidelines for the use of non-invasive assessment of liver fibrosis suggest a number of distinct algorithms for different liver disease. The proposed algorithm for HCV patients is shown in Figure 1. The basic principle is that TE is combined with a serum marker test for liver fibrosis. Concordant results may reduce the need for biopsy while inconclusive results may be a reason for biopsy. The experience of the authors of this chapter is however that for the sake of determining the stage of liver fibrosis it is hardly ever necessary to perform biopsy unless other information is needed (e.g., evidence for autoimmune hepatitis); furthermore, patients are reluctant to undergo biopsy due to the widespread information on non-invasive alternatives.

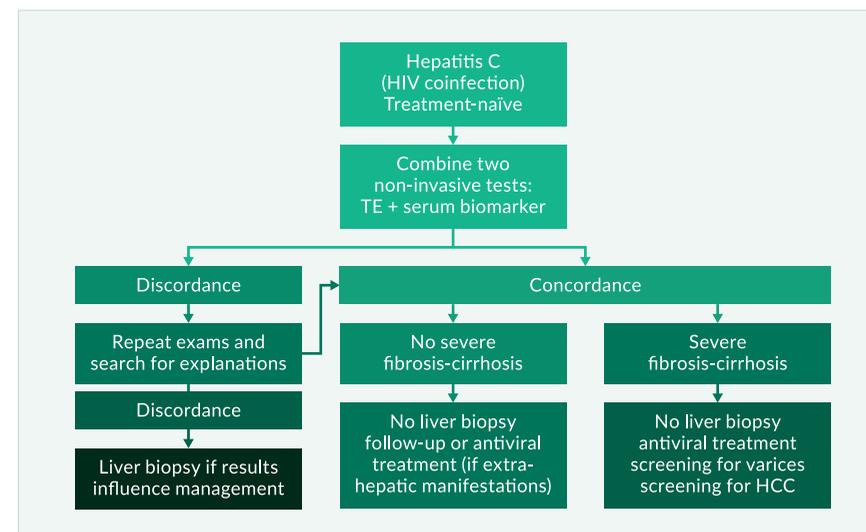


Figure 1. Assessment of liver fibrosis in chronic hepatitis C (EASL 2015)

Acoustic radiation force imaging (ARFI) and shear wave imaging (SSI)

Besides TE as the primary tool, shear wave technology to assess liver fibrosis, ARFI and SSI have now been more intensively studied for the assessment of fibrosis, cirrhosis and complications. ARFI and SSI both use a region of interest that can be adapted by the investigator. ARFI is implemented in Philips and Siemens ultrasound machines. Ideally, the region of interest (10×5 mm) is set 1–2 cm below the liver capsule. As in TE, ten sequential measurements are performed and the interquartile range is used to assess the accuracy of fibrosis evaluation. Although ARFI and SSI can be used in obese patients and patients with ascites, there is a subgroup of patients in whom reliable results may not be obtained (Cassinotto 2014), comprising up to 3% in ARFI cohorts and up to 11% in SSI studies (Cassinotto 2014). A recent meta-analysis reported that the accuracy for the prediction of fibrosis stages $\geq F_2$, $\geq F_3$ and cirrhosis were 0.87, 0.91 and 0.93, respectively (Friedrich-Rust 2012). A recently published head-to-head analysis comparing TE with ARFI showed comparable results for both methods (Colombo 2012). However, ARFI was less prone to methodological failure than TE. Both methods seem reliable for the detection of advanced fibrosis (Colombo 2012, Rizzo 2011, Sporea 2012). As with TE, many procedure- and patient-related factors may influence test results, in particular increased stiffness during hepatitis flares (Chen 2012, Karlas 2011).

Another shear wave-based technology has recently been introduced for the diagnosis of liver fibrosis (real-time SSI by Supersonic Imaging),

combining TE stiffness calculations with the possibility of defining regions of interest as in ARFI. While this method has not yet been widely used, early studies show a comparable diagnostic accuracy compared to TE (Ferraioli 2012a, Ferraioli 2012b). The comparison of all three methods (ARFI, TE, SSI) with liver biopsy in patients with fatty liver disease did not reveal substantial differences although SSI may be more reliable in the diagnosis of $>F_4$ fibrosis in these cases but no differences between SSI and TE or ARFI and TE have been reported in this context. Interestingly in this patient cohort the cut-offs were very close for SSI and TE and substantially lower than for patients with chronic viral hepatitis (6.3/6.2 kPa for $\geq F_2$, 8.3/8.2 kPa for $\geq F_3$, and 10.5/9.5 kPa for F_4 , respectively) (Cassinotto 2015). In principle these results were confirmed in unselected cases of patients with chronic liver disease (Gerber 2015).

Concerning data on correlation with histological fibrosis stages, TE, ARFI and SSI all suffer from the same limitations with overlapping ranges of stiffness results for individual fibrosis stages. However, they all seem adequate in detecting the presence of fibrosis and cirrhosis. Numerous comparisons have been made in order to detect an advantage of one machine over another. In the end none of these studies identified substantial differences for choosing one method over the other. A detailed critical review on available ultrasound methods with all pros and cons of each single methods has been published recently and is recommended for further reading (Ferraioli 2015).

Computational methods may improve the specificity and sensitivity for the diagnosis of liver fibrosis stages. For 2D-Shear wave elastography a neuronal network was applied and predictions were compared to histology stages. This approach may maximise sensitivity and specificity (Wang 2018), but predictions for lower fibrosis stages were not as markedly improved as for advanced fibrosis. These techniques highlight the potential of smart computer algorithms (“artificial intelligence”) to support non-invasive assessment of liver phenotypes.

Other imaging techniques for the assessment of liver fibrosis

A number of different imaging techniques such as conventional ultrasound, real-time elastography, portal venous transit time, MR imaging have been used for the assessment of liver fibrosis. None of these methods has yet achieved an overall clinical acceptance regarding the assessment of liver fibrosis, either due to low sensitivity and/or specificity, or high costs.

Clinical decision algorithms

Non-invasive markers for the staging of liver fibrosis are at the edge of replacing liver histology as the gold standard, at least in hepatitis C. This is due to the fact that outcome studies with clear endpoints like mortality are available (Vergniol 2011, Pakres 2010, Naveau 2009, Mayo 2008) or under investigation (NCT01241227, NCT02037867 and others). The advantages of these non-invasive tests in comparison to liver biopsy are striking. In order to overcome test limitations and to benefit from their specific advantages, a frequent strategy is to combine different non-invasive tests, using liver biopsy only in case of doubt. However, algorithms vary greatly in performance and acceptance. Whereas some authors have estimated a reduction in liver biopsies of 30%, others have estimated reductions of up to 80% (Leroy 2007, Sebastiani 2004, Sebastiani 2006, Sebastiani 2007). New strategies with sophisticated algorithms may overcome these limitations and a combination of TE with FibroMeter give results that may be detailed and reliable on liver fibrosis stage without any need for histology. However, only one study from France has described this method, which needs to be cross-validated by independent groups (Boursier 2011a, Boursier 2011b).

Population based screening for advanced liver fibrosis

Patients not being in regular care are diagnosed in late stages when liver decompensation or liver cancer develops. The diagnosis is rarely made in early stages-when liver fibrosis is mild to moderate but cirrhosis is not yet established-because the disease is asymptomatic. Liver biopsy is not a suitable procedure for population-based screening for fibrosis and/or fatty liver but non-invasive methods might be. A comprehensive survey on the frequency of liver fibrosis in population-based studies revealed progressive disease in up to 25.7% of participants (Harris 2017). In a population-based screening study using transient elastography, prevalence estimates of increased liver stiffness (≥ 6.8 , ≥ 8.0 , and 9.0 kPa) were 9.0%, 5.8%, and 3.6%, respectively. Elastography was more accurate than alanine aminotransferase, NAFLD fibrosis score, or FIB-4 (Caballería 2018). The minimum acceptable accuracy of non-invasive tests to diagnose cirrhosis with equivalence to liver biopsy depends on cirrhosis mortality and prevalence (Majumdar 2019): At 5% and 20% cirrhosis prevalence, sensitivities and specificities are 89% and 88%, and 94% and 85%, respectively (if the physician found it acceptable to subject 20 patients to biopsy to find one correct case with cirrhosis). Using these criteria, TE alone and TE plus FibroTest are the only existing tests that were better than or equal to biopsy at diagnosing cirrhosis at 5% prevalence (Majumdar 2019). In a recent cost-effectiveness guided approach it could

be demonstrated that a 9.1 kPa TE cut-off provided the best accuracy for the diagnosis of significant fibrosis (\geq F2) in general population settings, whereas a threshold of 9.5 kPa was optimal for populations at-risk of alcohol-related liver disease. TE with the proposed cut-offs outperformed fibrosis scores in terms of accuracy. Screening with TE was cost-effective with mean incremental cost-effectiveness ratios ranging from 2,570 €/QALY (95% CI 2,456-2,683) for a population at-risk of alcohol-related liver disease (age \geq 45 years) to 6,217 €/QALY (95% CI 5,832-6,601) in the general population. Overall, there was a 12% chance of TE screening being cost saving across countries and populations (Serra-Burriel 2019).

Non-alcoholic fatty liver disease

Nowadays virus-induced chronic liver diseases can be effectively treated by well tolerated antiviral drug regimens, but the epidemic of non-alcoholic fatty liver disease (NAFLD) demonstrates an exponential increase in burden of disease. The mechanisms contributing to hepatic steatosis and the development of an inflammatory state with progression to liver disease are under intense investigation. Patients with NAFLD, in particular with non-alcoholic steatohepatitis (NASH), suffer from an increased risk of advancing to progressive liver disease with fibrosis, eventually resulting in cirrhosis and the need for liver transplantation.

The challenges for the development non-invasive diagnostic strategies are:

1. to detect steatosis
2. to distinguish an indolent steatotic from the necoinflammatory state in NASH
3. to determine the stage of damage measured as fibrosis

As for liver fibrosis serological markers either as single markers or combined in scores are being evaluated in comparison to liver histopathology. It is debated whether non-invasive tests are comprehensive for the detection and classification of disease severity of NAFLD (Bedossa 2018; Castera 2018). It can be expected that non-invasive techniques will play a more important role in the diagnostic work-up of patients with fatty liver disease.

Serological markers of steatosis in NAFLD are also being evaluated. Similarly to the history of non-invasive fibrosis assessment, attempts are made to established steatosis-specific markers and scores that aim to diagnose steatosis by the combination of different parameters with more or less specificity for steatosis and inflammation in fatty liver disease.

Studies also aim to distinguish the necoinflammatory state, i.e. NASH,

from simple steatosis. A comprehensive review on this topic has been published by Vilar-Gomez and Chalasani (Vilar-Gomez 2018). Examples of specific markers for NAFLD/NASH are cytokeratin 18 and fibroblast growth factor 21, which are released into the circulation in response to oxidative stress, hepatocyte apoptosis and inflammation. However, a recent meta-analysis came to the conclusion that current non-invasive tests do not accurately differentiate NASH from simple steatosis (Verhaegh 2018). Further efforts are needed to identify more sensitive and specific markers and scores.

As for liver fibrosis, several studies aim to increase the diagnostic accuracy by combining different tests in NAFLD. In the case of NAFLD-associated fibrosis, the sequential use of liver stiffness measurement, NFS and FIB-4 has led to an improvement of correct classification. However, the best combination and sequence of markers is yet to be defined (Petta 2017). In the current German guideline, two scores that combine a number of factors have been included (Table 5). The guideline has also suggested an algorithm for the diagnostic work up of NAFLD (which needs more evaluation). It concludes that non-invasive steatosis assessment may be done by applying the FLI or MRI criteria. For the assessment of advanced fibrosis in NAFLD, the NFS score is applicable.

<https://www.dgvs.de/wissen-kompakt/leitlinien/leitlinien-der-dgvs/nash/>

Whereas the detection of liver fibrosis via TE is hampered by multiple factors, this may also be the case for serum markers of NAFLD. For instance, it could be shown that the NAFLD fibrosis score (and the FIB-4 score) is inaccurate, especially in patients aged 65+ (McPherson 2017). This may explain the results of a recent meta-analysis (Verhaegh 2018) that showed that the majority of non-invasive markers have no sufficient diagnostic value to reliably discriminate steatosis and NASH. As in liver fibrosis authors aim to further increase the diagnostic accuracy by combining different tests in fatty liver disease. In the case of liver fibrosis in NAFLD, the sequential use of liver stiffness measurement, NFS and FIB-4 lead to better classification. However, the best combination and sequence of markers is yet to be defined (Petta 2017).

Table 5. Non-comprehensive list of serological marker indices of steatosis

Index	Variables	Formula for calculation	Interpretation
Fatty liver Index (FLI)	BMI, GGT, triglycerides, waist circumference	$(e^{0.953 \cdot \log_e(\text{triglycerides})} + 0.139 \cdot \text{BMI} + 0.718 \cdot \log_e(\text{GGT}) + 0.053 \cdot \text{waist circumference} - 15.745) / (1 + e^{0.953 \cdot \log_e(\text{triglycerides})} + 0.139 \cdot \text{BMI} + 0.718 \cdot \log_e(\text{ggt}) + 0.053 \cdot \text{waist circumference} - 15.745) \cdot 100$	Cut off <30: Likelihood-ratio (LR) of 0.2 with a sensitivity of 82% that no steatosis is present Cut off had specificity of 86% and positive LR of 4.3 that steatosis is present
NAFLD fibrosis score (NFS)	Age, BMI, diabetes, AST, ALT, plt, albumin	$-1.675 + 0.037 \times \text{age (years)} + 0.094 \times \text{BMI (kg/m}^2) + 1.13 \times \text{IFG/diabetes (yes = 1, no = 0)} + 0.99 \times \text{AST/ALT ratio} - 0.013 \times \text{platelets (} \times 10^9/\text{l)} - 0.66 \times \text{albumin (g/dL)}$	Cut off < -1.455: Absence of significant fibrosis (93% certainty) Cut off > 0.676: Presence of significant fibrosis (90% certainty)

Plt = platelets

Can elastography reliably assess fibrosis in these patients and what are the precautions we need to consider?

Up to date there is no conclusive evidence that increased liver stiffness is a good predictor for the presence and severity of lipid storage in the liver in patients suspected of NAFLD. In addition, TE and other shear wave elastography methods can reliably predict cirrhosis (F4: sensitivity 92%, specificity 92%), but the detection of early fibrosis stages appears to be limited (Kwok 2014). Finally, elastography is not a reliable tool to detect necroinflammatory changes in the liver.

Controlled Attenuation Parameter (CAP)

Liver fat content has been historically assessed by ultrasound using a semi-quantitative estimate or by liver histology. Steatosis is a growing problem whether in the context of non-alcoholic steatohepatitis or as a co-factor in the metabolic syndrome and other liver diseases. Until recently reliable quantitative measures of the degree of steatosis were missing. A novel tool to overcome this diagnostic gap may be CAP. This analysis is available in TE machines from Echosens that measure the attenuation of the intensity of the echo from the ultrasound signal in the liver (available for the M-probe and the XL-probe). It is calculated only for reliable TE measurements and does not lengthen the TE procedure. The measurement is expressed in dB/m. Korean investigators defined a normal upper range of 266 dB/m in potential liver donors, all with histology proven fat contents <5%. However, in a less

strictly selected patient population defined as a “health check-up” cohort, a higher upper limit of normal of 288 dB/m was defined, which may be due to the inclusion of patients with diabetes (Chon 2014). A recent study defined histopathological categories of liver steatosis (S) grades (So: ≤10%, S1: 11 – 33%, S2: 34 – 66%, S3: ≥67%) and correlated these with CAP results. Using receiver operating statistics, the authors defined cut-offs with a sensitivity >90% for all grades of steatosis (215 dB/m for S ≥1, 252 dB/m for S ≥2, 296 dB/m for S3) (de Ledinghen 2012). In patients with chronic hepatitis C, corresponding cut-off values of 222 dB/m, 233 dB/m and 290 dB/m were identified for discriminating the steatosis grades (Sasso 2012).

As with TE, CAP results are also influenced by multiple factors and vary with the cause of the disease (de Ledinghen 2014). A recent meta-analysis focused on the confounders of high CAP values and found that besides NAFLD, diabetes and BMI are independently influencing CAP values. The authors also point out that the influence of these confounders may change according to the prevalence of steatosis in the studied population. According to their results in 2,735 patients, they determined the following cut-offs for >So, >S1 and >S2: 248, 268, and 280 dB/m, respectively (Karlas 2017). In contrast to TE, CAP measurements seem not to be influenced by recent meals.

Summary

Non-invasive tests have still not completely replaced liver biopsies, but smart combinations of non-invasive tools avoid this more invasive procedure in many patients. Whatever the current standard of care, the patient should be informed about the non-invasive tests, their applicability, and their limitations. The decision to perform a liver biopsy should ultimately be made together with the informed patient.

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18. Diagnosis, prognosis & therapy of hepatocellular carcinoma

Ulrich Spengler

Classification of HCC

Tumours are classified to stratify patients with respect to their survival prognosis, in order to select and offer optimised therapeutic options at any tumour stage. For hepatocellular carcinoma (HCC) the Barcelona Clinic Liver Cancer (BCLC) classification has been adopted as the international standard, which is recommended by both the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) (Table 1). The BCLC classification takes into account several aspects of the disease: the patient's general state of health, the severity of the liver disease as well as the extent of tumour spread (Llovet 1999). Patients in stages BCLC O and A have a considerably better prognosis than patients in advanced stages of liver cancer (Mazzaferro 1996). But roughly only 25% of patients with liver cancer are diagnosed at an early stage. Both EASL (EASL 2012) and AASLD guidelines provide recommendations regarding which therapy is best suited to treat patients at each stage of the BCLC classification. Unlike classification schemes in other types of malignancies, the BCLC classification is particularly helpful because it is entirely based on clinical parameters – molecular characteristics are not yet able to reliably assess individual prognosis of patients with HCC.

The BCLC classification seems to assess prognosis less accurately in Asian patients, where hepatitis B is a prevailing cause of liver cancer. An alternative classification, the Hong Kong Liver Cancer Staging System (HKLC), has been proposed recently, which had significantly better ability in Asian patients to distinguish subgroups with specific overall survival times (Yau 2014). Importantly HKLC identified subsets of patients with intermediate and advanced stages of liver cancer, who might benefit from more aggressive therapy (resection in intermediate stage, chemoembolisation in advanced stage). Nevertheless thus far, the HKLC classification is based exclusively on retrospective data from Asian patients in a single centre and still awaits confirmation by prospectively controlled studies and in non-Asian patients.

Table 1. Barcelona Clinic Liver Cancer (BCLC) Classification

Tumour stage	General state of health	Tumour characteristics	Child stage
0 Very early	Good	Single nodule <2 cm	A & B
A Early	Good	Single nodule <5 cm, 3 nodules <3 cm	A & B
B Intermediate	Good	Large, multiple nodules	A & B
C Advanced	Reduced	Vascular invasion, extrahepatic secondaries	A & B
D Terminal	Severely reduced	Any form	C

The Cancer of the Liver Italian Program (CLIP) has derived another widely used prognostic tool for HCC. The CLIP score combines features of macroscopic tumour morphology (unimodular versus multimodular with limited extension < 50% versus massive with extension > 50%), serum alpha-fetoprotein (AFP <400 ng/mL versus > 400 ng/mL), the Child-Pugh stage, and the presence of portal vein thrombosis to determine a prognostic score ranging from 0-6 (Anonymus 2000). Patients with advanced HCC and low serum levels of vascular endothelial growth factor (VEGF) or high levels of insulin-like growth factor I (IGF-I) have better survival at each disease state than those with serum levels in the opposite range. Thus, VEGF and IGF-I can be added to the CLIP score as an additional component referred to as V-CLIP or I-CLIP, respectively (Kaseb 2011a and 2011b).

The latest prognostic classification combines serum albumin and bilirubin alone (the ALBI score) and provides an easy-to-use, objective and discriminatory method for assessing liver functions in patients with HCC. Its validity has been confirmed in geographically distinct cohorts of patients with HCC either undergoing liver surgery for localised disease and sorafenib treatment for advanced disease (Johnson 2015).

Epidemiology

HCC constitutes the fifth most frequent form of cancer worldwide, and it holds the second place in malignancy-related mortality (Jemal 2011). Incidence and death rates of HCC are steadily rising in most parts of the world (about 2-3% per year). It occurs two to six times more frequently in men than in women. The key risk for HCC is liver cirrhosis, approximately 80% of which are related to hepatitis B and C on a global scale.

Chronic hepatitis B is the major risk factor for developing HCC in Africa and Asia, while in the US, Europe and Japan chronic hepatitis C, alcohol and non-alcoholic steatohepatitis (NASH) are leading causes of HCC. Eighty

percent of liver cancers are found in cirrhotic livers, which themselves carry a high risk for HCC. Chronic carriers of hepatitis B virus (HBV) have a 100-fold increased risk as compared to a non-infected healthy reference population. Recent reports from Taiwan indicate a direct link between HBV viral loads and the risk of developing liver cancer within 10 years (Chen 2006, Iloeje 2006). The risk of HCC is significantly increased once HBV-DNA exceeds 2000 IU/mL irrespective of the degree of hepatic inflammation. Quantitative HBsAg ≥ 1000 IU/mL is a further biomarker of increased HCC risk in patients with low or intermediate levels of HBV-DNA (Tseng 2013). The risk to develop HCC is higher in infection with HBV genotype C than B and also in infection with genotype D than A. Co-infection with HCV and HDV and/or exposure to environmental toxins such as aflatoxins and the algal toxin microcystin in drinking water further increase the risk of HCC.

Approximately 70 million people are infected with the hepatitis C virus worldwide, 20 to 30% of whom will develop liver cirrhosis, which carries a 3-5% annual risk of ultimately progressing to liver cancer. Unlike hepatitis B, a close relationship between HCV-RNA and the risk of developing HCC apparently does not exist (Bralet 2000). As a general rule patients will not develop liver cancer in chronic hepatitis C before their disease has progressed to advanced fibrosis and cirrhosis (Lok 2009). It appears that the risk of HCV-induced HCC related to the degree of inflammation and necrosis, while HBV-related HCC does not correlate well with inflammation and seems rather to involve activation of specific oncogenes by the virus.

Consumption of alcohol or tobacco enhances the risk of HCC (Donato 2002, Gelatti 2005). Beyond that, obesity (Calle 2003) and diabetes mellitus (Davila 2005) must be considered pivotal risk factors that can independently lead to liver cancer in Western countries and result in 4- to 40-fold increased HCC rates among patients with chronic viral hepatitis (Starley 2010). In patients with steatohepatitis, liver cancer can occur before cirrhosis has developed. Importantly, the risk of HCC is substantially reduced in diabetic patients who are treated with metformin (Lai 2012).

Finally, certain hereditary diseases such as haemochromatosis and alpha₁-antitrypsin deficiency predispose to HCC. Also genetic polymorphisms in the adiponutrin gene (rs 738409 C>G), in the KIF1B gene (rs 17401966), and the MICA gene (rs 2596542) seem to predispose patients with alcoholic and non-alcoholic fatty liver disease, chronic HBV and HCV infection, respectively, to develop cirrhosis and HCC (Fallet 2011, Nischalke 2011, Trepo 2013, Zhang 2010, Kumar 2011).

Surveillance of patients at high risk and early HCC diagnosis

Surveillance is cost effective if the expected HCC risk exceeds 1.5% per year in hepatitis C and 0.2% per year in hepatitis B. Simple clinical scores have been developed in hepatitis B (e.g., the REACH-B score) and hepatitis C (e.g., the HALT-C score) to assess when HCC surveillance becomes cost-effective (Chen 2013, Yuen 2009, Lok 2009). Surveillance has to be based on ultrasound examination at 6-month intervals. When 3- versus 6-month surveillance intervals were compared in a randomised study involving 1200 patients, there was no evidence that the shorter interval improved rates of early diagnosis and therapeutic outcomes. However, if patients with cirrhosis harbor nodular lesions, the 3-monthly control interval is preferred due to the high potential of malignancy and growth characteristics of such lesions (Yao 2006). Thus, nodules <1 cm, which usually are not HCC, should be monitored in 3-4 month intervals until they are proven to be stable or disappear (for up to 24 months). Nodules >1 cm should be evaluated with either 4-phase computed tomography (CT) or dynamic contrast-enhanced magnetic resonance imaging (MRI) as outlined in the section on diagnosis. Alpha-fetoprotein (AFP) has insufficient sensitivity and specificity, and thus is no longer recommended for HCC surveillance. Des-gamma-carboxy prothrombin (DCP), glycosylated AFP (AFP-L3), and glypican-3 are being evaluated with respect to HCC surveillance, and integrated as components of the GALAD score have outperformed ultrasound in a recent study suggesting that their combination with ultrasound might result in improved HCC surveillance of high risk patients (Yang 2019). The consistent use of ultrasound in patients with high risk for HCC enables us to diagnose carcinoma early in 30% of patients who then have a reasonable chance of curative therapy. On the other hand, Caucasian patients with low or no HBV activity are at low-risk for HCC, and surveillance is generally not recommended in such patients.

Diagnosis

Patients who develop HCC usually have no symptoms other than those related to the underlying chronic liver disease. However, in patients with sudden hepatic decompensation such as ascites, jaundice, hepatic encephalopathy or variceal bleeding often caused by portal vein thrombosis there is an increased likelihood of HCC. Occasionally patients develop paraneoplastic syndromes (hypoglycaemia, erythrocytosis, hypercalcaemia, severe watery diarrhoea, dermatomyositis and various

types of skin lesions), which apart from erythrocytosis herald a poor prognosis (Luo 2002). Plasma micro-RNAs are currently under evaluation as biomarkers for the non-invasive diagnosis of HCC at any stage (Borel 2012).

The diagnosis of HCC is made by detecting malignantly transformed hepatocytes in a liver biopsy or by dynamic contrast-enhanced radiological imaging techniques demonstrating intense arterial uptake followed by wash-out of contrast in the delayed venous phases reflecting arterialised perfusion of the tumour. Contrast-enhanced ultrasound may falsely suggest HCC in some patients with cholangiocarcinoma, and it should not be used as the only diagnostic tool for HCC (Vilana 2010). Nevertheless, novel diagnostic algorithms enable the diagnosis of HCC in a cirrhotic liver without histopathology or reference to elevated tumour markers.

The revised WHO classification distinguishes new specific subtypes of hepatocellular carcinoma (steatohepatic, clear cell type, macrotrabecular massive, chromophobefibrolamellar, scirrous, neutrophil-rich, lymphocyte-rich (WHO 2019). In particular the distinction between a dysplastic nodule and early HCC poses a particular challenge for the pathologist. Staining for glypican-3, heat shock protein 70, and glutamine synthetase is advised in this situation, and positivity for any two of these three markers confirms the presence of HCC (International Working Party 2009). Differentiation of HCC from cholangiocarcinoma may also require cell-type specific markers such as keratin-7, keratin-19, or CA 19-9.

Radiological diagnosis of HCC uses detection of hyper-vascularised nodular lesions. Contrast-enhanced computed tomography (CT) or nuclear magnetic spin resonance tomography (MRI) are considered to be equivalent diagnostic tools, and international consensus guidelines accept a diagnosis of HCC without histopathology, if the patient with a nodular lesion in a cirrhotic liver exhibits the following sequence of events: in the arterial phase, HCC enhances more intensely than the surrounding liver, because arterial blood in the liver is diluted by venous blood from the portal venous circulation, whereas HCC contains only arterial blood. In the venous phase, HCC enhances less than the liver, reflecting the fact that HCC does not have a portal venous blood supply and that the arterial blood flowing into the lesion no longer contains contrast. This phenomenon is termed “washout”. In the delayed phase “washout” persists, and occasionally HCC can only be detected in this phase of a dynamic study. Thus, a four-phase dynamic study is needed to reliably make a diagnosis of HCC (unenhanced, arterial, venous and delayed venous phases). Contrast enhancement in the early arterial phase, which disappears in the late venous phase, is highly specific for HCC.

Diffusion-weighted imaging (DWI) in MRI reflects water mobility in tissues, which is impeded in HCC tissue. Thus HCC results in signal hyperintensity within the tumour relative to the liver parenchyma. A recent meta-analysis provided evidence that DWI combined with dynamic

contrast-enhanced MRI performed significantly better than any of the two imaging techniques alone (Wu 2013). Hepatocyte-specific contrast agents such as gadoxate disodium and gadobenate dimeglumine are taken up by normal hepatocytes. Since most HCCs do not contain functional hepatocytes, signal hypointensity relative to the surrounding liver is observed in the hepatobiliary phase. As a consequence, hepatobiliary phase images are highly sensitive for HCC. However, this technique has only poor specificity (Bartolozzi 2013). Nodules with a hypointense signal in the hepatobiliary phase but without diagnostic features of HCC in the other phases may represent highly dysplastic nodules or early HCC and carry a high risk of progressing to conventional hypervascular HCC.

The current recommendations for diagnosis of HCC are summarised in Figure 1. For lesions smaller than 1 cm, detailed investigation is not recommended because most lesions will represent regenerative nodules rather than HCC. However, close follow-up in 3-month intervals should be offered using the same imaging technique that detected the lesion in the first place.

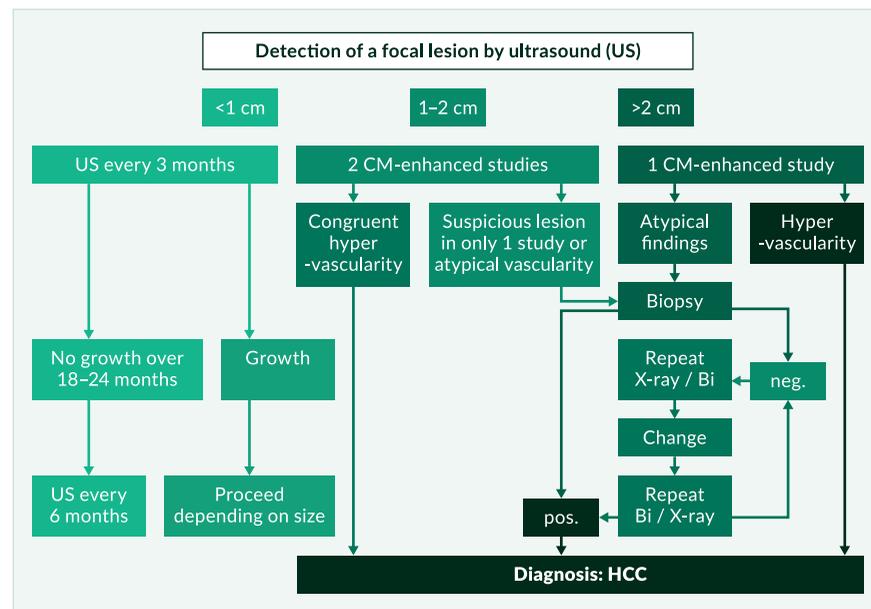


Figure 1. Diagnostic algorithm for the diagnosis of hepatocellular carcinoma depending on tumour size

For lesions larger than 1 cm, a guided biopsy of the lesion should be performed because diagnostic accuracy of radiological procedures declines with smaller liver tumours, while high (>90%) diagnostic sensitivity and specificity is maintained by histological analysis of biopsy specimens (Serste 2012). Alternatively, either dynamic MRI or multidetector CT scans

can be performed. If radiological findings are characteristic for HCC as described above, a firm diagnosis of HCC can be made and no further steps are necessary.

Contrast-enhanced CT and MRI exhibit excellent diagnostic sensitivity and specificity if the rules regarding early hypervascularity and washout are strictly applied. The presence of arterial hypervascularisation alone is not sufficient for a diagnosis of HCC, which requires the presence of venous washout as an essential second diagnostic component. In equivocal situations the diagnosis must be clarified by biopsies, which may have to be repeated within a short period of time.

Radiological assessment of treatment responses should not be based on tumour size alone but apply modified Response Evaluation Criteria in Solid Tumours (mRECIST) (Lencioni 2010). High quality arterial-phase imaging is required for this purpose. In general, MRI is preferred over CT owing to its superior tissue contrast resolution and sensitivity to detect both the tumour and post-treatment changes. Using contrast-enhanced techniques, absence of uptake within the tumour is considered to reflect necrosis, while persisting uptake indicates vital tumourous tissue. Rim contrast enhancement after ablative loco-regional therapy is not indicative of viable tumour, unless contrast enhancement also reveals nodular or thick uptake along the tumour margins or a clear wash-out (Chung 2012, Riaz 2009). Tumour recurrence is signaled by the re-appearance of vascular enhancement.

Stage-adapted therapy for liver cancer

The two key factors that are most important in determining a patient's prognosis and potential treatment options are the tumour mass and hepatic functional reserve. Patients with early HCC have excellent chances for curative cancer treatment. They can achieve 5-year survival rates of 50-70% by surgical resection, liver transplantation or percutaneous ablative procedures. With more advanced HCC, local transarterial embolisation and multikinase inhibitor therapy can still prolong life. Figure 2 gives a summary and concise overview of stage-adapted therapy for hepatocellular carcinoma.

Potentially curative therapy in BCLC stages 0-A

Surgical resection constitutes the backbone of curative treatment in patients with early HCC. It is the treatment of choice in patients with localised tumour spread and small-sized cancers and tumours in a non-cirrhotic liver

(evidence grade IIIA). Prognosis after surgical resection is excellent, if the tumour is not larger than 2 cm in diameter (5-year survival rates 70-90% with rates of tumour recurrence below 10%). Excluding patients with poor liver function keeps perioperative mortality below 5%. Favourable criteria for surgical resection comprise single nodules less than 5 cm in size or a maximum of 3 nodules in a single liver lobe. Patients should be carefully selected to diminish the risk of postoperative liver failure. Patients should have only moderately impaired liver function (Child's stage A cirrhosis), should not have portal hypertension (hepatic-portal-vein pressure gradient >10 mm Hg, presence of oesophageal varices or splenomegaly together with reduced platelet counts <100,000/ μ l) and should have a serum bilirubin in the normal range. Patients with tumour invasion of a major portal or hepatic vein, direct invasion of neighboring organs other than the gallbladder, peritoneal disease, and nodal or distant secondaries are not candidates for surgery.

Potentially curative partial hepatectomy is the optimal treatment for HCC in patients with adequate hepatic functional reserve. Right hemihepatectomy in cirrhotic patients has a higher risk of inducing hepatic decompensation than left hemihepatectomy. Non-anatomic resection may be necessary to minimise loss of functional liver parenchyma. Operative mortality for HCC is related to the severity of liver disease, and patients with complications of cirrhosis such as marked portal hypertension, ascites or bleeding have insufficient hepatic reserve to withstand resection. Most deaths are due to postoperative liver failure and < 10% are related to complications of bleeding. Ninety-day mortality rates appear a more reliable indicator of outcomes than 30-day perioperative mortality, especially in patients with extended resections and resections of cirrhotic livers, since progressive jaundice, ascites and eventually death develop slowly and well after 30 days in patients with marginal residual liver function. Of note, common prognostic tools, e.g. the Child-Pugh Classification or the Model for End-stage Liver Disease (MELD) score, are not adequate to identify patients with insufficient hepatic functional reserve after resection. Volume and function of the residual liver remnant can be determined by hepatic volumetry which is best performed before and after portal vein embolisation. Also CLIP and ALBI scores help to assess the hepatic functional reserve and risk of surgical resection. Because hepatic regeneration is impaired in cirrhosis, resection in general should not exceed 25% of the liver parenchyma. Preoperative portal vein embolisation can be used in selected patients to increase the volume of the liver remnant prior to major liver resections, particularly for right-sided tumours, because it initiates hypertrophy and allows for more extensive resections (Abulkhir 2008, Leung 2014). Selective arterial chemoembolisation (TACE) has been recommended as a complementary procedure prior to portal vein

embolisation because it reduces arterial blood supply to the tumour and also embolises potential arterioportal shunts (Yoo 2011).

Liver transplantation is an alternative therapeutic option, if the liver cancer cannot be cured by local resection due to anatomical reasons, if residual liver function after resection is anticipated to be poor, or if there is multi-nodular tumour spread into both liver lobes (grade IIIA evidence). Virtually all patients considered for liver transplantation are unresectable due to the degree of liver dysfunction rather than tumour extent. Commonly, patients with HCC are selected for liver transplantation according to the so-called Milan criteria, i.e., the patient has a single nodule of less than 5 cm in diameter or at most 3 nodules, none of which exceeds 3 cm in diameter (Mazzaferro 1996). Patients who meet the Milan criteria usually achieve survival rates of 80% and 70% one and five years after liver transplantation. However, it has been demonstrated that selected patients with more extensive stages of liver cancer can be transplanted with reasonable long-term outcomes (Yao 2001). Selection of patients according to the San Francisco criteria comprises solitary large nodules up to 6.5 cm as well as multi-nodular HCC with a maximum of 3 nodules, each of which must be smaller than 4.5 cm with a total sum of all nodule diameters less than 8 cm. Patients who remain within these extended selection criteria can still reach 70-80% five-year survival rates after liver transplantation. However, there is very limited data to support extending selection criteria for liver transplantation any further (Pomfret 2010).

A central issue in liver transplantation is the process of fair organ allocation. Shortage of donor organs is particularly critical in patients with liver cancer, because the tumour will continue to expand while the patient is on the waiting list, and can ultimately reach a stage that makes liver transplantation a futile option. It has been estimated that after one year on the waiting list, approximately 40% of patients can no longer be cured by liver transplantation (Poon 2007). In the Eurotransplant registry donor livers are allocated to patients according to their MELD scores. To circumvent the problem that patients with early HCC who are eligible for liver transplantation have rather low MELD scores, Eurotransplant accepts the diagnosis of HCC within the Milan criteria as so-called standard exemption, allocating additional points on top of the patient's lab MELD score in an incremental time-dependent fashion.

EASL/EORTC guidelines recommend to treat liver cancers locally when the expected time on the waiting list exceeds 6 months (EASL/EORTC 2012). Bridging therapy can be done by transarterial chemoembolisation, radiofrequency ablation or partial resection. This strategy probably also facilitates patient selection for liver transplantation, because those with stable disease after chemoembolisation achieve a greater than 90% five-year survival rate after liver transplantation, while only 35% of patients in

the group with progressive tumour expansion survive five years post-liver transplantation (Otto 2006).

Sirolimus, an inhibitor of mammalian target of rapamycin (mTor inhibitor) seems to be a promising immunosuppressive agent in liver transplantation of HCC, because it has antiproliferative activity against HCC *in vitro* and *in vivo* and can interfere with vascular endothelial growth factor (VEGF). Several early reports suggested a lower risk of posttransplant HCC recurrence with the use of sirolimus, and a registry-based comparison of 2491 adult patients with liver cancer, who underwent transplantation, versus 12,167 liver transplantations for other diagnoses suggested a posttransplant survival benefit for the use of sirolimus, that was specific to patients transplanted for HCC (Toso 2010). In support, a recent meta-analysis suggested that sirolimus-based regimens significantly decreased overall tumour recurrence rates and recurrence-associated mortality (Menon 2013). Although these data are encouraging, the International Consensus Conference on Liver Transplantation for HCC does not yet generally recommend sirolimus for transplantation in HCC, since available data are entirely derived from retrospective studies (Clavien 2012). Everolimus, a semisynthetic form of sirolimus may have similar effects as sirolimus but has not been studied adequately in patients with HCC. Side effects of sirolimus comprise thrombosis of the hepatic artery, delayed wound healing, incisional hernias, hyperlipidaemia, bone marrow suppression, mouth ulcers, skin rashes, albuminuria, and pneumonitis. Because of their side effect profile, in particular hepatic artery thrombosis, mTor inhibitors should not be used in the first three months after liver transplantation.

Non-surgical local procedures: Image-guided ablation is recommended for patients with early HCC when surgical options are precluded.

Radiofrequency ablation (RFA) is currently considered the standard technique, because most clinical data are available for RFA: A cohort study on percutaneous radiofrequency ablation demonstrated that complete ablation of lesions smaller than 2 cm is possible in more than 90% of patients with local recurrence in less than 1% (Livraghi 2008). In larger tumours, five-year survival rates are somewhat lower, at 70–80% for nodules less than 3 cm in diameter, and 50% for tumours between 3 and 5 cm (Lopez 2006). A cumulative meta-analysis has suggested that survival is better after radio frequency ablation than after ethanol injection (Cho 2009). In up to a third of patients a self-limited postablation syndrome has been reported after RFA which was associated with fever, malaise, chills, right upper quadrant pain, nausea and elevated liver enzymes (Dodd 2005). RFA is avoided for lesions in the hepatic dome or along the inferior liver edge to avoid diaphragmatic injury or intestinal perforation. In addition to size the local efficacy is also affected by the proximity of a lesion to large

blood vessels (Lu 2005), probably because the blood flow carries away heat from the lesions (the “heat sink” phenomenon). Following RFA gas bubbles may form in the liver as a result of treatment and should not be mistaken for infection or infarction (Park 2008). Although RFA is relatively well tolerated, severe and potentially fatal complications can occur, e.g. liver abscess, pleural effusion, pneumothorax and skin burns, subcapsular hepatic hematoma and needle tract seeding of tumour cells (Takaki, 2013). Outcomes of RFA are superior to percutaneous ethanol injection and may be equivalent to surgery in small tumours.

Some alternative treatment modalities have recently attracted attention because they may overcome some of the limitations associated with RFA.

Microwave ablation (MWA) can generate very high temperatures in the tumour tissue in a very short time. This can potentially lead to enhanced treatment efficacy and larger ablation zones and can reduce susceptibility to heat dispersion by blood flow in major vessels (Boutros 2010).

Cryoablation refers to methods, which destroy tissue by local freezing or alternating freezing and thawing. Rapid tissue freezing and thawing produce a cytotoxic effect by disrupting cellular membranes and inducing cell death. The cryolesion is hypochogenic and can be visualised and monitored by intraoperative ultrasound. Cryoablation can lead to equivalent treatment outcomes as RFA (Wang 2015). However, meanwhile most centres have abandoned cryoablation, because other techniques, e.g. RFA are technically easier to do, may potentially be associated with less local recurrence and lower complication rates.

Irreversible electroporation (IRE) induces cell death by repeated application of short-duration high-voltage electrical pulses, which irreversibly injure cellular membranes. Although hyperthermic effects may occur with high power applications, cell death associated with IRE is induced non-thermally. Hence, cooling owing to high perfusion is not a problem with this technique (Scheffer 2014). However, general anesthesia with neuromuscular blockade and cardiac gating to prevent arrhythmias are required. Other energy-based ablation treatment approaches comprise **laser induced thermal therapy (LITT)** and **high-intensity focused ultrasound (HIFU)**. Efficacy and safety of HIFU for primary or recurrent HCC has been predominantly studied in Hong Kong and appeared similar to outcomes with RFA. However, clinical experience outside of China is rather limited, since only a few centres worldwide have adopted these techniques. Thus, the place of HIFU is currently undefined.

Adjuvant therapy, in the context of resection, liver transplantation or local-ablative procedures, does seem to offer additional benefits. Thus far, antiviral treatment of hepatitis B with nucleos(t)ide analogs remains the single approved treatment after removal or local destruction of HCC. Interestingly, one study (Su 2014) reported that recurrence-free survival

and overall survival were significantly better in 9,461 Taiwanese patients who had liver resections for HBV-associated HCC between 1997 and 2011, when they were on anti-platelet therapy.

A randomised phase 3 trial involving 1,114 HCC patients after liver resection or local ablation, who were randomised to receive either sorafenib or placebo for 4 years or until tumour recurrence (STORM trial), did not meet its primary and secondary endpoints of recurrence-free survival, time to recurrence or overall survival (Bruix 2015). Positive reports are available from phase 2 trials with transarterial radioactive ¹³¹-iodine, capecitabine, heparanase and thalidomide. However, confirmatory phase 3 data are not yet available for any of these agents.

Tumour recurrence is frequent after putatively curative treatment of HCC. Although there is no generally accepted consensus on posttreatment surveillance, most centres apply CT or MRI imaging every 3 to 6 months for first two years after therapy, then annually, and if initially elevated, also recommend monitoring serum AFP every 3 months for first two years, then every 6 months (Clavien 2012). Most HCC recurrences are intrahepatic and reflect local recurrence or a new second primary lesion (Hatzaras 2014). The best predictors of HCC recurrence are high serum alpha-fetoprotein levels (AFP >500 ng/mL), microvascular invasion and/or additional tumour sites besides the primary lesion. Solitary nodules might be amenable to repeat resection, but HCC recurrence is frequently multifocal owing to intrahepatic dissemination of the tumour. Some patients with HCC recurrence after primary resection might benefit from salvage transplantation. The role of HBV infection for HCC recurrence after resection is under debate (Sun 2007, Cescon 2009, Char 2014), and early HCC recurrence has been reported to be even greater in hepatitis C infected patients than HBV infected patients (Utsunomiya 2015). Therapy with antiviral drugs seems to reduce late (\geq 2 years) HCC recurrence in chronic hepatitis B and C but does not seem to have much effect on early HCC recurrences (Yin 2013, Huang 2015). The effects of direct antiviral therapy in patients with HCV-related HCC is not yet clear, since rapid recurrence and expansion of HCCs have been reported to occur shortly after DAA therapy, even when the primary HCC had been “cured” quite some time before (Conti 2016, Kozbial 2016, Reig 2016).

Palliative therapy in BCLC stages B and C

Palliative treatment remains the only therapeutic option for patients with advanced stages of liver cancer that cannot be controlled by local therapy.

Arterial chemoembolisation is the most frequent palliative intervention offered to patients with HCC and is considered for patients with

non-surgical HCC who are also not suited for percutaneous ablation and do not have extrahepatic tumour spread. HCC exhibits intense neoangiogenic activity, so that even well-differentiated HCCs become highly dependent on arterial blood supply. Thus, hepatic arterial obstruction is performed either by angiographic transarterial embolisation or transarterial chemoembolisation. Usually lipiodol combined with an embolising agent such as gelatin or microspheres is mixed with cytostatic drugs and applied to the liver via an intra-arterial catheter. Suitable cytotoxic agents are doxorubicin, mitomycin and cis-platinum, but the optimal combination of drugs and treatment schedules has not been established. In randomised studies demonstrating a benefit of chemoembolisation, doxorubicin or cis-platinum was administered in 3-4 angiographic sessions per year. Chemoembolisation carries the risk of ischemic damage to the liver, potentially leading to fulminant liver failure. To minimise this risk chemoembolisation should be offered only to patients with good residual hepatic function, who have asymptomatic multi-nodular liver cancer without vascular invasion or extrahepatic tumour spread. Vice versa patients with decompensated liver disease (liver cirrhosis, Child's B or C) or imminent hepatic failure should not undergo chemoembolisation. Table 2 lists absolute and relative contraindications for chemoembolisation.

Table 2. Contraindications for transarterial chemotherapy for HCC

Absolute contraindications:
Macrovascular invasion of the portal vein with thrombus in the main portal vein and/or portal vein obstruction
Hepatic encephalopathy
Biliary obstruction
Liver cirrhosis stage Child-Pugh C
Relative contraindications:
Serum bilirubin > 2 mg/dL
Lactate dehydrogenase > 425 U/L
Aspartate aminotransferase > 100 U/L
Tumour mass > 50% of the liver
Cardiac or renal insufficiency
Severe thrombocytopenia
Transjugular intrahepatic portosystemic shunt TIPS
Complications of portal hypertension such as ascites or gastrointestinal bleeding

The side effects of interarterial chemoembolisation are the same as for systemic chemotherapy and consist of nausea, vomiting, bone marrow depression, alopecia and renal damage. TACE is a risk factor for hepatitis

B virus reactivation and antiviral prophylaxis is recommended in HBsAg positive patients. Common ischemic complications comprise a hepatic abscess, acute cholecystitis and damage to biliary tracts. Interstitial pneumonitis and gastrointestinal ulcerations due to abnormal shunting may occur owing to radiation injury. Pulmonary or cerebral lipiodol embolisations are rare but potentially fatal complications. Overall, treatment-related mortality rates are about 2%. As a frequent complication of hepatic ischaemia, more than 50% of patients also develop a so-called post-embolisation syndrome with fever, abdominal pain and a moderate degree of ileus. Fasting and fluid replacement is mandatory, but the post-embolisation syndrome is usually self-limited and patients can be discharged safely after 2 days.

Objective response rates vary between 16% and 60%, but less than 2% of patients achieve complete remission. Residual tumour cells recover their blood supply and the tumours continue to grow. Thus, repeated therapy may be needed. However, multiple courses can increase death from liver failure despite good tumour reduction; thus, counterbalancing the potential survival benefits from repeated treatment. TACE should be limited to the minimum number of interventions needed to control tumour growth.

Chemoembolisation is currently considered to significantly improve survival in suitable palliative patients (Llovet 2002). Beyond that, combination therapy with TACE and RFA appears to be the most efficient treatment of early HCC (Lan 2016) and is used as bridging therapy for HCC patients on the waiting list for liver transplantation. However, its use in patients allocated to curative resection it is not recommended, because surgical complication rates are increased thereafter.

DEB TACE (transarterial chemoembolisation using drug eluting beads) constitutes a modification of chemoembolisation, where embolising particles act as carriers and are loaded *in vitro* with cytotoxic agents such as doxorubicin. In addition to their ischemic effects, drug-eluting beads release the drug into the tumour microenvironment in a slow and controlled fashion, thus potentially enhancing their antitumoural activity. While the clinical response to DEB chemoembolisation is rather similar to conventional chemoembolisation, systemic exposure to chemotherapy is apparently reduced; in particular, biliary side effects are less frequent and left ventricular function better preserved (Vogl 2011). Conversely, treatment-associated gastrointestinal adverse effects appear to be more frequent in DEB-TACE than in conventional chemoembolisation. Meanwhile, also irinotecan-eluting beads are being studied.

Radiotherapy with Yttrium-90 microspheres has been developed as a novel alternative palliative treatment of liver cancer with unexpectedly impressive anti-tumoural activity in selected individual cases (Sangro 2006, Jacobs 2007, Salem 2006, Liu 2004). Very small particles (25 – 45 µm) made

of glass (TheraSpheres®) or resin (SIR-Spheres®) serve as sealed sources of the radio-emitting isotope 90-yttrium (90Y). Microspheres are injected during hepatic angiography and ultimately lodge in the abnormal tumour vessels. To avoid misplacement of microspheres into extrahepatic territories a thorough angiographic evaluation comprising injection of 99Tc macro-aggregated albumin is necessary prior to treatment in order to detect and eventually occlude aberrant vessels, and to also assess hepatopulmonary shunting. Of note, unlike chemoembolisation, small microspheres do not occlude the blood vessels and can also be applied in the presence of portal vein thrombosis. Radioembolisation potently induces tumour necrosis. However, the therapeutic response to 90Y-radiotherapy is delayed; the median time to develop necrosis (reduced contrast enhancement) and tumour shrinkage are approximately 30 and 120 days, respectively (Keppke 2007). Furthermore, heterogenous contrast enhancement in a perivascular distribution of a 90Y-treated liver segment or lobe reflects radiation injury and should not be interpreted as tumour progression (Riaz 2009). In a randomised controlled, prospective phase 2 study on 45 HCC patients in BCLC stages A and B, 90Y radioembolisation resulted in significantly longer times to progression than chemoembolisation (>26 months versus 6.8 months, Salem 2016).

Randomised controlled trials comparing radioembolisation to other treatment strategies are not yet available. However there is accumulating good evidence from several well-characterised large cohort studies (Hilgard 2010, Salem 2010, Sangro 2011, Mazzaferro 2013). Taking into account tumour stage, intermediate tumour stage patients treated by radioembolisation achieve 16 to 18 months of median survival time (Salem 2010, Sangro 2011, Mazzaferro 2013). Adverse events, response rates and time to progression appeared improved while overall survival was equivalent when radioembolisation was compared to chemoembolisation (Salem 2011). When downstaging to transplantation is allowed by local regulations, radioembolisation outperforms chemoembolisation (Lewandowski 2009). Finally, a randomised controlled phase 3 trial in 467 patients comparing radioembolisation to sorafenib chemotherapy did not reveal any significant survival difference between the two treatment arms (SIRT: 8.0 months versus sorafenib 9.9 months; $p=0.18$) (Villain 2017).

Systemic chemotherapy with conventional anti-cancer drugs does not seem to offer survival benefits, whether given as a single agent or as part of combination chemotherapy (Llovet 2003). Likewise, anti-hormonal therapy with tamoxifen or octreotide has not provided improved patient survival when studied under controlled conditions (Gallo 2006, Yuen 2002).

Systemic palliative HCC therapies

Molecular-targeted therapeutic strategies offer new hope for effective palliative therapy in liver cancer. **Sorafenib (Nexavar®)** is an orally available multi-kinase inhibitor acting on several distinct tyrosine kinases (VEGFR2, PDGFR, c-kit receptor) as well as on serine/threonine kinases (b-Raf and p38). Thus, by inhibiting angiogenesis and cellular proliferation, sorafenib can block two of the major signalling pathways of HCC expansion. In a phase 3 study (the SHARP trial) involving 602 patients, sorafenib 400 mg BID was moderately well-tolerated and associated with improved survival in 44% of patients resulting in 3 months extended survival in treated patients (10.7 months in the sorafenib arm versus 7.9 months in the control arm). The efficacy of sorafenib has been confirmed in a second randomised placebo-controlled trial, mostly involving patients with HBV-associated HCC (Cheng 2009) and in 1586 patients of the GIDEON (Global Investigation of Therapeutic Decisions in Hepatocellular Carcinoma and of its Treatment with Sorafenib) prospective database (Lencioni 2012). Sorafenib has established itself as the first option in patients with HCC who can no longer be treated with local therapies. The SHARP trial largely included patients with preserved liver function. Although the pharmacologic profile is favourable, data in Child-Pugh class B patients are scarce (Abou Alfa 2011). Patients with liver cirrhosis Child class C, however, do not achieve a survival benefit from sorafenib and should only receive best supportive care. Diarrhea, weight loss, hand-foot syndrome and rash, hypertension, renal toxicity with hypophosphataemia, thromboembolism, bleeding, cardiotoxicity, thyroid dysfunction, pruritus, alopecia, impaired wound healing and hepatotoxicity are important side effects of sorafenib. Sorafenib has also been associated with fulminant hepatic toxicity, which is characterised by elevated aminotransferases, coagulopathy and hyperbilirubinaemia. Sorafenib is apparently particularly effective in HCC related to chronic hepatitis C. However, its role for treatment of recurrent HCC after liver transplantation currently remains still undefined. Sorafenib can be safely combined with chemoembolisation therapy (Pawlik 2011) but this combination apparently does not provide any clinical benefit. Likewise, in the SORAMIC study the combination of sorafenib with 90 Yttrium radiotherapy (SIRT) did not result in better survival than sorafenib alone. However, certain patient subgroups, e.g. young patients, non-cirrhotic patients or those with a non-alcoholic aetiology, may still benefit from a SIRT/sorafenib combination treatment.

Lenvatinib (Lenvima®), is an inhibitor of VEGF receptors 1-3, FGF receptors 1-4, PDGF receptor α , RET and KIT and shows activity in hepatocellular carcinoma. The phase III REFLECT study comparing lenvatinib (8-12mg/d) to sorafenib (400 mg twice daily) in untreated

patients with advanced hepatocellular carcinoma revealed that lenvatinib was not inferior to sorafenib and improved survival to 13.6 (12.1-14.9) months (sorafenib 12.3 (10.4-13.99 months n.s.) (Kudo 2017). Quality of life scores deteriorated in both treatment groups after treatment with rather similar toxicity profiles: However, patients, who received lenvatinib, experienced fewer instances of palmar-plantar erythrodysesthesia, diarrhoea and alopecia but more instances of arterial hypertension, proteinuria, dysphonia, and hypothyroidism. In summary, lenvatinib has been approved in Japan, Europe and the US as a second, first-line treatment option in patients with advanced hepatocellular carcinoma. The safety of lenvatinib and its use in combination regimens is further evaluated in multiple ongoing studies.

Other antagonists targeting VEGFR, EGFR, ERBB2, Akt-mTor or Wnt/ β -catenin signal transmission pathways have been evaluated in HCC. However, sunitinib, brivanib, linifanib, tivantinib, or the combination of erlotinib with sorafenib, everolimus and ramucirumab, all have failed to demonstrate relevant survival benefits.

Regorafenib (Stivarga®) is a small molecule multikinase inhibitor with structural analogy to sorafenib. Regorafenib targets VEGF receptors 1-3, TIE2, PDGFR β , FGFR, RET, KIT, RAF kinase and MAPK thus intensively inhibits several pathways involved in angiogenesis, oncogenesis, metastasis and tumor immunity. In the RESORCE phase 3 trial regorafenib met its primary study endpoints and revealed prolonged survival (10.6 versus 7.8 months) and better disease control than placebo in patients who had failed on sorafenib (Bruix 2016). Thus, regorafenib has recently been licensed for HCC patients progressing on first-line drug treatment. The most common adverse effects of regorafenib were rash and hand-foot syndrome, hypertension, increased AST, and hyperbilirubinemia. Similar to sorafenib skin toxicity with regorafenib was associated with improved overall survival (Bruix 2018). Of note, re-analysis of the data from the REFLECT study, where 75% of patients subsequently were treated with sorafenib, suggests that sorafenib may offer an alternative second-line treatment strategy for patients with HCC who had received lenvatinib as a first-line drug.

The multikinase inhibitor **cabozantinib (Cabometyx®)** is active against VEGFR2, c-MET, and AXL, as well as RET, Kit and FLT3. Beyond angiogenesis and oncogenesis inhibited kinases are implicated also in pathways of resistance to VEGFR inhibitors such as sorafenib. Consequentially cabozantinib was tested as a second-line treatment versus placebo in 707 patients with advanced HCC who received up to 2 prior system treatment regimens (including sorafenib) and who had disease progression (CELESTIAL trial) (Abou-Alfa GKI 2018). In this study Cabozantinib substantially improved overall survival versus placebo (median 10.2 versus 8 months) and the benefit was more pronounced when patients had received

sorafenib as the only prior therapy (11.3 versus 7.2 months). Cabozantinib also achieved greater progression-free survival (5.2 versus 1.9 months), and thus has become licensed as a second-line treatment option for patients failing on or intolerant to sorafenib. Dose reductions were frequent in the treatment arm (63%), often caused by side effects (16%) such as hand-foot skin reaction, hypertension, elevated liver enzymes, fatigue, diarrhoea, asthenia and decreased appetite. Thus, poor tolerability of cabozantinib may limit its use in clinical practice.

Ramucirumab (**Cyramca**[®]) is a humanized monoclonal antibody which selectively inhibits VEGFR2 and showed activity against HCC in early trials. Of note, the REACH trial of ramucirumab against placebo indicated a survival benefit particularly for the subgroup of HCC patients with elevated AFP levels (Zhu 2015). This observation formed the basis for the biomarker-driven REACH-2 trial, which evaluated ramucirumab versus placebo in advanced HCC patients with failure of or intolerance to sorafenib and high AFP (≥ 400 ng/ml) (Zhu 2019). REACH-2 met its primary study endpoint and confirmed that ramucirumab improved overall survival (8.5 versus 7.3 months, $p=0.02$) and progression free survival (2.8 versus 1.5 months, $p<0.0001$). A pooled safety and efficacy analysis of the REACH-2 trial with the patients who had AFP levels ≥ 400 ng/ml in the REACH study confirmed these findings (Zhou 2018), so that ramucirumab has been licensed as a biomarker-controlled second-line treatment for the subgroup of HCC patients with high AFP. Ramucirumab has a manageable safety profile with hypertension and hyponatremia as the most common side effects, and on the other hand revealed declines in disease-related symptoms, making it a second-line drug demonstrating both improved survival and quality of life.

Immune-based therapy. Currently cancer immunotherapy has become encouraging because monoclonal antibodies (mAbs), which block molecules that negatively regulate T-cell responses, can reverse T-cell exhaustion and reconstitute anti-tumour immunity (Prieto 2015). Immune checkpoint inhibitors, such as ipilimumab (anti-CTLA-4), nivolumab (anti-PDL-1) and pembrolizumab (anti-PD-1) have already received approval from regulatory agencies for therapy of malignant melanoma, lung and renal cancer. Checkpoint inhibitors reactivate the exhausted antitumour response and can result in an objective and maintained immune control of tumour growth. Initial data from the CheckMate 040 study, an open-label phase 1/2 dose escalation and expansion trial with intravenous bi-weekly application of the PD-L1 antagonist nivolumab, reported 20% objective response rates across all underlying etiologies of liver cancer (El-Khoueiry 2017). However, the results of a randomised controlled phase 3 trial, CheckMate 459, did not reach its primary study endpoint. The PD-1 inhibitor pembrolizumab induced complete remission, in 1% and partial remission in 16.3% of 104 patients with advanced liver cancer, who had disease progression on

sorafenib in the KEYNOTE-224 phase 2 study but likewise failed to reach its primary study endpoint in the KEYNOTE-240 trial. Based on their phase II data both nivolumab (**Opdivo**[®]) and pembrolizumab (**Keytruda**[®]) were licensed for patients with advanced HCC in the US but not in Europe. However, both antibodies are further evaluated as components in various combination rescue studies for patients with progressive HCC.

The spectrum of adverse effects associated with nivolumab and pembrolizumab comprises a variety of autoimmune and graft-versus-host-disease like reactions such as skin disease, diarrhoea, thyroiditis and autoimmune-like hepatitis but overall side effects appear still to be acceptable.

Recently, a phase III study comparing the combination of monoclonal antibodies atezolizumab and bevacizumab versus sorafenib (IMbrave150 study; Cheng AL et al. ESMO Asia 2019) has created new hope, because the combination resulted in substantially improved survival, delay of disease progression and quality of life across almost all groups of patients at acceptable adverse effects. However, patients with liver cancer of non-viral etiology appeared to have less benefit from this novel systemic treatment option.

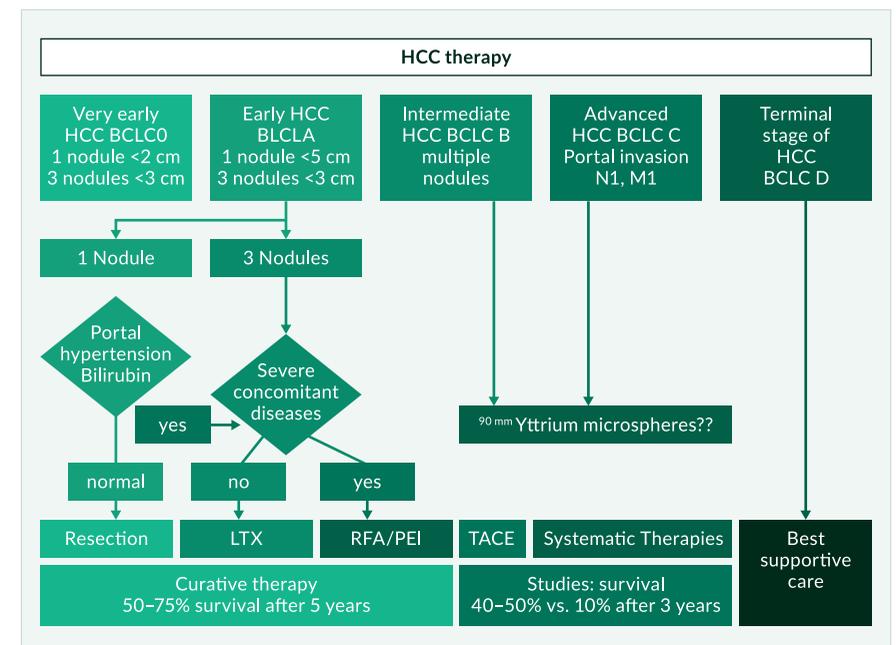


Figure 2. Overview of stage-adapted therapy of liver cancer relative to the BCLC criteria. *Systemic therapy comprises Sorafenib and Lenvatinib as first line options, Regorafenib, Cabozantinib, and Ramucirumab as further options after failure or intolerance of first line drug therapies.

Prophylaxis of liver cancer

Despite conspicuous progress in the diagnosis and therapy of HCC, the prognosis of HCC has not improved very much over time. Thus, prophylactic measures are of pivotal importance. HBV vaccination, now recommended by many national vaccination councils, has been proven in Taiwan to markedly reduce HBV infection rates along with the incidence of HCC as a complication of chronic hepatitis B in later life (Lok 2004).

Patients with chronic HBV and patients with chronic hepatitis C should be offered antiviral therapy as effective secondary prophylaxis of HCC. Although HBe antigen positive (van Zonneveld 2004) and HBe antigen negative patients with chronic hepatitis B showed reduced incidence rates of HCC when successfully treated with interferon (Papatheoridis 2001, Brunetto 2002, Lampertico 2003), antiviral therapy with nucleos(t)ide analogs seems to reduce the risk of HCC less convincingly (Papatheoridis 2010, Papatheoridis 2011). Newer, more potent nucleos(t)ide analogs such as entecavir seem to reduce the risk of HBV-associated liver cancer more potently, particularly in high risk patient groups (Hosaka 2012). Systematic analysis of the available data suggests that HBV-treatment can reduce the relative HCC risk by about 60%. Also, several meta-analyses suggest that successful interferon therapy will reduce the risk of HCC in chronic hepatitis C (Camma 2001, Papatheoridis 2001a, Veldt 2004). Despite some initial confusion on the role of the newly available directly acting antiviral drugs in hepatitis C concerning HCC prevention it has meanwhile become clear that rates of HCC development are substantially diminished after DAA therapy (Carrat 2019). Nevertheless, patients who have cirrhosis and/or long disease duration prior to antiviral therapy should be followed in HCC surveillance programs, since their risk of liver cancer remains still high even after achieving a sustained virological response (Yu 2006, Van der Meer 2012, Aleman 2013).

Improving additional risk factors such as obesity and poorly controlled diabetes mellitus may further reduce the risk of HCC development: weight reduction and exercise improve the prognosis of steatohepatitis, and metformin and thiazolidinedione should be favoured over sulfonylurea drugs in the treatment of diabetes (Greten 2013). The use of aspirin but not other nonsteroidal antiinflammatory drugs was associated with a decreased risk of HCC in a US Diet and Health study (Sahasrabudde 2012), and several studies suggest that use of statins leads to a lower risk of HCC (Singh 2013, Shi 2014, Hsiang 2015). Finally, daily consumption of two or more cups of coffee reduces the risk of HCC by 40-50% in patients with chronic viral hepatitis (Gelatti 2005, Bravi 2007, Larsson 2007, Wakai 2007).

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19. Transplant hepatology: a comprehensive update

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Introduction

Over the past 30 years major advances have been made in the field of organ transplantation due to improvements in surgical techniques and organ conservation as well as optimisation of intensive care and immunosuppressive management. This chapter focuses on important issues in the field of transplant hepatology and may provide helpful information to physicians involved in the care of adult liver transplantation (LT) recipients. It includes indications for LT, current organ allocation policy, pretransplant evaluation, management while on the waiting list, living donor liver transplantation (LDLT) and management of early and long-term complications post-LT.

Timing and indications for liver transplantation

Appropriate selection of candidates and timing of LT is crucial in reducing mortality and improving outcomes in LT recipients. A patient is considered too healthy to undergo LT if the expected survival is longer without surgery. Therefore, criteria are needed in order to select patients with priority for LT who can most benefit from transplantation. In 2002, the Organ Procurement and Transplantation Network along with the United Network of Organ Sharing (UNOS) developed a system based on the model for end-stage liver disease (MELD) (Table 1) to prioritise patients on the waiting list. In the Eurotransplant countries, the Child-Pugh Turcotte (CPT) score was replaced by the MELD score in December 2006.

The lab MELD score using the three laboratory parameters depicted in

Table 1 ranges from 6 (less ill) to 40 (severely ill). It estimates mortality in patients with end stage liver disease within 90 days (Kwong 2015). The MELD score is used for candidates 12 years of age or older and the Paediatric End Stage Liver Disease Model (PELD) score is used for patients <12 years of age. In a large study (Merion 2005) looking at the survival benefit of LT candidates, those transplanted with a MELD score <15 had a significantly higher mortality risk as compared to those remaining on the waiting list, while candidates with a MELD score of 18 or higher had a significant transplant benefit.

Table 1. Calculation of the MELD* Score

MELD Score =	$10 \times (0,957 \times \ln [\text{creatinine mg/dL}] + 0,378 \times \ln [\text{total bilirubine mg/dL}] + 1,12 \times \ln [\text{INR}^{**}] + 0,643)$
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*Model of End-stage Liver Disease, **International Normalized Ratio

The MELD score does not accurately predict mortality in approximately 15–20% of patients. Therefore MELD-based allocation allows exceptions for patients whose score may not reflect the severity of their liver disease. These exceptions include hepatocellular carcinoma (HCC), non-metastatic hepatoblastoma, adult polycystic liver degeneration, primary hyperoxaluria type 1, small-for-size syndrome, cystic fibrosis, familial amyloid polyneuropathy, hepatopulmonary syndrome, portopulmonary hypertension, urea cycle disorders, hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu disease), hemangioendothelioma of the liver, biliary sepsis, primary sclerosing cholangitis (PSC) and cholangiocarcinoma. Patients with standard exceptions will be assigned a higher MELD score (match MELD) than that assigned by the patient's laboratory test results (lab MELD). Consequently, this resulted in an increasing proportion of patients transplanted for HCC and other exceptions over time (Massie 2011).

MELD has proved to be accurate as a predictor of waiting list mortality, but has shown to be less accurate in predicting posttransplant outcome (Kaltenborn 2015). For instance, MELD allocation resulted in decreased waiting list mortality; whereas posttransplant morbidity has increased due to transplantation of a higher proportion of sicker recipients with MELD scores >30 (Dutkowski 2011). Moreover, the quality of donor organs has been impaired over the last two decades (Schlitt 2011).

Creatinine values exert a systematic bias against women due to their lower creatinine values conditioning a longer waiting time for an organ (Rodríguez-Castro 2014). Thus women are disadvantaged by use of MELD score in terms of access to LT. The question has been raised whether additional candidate characteristics should be explicitly incorporated into the prioritisation of waiting list candidates (Sharma 2012). It has also been suggested to take into account not only pretransplant mortality but also

donor-related factors for estimation of the donor risk index (DRI) (Feng 2006) and posttransplant mortality. Furthermore, standardisation of laboratory assays and variants of MELD including incorporation of parameters such as sodium or cholinesterase have been proposed to overcome the limitations of the current scoring system (Choi 2009, Weissmüller 2008, Vitale 2012). The Hong Kong transplant group aimed to establish additional criteria to predict short-term mortality in severe flares of chronic hepatitis B virus (HBV) infection (Fung 2019). Their results revealed that HBV-infected patients with MELD ≥ 28 should be worked up for LT, and those with MELD 28–32 with 3–4 at-risk criteria (age ≥ 52 years, ALT >217 U/L, platelets <127, and abnormal baseline imaging), or MELD ≥ 32 should be listed.

UNOS made a policy change and revised the MELD scoring system on January 11, 2016 by incorporating the serum sodium value because patients with hyponatremia have significantly higher mortality rates compared with those with normal serum sodium levels.

Candidates for LT must have irreversible acute or chronic end-stage liver disease. Hepatitis C virus (HCV) or alcohol-induced liver (ALD) disease have been the most common disease indications in adults with liver cirrhosis (<https://www.eltr.org>) during the last decades (Figure 1). Non-alcoholic fatty liver disease (NAFLD) is a frequent aetiology of liver disease in western countries and has become a leading indication for LT in the United States (US) and Europe; whereas the proportion of transplant waitlist additions for HCV-associated disease has declined since the introduction of interferon-free, direct-acting antiviral (DAA) therapy (Cotter 2019). Data from the UNOS and Organ Procurement and Transplantation Network registry from 2004 through 2013 revealed that the number of adults with non-alcoholic steatohepatitis (NASH) awaiting LT has almost tripled since 2004 (Wong 2015).

Other indications include cholestatic liver disorders (primary biliary cirrhosis [PBC], PSC), HBV infection, autoimmune hepatitis (AIH), inherited metabolic diseases (Wilson's Disease, haemochromatosis, α -1-antitrypsin deficiency), HCC, and acute or acute-on-chronic hepatic failure. In children, biliary atresia and metabolic liver diseases are the most common indications. Contraindications for LT include active alcohol and drug abuse, extrahepatic malignancies, sepsis, uncontrolled pulmonary hypertension, and coexistent medical disorders such as severe cardiopulmonary condition, technical or anatomical barriers such as thrombosis of the entire portal and superior mesenteric venous system. Previous malignancy history must be carefully considered and likelihood of recurrence estimated.



Figure 1. Indications for liver transplantation (LT). Primary diseases leading to LT in Europe, 1988–2015 (Data kindly provided from European Liver Transplant Registry, <https://www.eltr.org>)

PBC = primary biliary cholangitis **SBC** = secondary biliary cirrhosis

Patient evaluation

Evaluation of a potential transplant candidate is a complex and time-consuming process that requires a multidisciplinary approach. Requirements for evaluation may differ slightly between transplant centres. The evaluation process must identify extrahepatic diseases that may exclude the patient from transplantation or require treatment before surgical intervention. The protocol we use for evaluation of potential transplant candidates is shown in Table 2.

Pre-transplant management issues

In cases of recurrent variceal hemorrhage despite prior interventional endoscopic therapy (and non-selective beta-blockade) or refractory ascites, transjugular intrahepatic portosystemic shunts (TIPS) have been used to lower portal pressure and as bridging therapy for transplant candidates. The identification of predisposing factors and medication such as lactulose and rifaximin, a minimally absorbed antibiotic, are effective for prophylaxis and management of hepatic encephalopathy (HE) (Mullen 2014).

Hepatorenal syndrome (HRS) represents a complication of end-stage liver disease and is a risk factor for acute kidney injury (AKI) in the early post-operative phase (Saner 2011, Saner 2012). It is classified into type 1 HRS characterised by a rapid impairment of renal function with a poor prognosis; type 2 HRS is a moderate steady renal impairment. Vasoconstrictors including terlipressin in combination with volume expansion are commonly used and have been shown to be effective for restoration of arterial blood flow and serve as bridging therapy to LT (Hinze 2013). Extracorporeal liver support systems based on exchange or detoxification of albumin have been successfully employed in indicated cases. In case a recipient is on Molecular Adsorbent Recirculation System (MARS) therapy the centre uses the bilirubine and creatinine values measured most prior to initiation of MARS treatment (<https://eurotransplant.org/cms/mediaobject.php?file=H5+ELAS+MELD+Oct+20161.pdf>). This lab MELD under MARS therapy is valid for 7 days irrespective of the height of the lab MELD. After 7 days, a reconfirmation can be made.

Beyond MELD, other parameters such as frailty and sarcopenia might be essential to consider suitable patients for the waiting list. Sarcopenia is part of the frailty complex present in cirrhotic patients. According to the operational definition by the European Working Group on Sarcopenia in Older People (EWGSOP), the diagnosis of sarcopenia comprises the presence of both low muscle mass and low muscle function in terms of low muscle strength or low physical performance. Muscle wasting is considered one of the major complications of end-stage liver cirrhosis and may be caused by a variety of factors such as reduced nutrient intake, dietary restrictions in sodium and water in decompensated liver disease, reduced protein intake for hepatic encephalopathy, reduced intestinal absorption secondary to maldigestion caused by pancreatic exocrine insufficiency or to intestinal bacterial overgrowth due to small bowel motility disorders and a hypermetabolic state with increased energy consumption and high protein catabolism.

Sarcopenia was highly associated with waitlist mortality and negative perioperative outcome (Kahn 2018; Meeks 2017). This was in particular an issue in patients who were listed with low priority based on a low MELD score (van Vugt 2017).

After waitlisting, laboratory values must be updated according to the recertification schedule shown in Table 3.

Table 2. Basic (not exhausted) evaluation protocol for potential transplant candidates

Physical examination
Diagnostic tests (baseline laboratory testing; serologic, tumour/virologic, and microbiological screening; coagulation tests, autoantibodies; thyroid function tests)
Abdominal ultrasound with vascular Doppler/Duplex
Abdominal MRI or computer tomography (CT) scan
Chest X-rays
Electrocardiogram (ECG), cardio CT in patients ≥ 50 years or > 2 cardiological risk factors, coronary angiography only if indicated and after cardio CT, Swan-Ganz catheterisation, Doppler/Duplex carotid arteries
Upper and lower endoscopy
Pulmonary function testing
Mammography (in females > 50 years)
Physician consultations (anesthesiologist, gynecologist, urologist, cardiologist, neurologist, dentist, ear, nose, and throat specialist)
A meticulous psychosocial case review (medical specialist in psychosomatic medicine, psychiatry or psychology)

Table 3. Recertification schedule of MELD data

Score	Recertification	Lab values
≥ 25	every 7 days	≤ 48 hours old
24–19	every 30 days	≤ 7 days old
18–11	every 90 days	≤ 14 days old
≤ 10	every year	≤ 30 days old

Special attention regarding specific, disease-related therapy prior to surgery should be given to transplant candidates undergoing LT for HCC or virally-related liver diseases.

Waiting list monitoring of patients with ALD

ALD is currently the most common indication for LT in many European and US LT centers. The 6-month abstinence requirement (the so-called '6-month rule') is a common practise requiring candidates abstinent from alcohol for at least 6 months to be eligible for transplant.

ALD is associated with a lower risk of waitlist removal for deterioration (HR 0.84, 95%CI 0.81–0.86, $p < 0.001$) and a higher risk of waitlist removal for improvement (HR 2.91, 95%CI 2.35–3.61 $p < 0.001$) as compared to non-ALD (Giard 2019).

Alcoholic hepatitis (AH) represents a subpopulation of patients with ALD

with short term mortality approaching 70% in severe cases. The thresholds for amount and duration of alcohol use leading to severe AH (SAH) are not clearly defined. However, an average consumption of more than 40 g per day for women and 50–60 g per day for men are estimated minimum thresholds for the diagnosis of SAH. Heavy alcohol use has usually occurred for > 6 months (typically for several years) with < 2 months of abstinence before clinical presentation of jaundice.

Until recently, LT as a treatment for SAH has been a taboo in most transplant centers owing to concerns about the limited organ supply and the risk that the SAH liver recipient will return to harmful drinking. Moreover, there has been a controversial discussion in literature about LT in SAH (Fung 2017, Lucey 2017, Barosa 2017, Daswani 2018, Kubiliun 2018, Lee 2018, Marroni 2018, Zhu 2018, Mitchell 2019, Thursz 2018), and this issue has been debated in national and international conferences and liver societies (Addolorato 2016, Martin 2014, EASL CPG 2018: management of alcohol-related liver disease, Graziadei 2016).

The change in attitude has been launched by a French-Belgian study group (Mathurin 2011) which favoured early LT in SAH as a reasonable rescue option for patients who failed to respond to conservative therapy. The authors selected patients who had no prior episodes of AH and had scores ≥ 0.45 according to the Lille model or rapid deterioration of liver function despite medical therapy. Only patients were selected who had family support, no severe comorbidities and were committed to alcohol abstinence. Only 2.9% of available grafts were considered for this indication. The cumulative 6-month survival rate (\pm SE) was significantly higher among patients undergoing early LT than among those who were not placed on the waiting list ($77 \pm 8\%$ vs. $23 \pm 8\%$, $P < 0.001$). This was also true through 2 years of follow-up (hazard ratio, 6.08; $P = 0.004$). Three patients had an alcohol relapse at 720 days, 740 days, and 1140 days after LT.

A lively international debate about the selection criteria in patients with ALD was sparked in 2012. An advantage of the 6-month period of abstinence before listing is avoidance of unnecessary LT in patients who will spontaneously improve and a commitment of the patient to abstinence giving the opportunity to implement preventive strategies against future relapse episodes (Im 2019). Arguments in favour for LT is the risk of death in patients with severe ALD/AH, the fact that the 6-month rule as a single predictor of abstinence is debatable and may discriminate patients with favourable prognosis and low risk of recurrence.

Suggested predictors of recurrence include positive family history of substance use, alcohol-related comorbidity, failed rehabilitation attempts, history of prior alcohol-related legal issues, history of substance abuse (other than alcohol), lack of social support, lack of familiar support, denial of drug-related problems and addiction length and intensity of ALD. Prognostic

instruments used to predict future drinking after LT include the University of Michigan Alcoholism Prognosis score, the Alcohol Relapse Risk score, the High Risk Alcoholism Relapse (HRAR) score and the Stanford Integrated Psychosocial Assessment for Transplantation (SIPAT) (Im 2019). However these scores were not specifically developed for the LT setting. Therefore, Lee et al. (b) (2019) developed a new prognostic score (SALT score) using 4 pretransplant variables to identify AH candidates at low risk for alcohol relapse after early LT. A multidisciplinary approach including psychosocial and medical assessment and integration of an addiction specialist may be a crucial prerequisite to properly determine suitability of the ALD patient for LT.

Results of several studies and retrospective analyses resulted in a paradigm shift in therapy for highly selected patients with SAH who are not responding to medical therapy. The UNOS, the EASL Clinical Practice Guideline on alcohol-related liver disease (2018) and the American College of Gastroenterology (ACG) Clinical Guideline (Singal 2018) therefore suggest that the decision for waitlisting should not be based only on the 6-month abstinence rule. Presently, in case of non-response to conservative therapy, highly selected patients can therefore be considered for early LT in various European countries and the majority of US transplant centers (Thursz 2019, Antonini 2018, Testino 2014).

Addiction rehabilitation programs should be implemented prior to LT, and post-LT contracting, for alcohol after care and counseling should be considered in patients who are too sick to attend pretransplant rehabilitation treatment.

Management of patients with ALD in the context of LT is an ongoing debate in Germany. According to legally binding guidelines of the German Medical Association abstinence must be proven by negative urine ethyl glucuronide (uETG) tests (and hair-ETG/carbohydrate-deficient Transferrin (CDT) if applicable) during the 6 months before possible waitlisting (https://www.bundesaerztekammer.de/fileadmin/user_upload/downloads/pdf-Ordner/RL/RiliOrgaWIOvLeberTx20190924.pdf). Furthermore, a positive psychiatric assessment with potential recommendations for psychotherapeutic measures is mandatory before listing. As soon as a patient is on the waiting list due to ALD, ETG testing is required at every visit in the LT outpatient clinic (at least every 3 months).

The majority of patients with severe SAH already reveal cirrhotic changes of the liver in terms of acute on chronic liver failure and do not meet the 6-months rule. In exceptional urgent cases the transplant conference of the corresponding German LT center can deviate from the 6-months rule (https://www.bundesaerztekammer.de/fileadmin/user_upload/downloads/pdf-Ordner/RL/RiliOrgaWIOvLeberTx20190924.pdf). This presupposes a request by the transplant center for an alcohol audit which

is carried out by a committee of specialists nominated by the German Medical Association. Eurotransplant organizes the audit process consisting of 3 auditors who give an expert opinion (independently of each other). A positive vote is achieved if all 3 auditors agree to an exceptional listing. However, after completion of the audit process the transplant conference takes the final decision to list or not to list the patient

Psychosocial interventions should be routinely used in the medical management of ALD prior to and after LT (EASL CPG: Liver transplantation [2016]). Once listed, patients with ALD should be monitored for alcohol use by clinical interviewing and random biochemical testing. The specific biochemical test used in different countries and transplant centers will depend on availability, program resources and costs. Currently, anticraving drugs (except baclofen) and disulfiram are not recommended in patients with advanced ALD, because of the potential side effects and insufficient experience in this population.

Waiting list monitoring of hepatitis B liver transplant candidates

The goal of antiviral therapy in HBV patients on the waiting list is to achieve viral suppression to undetectable HBV DNA levels using sensitive tests (Figure 2) (Cornberg 2011, Beckebaum 2013a). Several studies have demonstrated clinical benefits in patients with decompensated cirrhosis with viral suppression as reflected by a decrease in CPT score, improvement of liver values and resolution of clinical complications (Kapoor 2000, Schiff 2007). Moreover, initiation of nucleos(t)ide analogue (NUC) treatment prior to LT has markedly reduced HBV recurrence posttransplantation.

Due to frequent emergence of mutations in the YMDD motif of the DNA polymerase during long-term lamivudine (LAM) therapy (Beckebaum 2008, Beckebaum 2009), potent nucleos(t)ide analogs (entecavir [ETV], tenofovir disoproxil fumarate [TDF]) or tenofovir alafenamide [TAF], the latter with reduced impact on markers of renal function and bone mineralisation) with a high resistance barrier have become the standard antiviral treatment.

Lactic acidosis has been reported to occur in NUC-treated patients (particularly ETV) with highly impaired liver function (Lange 2009). However, more recent studies have found that NUCs have been associated with low rates of lactic acidosis and other serious adverse events such as impairment of renal function, osteopenia and osteoporosis (Ridruejo 2012).

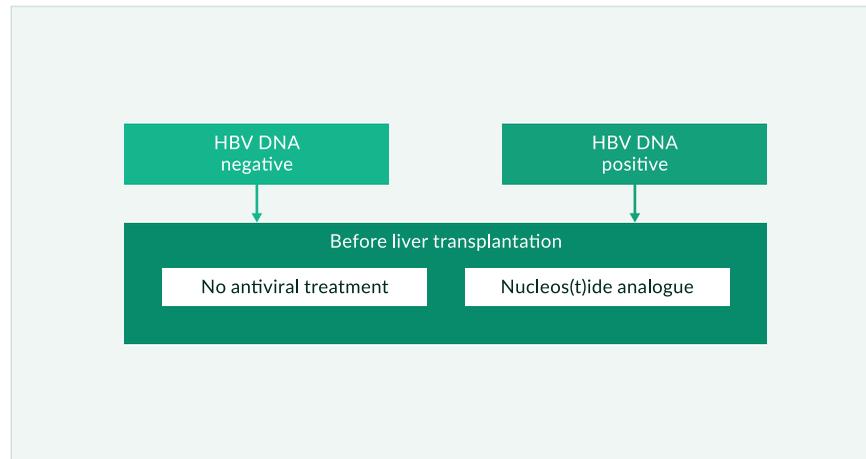


Figure 2. Management of HBV patients prior to liver transplantation (LT). In all viremic patients awaiting LT due to HBV-related liver damage, efficient antiviral therapy is required. Suppression of HBV DNA may lead to clinical stabilisation resulting in removal from the waiting list or in a delay in the need for LT (Beckebaum 2012).

Waiting list monitoring and treatment of hepatitis C liver transplant candidates

Waitlisted patients who have a viral response on antiviral therapy have a better outcome after LT (Picciotto 2007). The combination of direct-acting antivirals (DAAs) (Kumar 2014, Coilly 2016) has been a major step forward in transplant hepatology. NS5A inhibitors such as pibrentasvir (PIB), ledipasvir (LDV), velpatasvir (VEL), elbasvir (EBV), NS5B inhibitors such as sofosbuvir (SOF), and protease inhibitors (PI) such as grazoprevir (GZR), glecaprevir (GLE) and voxilaprevir (VOX) are well-tolerated DAAs for treatment of HCV patients on the waiting list and after LT. Daclatasvir combined with SOF is not costeffective and therefore not any more a treatment option in Germany. Discontinuation of simeprevir has been announced by the manufacturing company due to a significant decline in utilisation and the availability of more effective therapies, such as pangenotypic combination regimens. Paritaprevir and ombitasvir are no longer distributed in Germany.

High response rates, improved tolerability and fewer side effects of the new drugs allow antiviral therapy of patients who could not be treated in the interferon era or showed low response rates in interferon-based antiviral therapies. It has to be considered that HCC surveillance remains an issue in cirrhotic patients irrespective of whether a virological eradication was achieved or not.

Coordinators of European Liver and Intestine Transplant Association (ELITA) consensus statements determined serum HCV RNA negative status for at least 1 month before LT as a reliable virological endpoint for prevention

of recurrent HCV infection posttransplant (Belli 2017). Data collected by the ELITA showed that antiviral therapy allows for a long-term improvement of liver function and the delisting of one-third of treated patients (Perricone 2018). Risk of liver-related complications after delisting was low. A treatment period of 3 months was recommended as a minimum time period before inactivation and delisting. Process of delisting mainly depends on MELD score, albumin and clinical improvements after 12 weeks of therapy.

Improvement of hepatic function due to successful HCV treatment prior to LT may negate or delay the need for LT in some patients, which is crucial given the scarcity of donor organs and mortality on the waiting list. Data for primary LT candidates obtained from the Organ Procurement and Transplantation Network database showed decreasing mortality and disease severity in hepatitis C patients on the LT waiting list in the United States (Kwong 2017). Similar developments have been observed in Europe. However, there is a proportion of patients who show no change or worsening of their MELD score despite successful HCV therapy. This may result in lowering the priority of LT (MELD score reduction) without improving quality of life, thereby delaying potentially curative LT. Therefore, robust predictors of improvement in hepatic function and quality of life are currently being investigated to identify patients with decompensated cirrhosis who benefit from DAA therapy prior to LT. El-Sherif (2018) et al. performed a retrospective analysis of data from 4 trials determining the impact of SOF-based therapy in HCV patients with CPT class B (n=502) and CPT class C (n=120). Patient outcomes were tested at 36 weeks. The authors found that presence of ascites or encephalopathy, serum level of albumin <3.5 g/dL or alanine aminotransferase <60 U/L, and body mass index >25 kg/m² were related with an increased risk of not improving in CPT to class A, independent of SVR. Serum level of albumin <2.8 g/dL and increased bilirubin were associated with a higher risk of LT or death. The authors created a scoring system based on 5 baseline factors (body mass index, encephalopathy, ascites, serum levels of alanine aminotransferase and albumin), called the “BE3A score”, which is significantly related to patient outcomes and may be helpful as a decision-making tool.

HCV PI should not be used in patients with Child-Pugh B or C decompensated cirrhosis. In patients who receive combination therapy ribavirin (RBV) can be started at a dose of 600 mg daily and subsequently adjusted to daily weight-based RBV dose (1000 or 1200 mg in patients <75 kg or ≥75 kg, respectively). Drug-drug interactions are possible with the HCV DAAs, therefore a thorough drug-drug interaction risk assessment is required (www.hep-druginteractions.org).

According to updated European Association for the Study of the Liver (EASL) Recommendations on Treatment of Hepatitis C (2018), patients without cirrhosis and with compensated (Child-Pugh A) cirrhosis without

HCC awaiting LT with a MELD score <18–20 should be treated prior to LT; whereas those without HCC and a MELD score ≥18–20 should be transplanted first. Patients with decompensated cirrhosis (Child-Pugh B or C) without HCC awaiting LT with a MELD score <18–20 have an indication for antiviral treatment. In HCV transplant candidates with HCC, without cirrhosis or with compensated cirrhosis, optimal time point of antiviral treatment (before or after LT) is not clear. ELITA consensus statements recommend treatment of patients with a MELD ≥21–25 in a case by case decision; and not to treat pretransplant patients with MELD >25 (Belli 2017).

The need for RBV has been discussed controversially and remains to be studied. Addition of RBV is associated with a higher incidence of adverse events and might be of benefit to increase SVR. This may account in particular for decompensated cirrhotic as well as for treatment-experienced cirrhotic patients. For instance, an integrated analysis of patients with liver cirrhosis from the phase II and phase III clinical development programme of SOF/LDV demonstrated that only treatment-experienced patients treated for 12 weeks had increased SVR rates with addition of RBV (SVR: 90% vs. 96%, n.s.). In the TURQUOISE-II study SVR rates of patients with 12 or 24 weeks of treatment and addition of RBV were 92% vs. 96% (Poordad 2014). RBV dose reduction did not impact SVR rates. Genotype 1a patients with prior null-response to pegylated interferon+RBV therapy achieved higher SVR rates when treated for 24 instead of 12 weeks (93 vs. 80%). Of the patients (n=513) analysed in the SOF/LDV trial conducted by Reddy et al. (2015), 69% were not treatment-naïve and 47% had failed previous treatment with a PI regimen. Overall, 96% achieved SVR12 similar to treatment-naïve and previously treated patients (98% vs 95%, respectively). SVR12 rates were 95% in patients receiving 12 weeks and 98% in patients receiving 24 weeks of treatment, and did not vary substantially with or without RBV.

In a phase 3, double-blind, placebo-controlled study, naïve and previously treated patients with compensated cirrhosis (genotype 1, 2, 4, 5, or 6), receiving once-daily SOF-VEL for 12 weeks achieved high SVR rates of 99% (Feld 2015). In a phase 3, open-label study (Curry 2015) CPT class B cirrhotic patients (naïve and previously treated, HCV genotype 1, 2, 3, 4, or 6) were randomly assigned in a 1:1:1 ratio to receive SOF and velpatasvir once daily for 12 weeks, SOF-VEL plus RBV for 12 weeks, or SOF-VEL for 24 weeks. Overall SVR rates were 83% among patients who received 12 weeks of SOF-VEL, 94% among those who received 12 weeks of SOF-VEL plus RBV, and 86% among those who received 24 weeks of SOF-VEL. The response rates were not significantly different among the three study groups. The most common adverse events among all patients were fatigue (29%), nausea (23%), and headache (22%), whereas anaemia (31%) predominantly occurred in patients receiving RBV.

Real world data showed that efficacy, safety, and tolerability have been

very similar to those in clinical trials, reinforcing the value of these new DAA treatment options (Reddy 2017, Aql 2015). Patients infected with HCV genotype 3 can be efficaciously treated with the fixed-dose combination of SOF (400 mg) and VEL (100 mg). In a study conducted by Afdhal et al. (2017) impact of SOF and RBV on hepatic venous pressure gradient (HVPG) was investigated in patients with compensated and decompensated cirrhosis with portal hypertension. 72% of patients (33/46) achieved SVR12. Nine patients (24%) had ≥20% decreases in HVPG during DAA therapy. Four of the 33 (12%) patients with baseline HVPG ≥12 mm Hg had HVPG <12 mm Hg directly after treatment completion. Of nine patients with pretreatment HVPG ≥12 mm Hg who achieved SVR12 and completed 48 weeks of follow-up, eight (89%) had a ≥20% reduction in HVPG.

In a recently published cohort study using the Scientific Registry of Transplant Recipients (SRTR) database from 2003 to 2015 a total of 47,591 adults waitlisted for LT from HCV, HBV, and NASH were identified (Flemming 2017). The authors found that in the era of DAA therapy adjusted incidences of LT waitlisted for decompensated cirrhosis in HCV patients decreased by over 30%, whereas waitlisting for decompensated cirrhosis in NASH increased by 81%. Waitlisting for HCC in both the HCV and NASH populations significantly increased in both the PI and DAA eras ($P < 0.001$ for all); whereas HCC waitlisting in HBV remained stable.

Prior to DAA introduction, HCV positive donor LT has shown to be associated with an equivalent patient and graft survival as compared to HCV negative donor LT in HCV positive LT recipients. Use of HCV-infected organs in solid organ transplantation in an HCV-uninfected recipient has been discussed as a result of the advent of safe and highly effective pan-genotypic DAAs (Nangia 2019). An immediate treatment approach for HCV NAT-positive LT into uninfected recipients has shown to be efficacious in several studies (Bethea 2019). However, this promising strategy has been more widely investigated in the kidney transplant setting (Crismale 2019) and there is insufficient data about the use of DAA in recipients of HCV positive donor livers. Any use of HCV positive donors in HCV negative LT recipients requires a thorough informed consent. Recently published recommendations for liver recipient selection of hepatitis C viremic donors are available from an international consensus meeting (Burton 2020).

Based on available data and according to EASL recommendations (2018) the use of HCV-infected organs is acceptable in patients at high risk of death on the waiting list and should not be offered to non-infected recipients with a MELD score <20 if there is no access to anti-HCV therapy. Multi-centre studies are needed to determine whether widespread implementation of this strategy is a useful approach.

Adjunct treatment and staging of HCC transplant candidates

Under MELD allocation, patients must meet the Milan criteria (one tumour ≤ 5 cm in diameter or up to three tumours, all ≤ 3 cm) to qualify for exceptional HCC waiting list consideration. Diagnosis of HCC is confirmed if the following criteria are met according to the German Medical Association (https://www.bundesaerztekammer.de/fileadmin/user_upload/downloads/pdf-Ordner/RL/RiliOrgaWIOvLeberTx20190924.pdf): (1) liver biopsy-proven alone or (2) two contrast-enhanced (CE) imaging techniques (CE-magnetic resonance imaging [MRI], CE-computed tomography [CT] or CE-ultrasound [US]) in tumours 1 cm up to ≤ 2 cm; (3) one contrast-enhanced imaging technique (CE-MRI, CE-CT) in tumours > 2 cm; (4) arterial hypervascularisation with rapid venous wash out, displaying contrast reversal in comparison to the surrounding liver tissue in 3-phase cross-sectional imaging techniques. Patients are registered at a MELD score equivalent to a 15% probability of pretransplant death within 3 months. Patients will receive additional MELD points equivalent to a 10% increase in pretransplant mortality to be assigned every 3 months until these patients receive a transplant or become unsuitable for LT due to progression of their HCC. The listing centre must enter an updated MELD score exception application in order to receive additional MELD points.

Pre-listing, the patient should undergo a thorough assessment to rule out extrahepatic spread and/or vascular invasion. The assessment should include CT scan or MRI of the abdomen, pelvis and chest. We perform tri-monthly routine follow-up examinations (MRI or CT scan) of waitlisted HCC patients for early detection of disease progression. Underestimation of HCC burden before LT has shown to be frequent despite advanced imaging technologies. This has recently been reconfirmed in a study conducted by Ecker et al. (2018). The authors collected HCC patients who underwent LT after preoperative MRI in a prospective institutional database (January 2003 to December 2013). Patients were subdivided in those “within” or “outside” Milan criteria by both imaging and explant pathologic evaluation. Of 318 patients with HCC meeting Milan criteria by MRI at the time of LT, only 248 (78.0%) remained within Milan on explant examination.

Waiting list drop-out rates can be reduced by the application of bridging therapies such as transarterial chemoembolisation (TACE) or radiofrequency ablation (Roayie 2007). In patients treated with transarterial chemoembolisation before LT for HCC Response Evaluation Criteria in Solid Tumours (RECIST) have shown to be superior to EASL criteria at 1 month follow-up for predicting long-term survival (Shuster 2013). Transarterial radionuclide therapies such as Yttrium-90 microsphere transarterial radioembolisation (TARE) have been tested for bridging therapy in selected cases (Toso 2010, Memon 2013).

Kulik et al. (2018) aimed to investigate the effectiveness of locoregional therapy (LRT) in LT candidates with HCC on the LT waitlist. They conducted a systematic review and metaanalysis considering multiple databases from 1996 to April 25, 2016, for studies that enrolled adults with cirrhosis awaiting LT and treated with bridging or down-staging therapies before LT. LRT included TACE, transarterial radioembolization, ablation, and radiotherapy. The authors showed that in LT candidates with HCC, the use of LRT is associated with a nonsignificant trend toward improved waitlist and posttransplant outcomes. Bridging therapy should be considered in particular in patients outside the Milan criteria, with a likely waiting time of longer than 6 months, and those within the Milan criteria with high-risk characteristics of HCC. Sorafenib has been administered in a few studies before LT to investigate the safety and efficacy of this oral multikinase inhibitor in the neoadjuvant setting (Fijiki 2011, Di Benedetto 2011). A systematic review of the few available studies showed that perioperative use of sorafenib did not improve patient survival and could even lead to a worse prognosis (Qi 2015). Moreover, sorafenib is frequently associated with side effects such as fatigue, weight loss, skin rash/desquamation, hand-foot skin reaction, alopecia and diarrhoea, requiring dose reduction or treatment discontinuation. Accurate discrimination of HCC patients with good and poor prognosis by specific criteria (genomic or molecular strategies) is highly warranted to select appropriate treatment options (Bittermann 2014, Tournoux-Facon 2011).

Liver transplantation in autoimmune hepatitis and cholestatic liver diseases

Between 1988 and 2015, 4% of cirrhosis patients were transplanted due to AIH and 8% due to PBC, based on the data from the European Liver Transplant Registry (<https://www.ELTR.org>).

In a recently published international multicenter study of 3,902 PBC patients, Harms et al (2019) found that treatment with UDCA is associated with prolonged liver transplant-free survival.

PSC, accounting for approximately 5% of all transplant cases, is a rather small indication group on the waiting list. According to the Guidelines of the German Medical Association, patients with PSC who fulfill the standard exception criteria receive at listing a match MELD reflecting the sum of 3-month mortality according to lab MELD and a 20% 3-month mortality. A modified version of these guidelines became effective in March 2012: patients will be listed initially according to a 3-month mortality of 15% (equivalent to a MELD score of 22) and then are upgraded every three months following every 10% increase of the 3-month mortality (<https://www.>

bundesaerztekammer.de/fileadmin/user_upload/downloads/pdf-Ordner/RL/RiliOrgaWIOvLeberTx20190924.pdf). The previous point increment applied within the match MELD standard criteria was inadequate, and these revised guidelines more accurately reflect the urgency for LT.

Living donor liver transplantation: indications, donor evaluation, and outcome

LDLT was introduced in 1989 in a successful series of paediatric patients (Broelsch 1991). Adult-to-adult LDLT (ALDLT) was first performed in Asia where cadaveric organ donation is rarely practiced (Sugawara 1999, Kawasaki 1998). LDLT peaked in the US in 2001 (Qiu 2005) but thereafter the numbers declined by 30% over the following years (Vagefi 2011, Carlisle 2012). A decline over time was also observed in Europe, whereas LDLT activity increased in Asia.

In selected cases, LDLT offers significant advantages over deceased donor LT (Quintini 2013). The evaluation of donors is a cost-effective and time-consuming process. Clinical examinations, imaging studies, special examinations, biochemical parameters, and psychosocial evaluation prior to donation varies from centre to centre and has been described elsewhere (Valentin-Gamazo 2004). Using Germany as an example, the expenses for evaluation, hospital admission, surgical procedure, and follow-up examinations of donors are paid by the recipient's insurance. Due to the increasing number of potential candidates and more stringent selection criteria, rejection of potential donors has been reported in 69-86% of cases (Valentin-Gamazo 2004, Pascher 2002). The advantages of LDLT include the feasibility of performing the operation when medically indicated and the short duration of cold ischaemia time. In the absence of a prospective study comparing HCC patients undergoing LDLT vs. deceased donor LT (DDLTL), there is no evidence to support a higher HCC recurrence after LDLT vs. DDLTL (Akamatsu 2014).

LDLT is associated with surgical risks for the recipient AND donor (Baker 2016). The surgical procedures for LDLT are more technically challenging than those for deceased donor LT. In the recipient operation, bile duct reconstruction has proven to be the most challenging part of the procedure with biliary complications ranging from 15% to 60% (Sugawara 2005).

Regarding donor outcome, morbidity rates vary considerably in the literature (Patel 2007, Beavers 2002, Shiraz 2016). Possible complications include wound infection, pulmonary problems, vascular thrombosis with biliary leaks, strictures, and incisional hernia. A major concern related to LDLT is still donor safety because an operative procedure with potential

risks must be carried out on a healthy individual (Baker 2016). Biliary complications are the most common postoperative complication in LDLT and occur in up to 7% of donors (Perkins 2008, Sugawara 2005). Liver regeneration can be documented with imaging studies and confirmed by normalisation of bilirubin, liver enzymes, and synthesis parameters. Morbidity rates are strongly related to the experience of the surgical team and should be performed only by established transplant centres with appropriate medical expertise. The currently reported postoperative mortality rates for left and right hepatectomy are 0.1% and 0.5%, respectively. Outcome in patients undergoing LDLT is similar if not even better than in those undergoing deceased donor LT (Nadalin 2015).

Perioperative complications

Cardiac decompensation, respiratory failure following reperfusion, and kidney failure in the perioperative LT setting constitute major challenges for the intensive care unit. Acute kidney injury (AKI) has a major impact on short- and long-term survival in LT patients. For instance, Pulitano et al. (2018) found that AKI was associated with increased risk of early allograft dysfunction and chronic kidney disease stage ≥ 2 posttransplant.

There is no currently accepted uniform definition of AKI, which would facilitate the standardisation of care of patients with AKI and improve and enhance collaborative research efforts. Biomarkers such as neutrophil gelatinase-associated lipocalin or kidney injury molecule-1 have been developed for the prevention of delayed AKI treatment (Saner 2012). Moreover, genetic profiling of post-reperfusion milieu showed that endothelin-1 and interleukin-18 serum levels on postoperative day 1 were independent predictors of AKI in multivariate analysis (Pulitano 2018).

Early dialysis has been shown to be beneficial in patients with severe AKI (stage III according to the classification of the Acute Kidney Injury Network) (Bellomo 2004), whereas treatment with dopamine or loop diuretics have shown to be associated with worse outcome. Preventative strategies of AKI include avoidance of volume depletion and maintenance of a mean arterial pressure >65 mm Hg (Saner 2011).

Despite advances in organ preservation and technical procedures, postoperative complications due to preservation/reperfusion injury have not markedly decreased over the past several years. Typical histological features of preservation and reperfusion injury include centrilobular pallor and ballooning degeneration of hepatocytes. Bile duct cells are more sensitive to reperfusion injury than hepatocytes (Washington 2005) resulting in increased serum levels of bilirubin, gamma-glutamyl transpeptidase (GGT) and alkaline phosphatase (AP). Machine perfusion

preservation is an emerging technology that limits ischaemia/reperfusion injury associated with preservation and may result in improved outcomes after LT and expansion of the donor pool (Quillin 2018).

Vascular complications continue to have devastating effects. In deceased LT, overall vascular complications such as hepatic artery thrombosis (HAT) have been reported in 1.6-4% of patients. Shiraz et al. (2016) retrospectively analysed the trends observed in vascular complications with changing protocols in adult LDLT (A-LDLT) and paediatric LDLT (P-LDLT) over 10 years. Depending on the era of LT the authors stratified the cohort in Group I (n=391, Jan. 2006- Dec.2010) and Group II (n=741, Jan. 2011- Oct. 2013) patients. With a minimum follow up of 2 years, incidence of HAT in adults has reduced significantly from 2.2% in Group I to 0.5% to Group II, $p = 0.02$. In Group II non-significantly more adult patients (75%) with HAT could be salvaged compared to only 25% patients in Group I ($p=0.12$). Incidence of portal vein thrombosis (PVT) has been remained similar ($p=0.2$) in the two eras.

Yang et al. (2014) found that independent risk factors associated with early HAT were recipient/donor weight ratio ≥ 1.15 (OR=4.499), duration of hepatic artery anastomosis >80 min (OR=5.429), number of units of blood received intraoperatively ≥ 7 (OR=4.059) and postoperative blood transfusion (OR=6.898). After logistic regression, duration of operation >10 h (OR=6.394), retransplantation (OR=21.793) and rejection reactions (OR=16.936) were identified as independent risk factors associated with early HAT. Graft type (whole/living-donor/split), duration of operation >10 h, retransplantation, rejection episodes, recipients with diabetes preoperatively and recipients with a high level of blood glucose or diabetes postoperatively had a higher risk of late HAT in the univariate analysis. Doppler exams of the hepatic artery and portal vein are frequently performed in the early postoperative setting. HAT in the early postoperative period can be managed with thrombectomy. Late HAT with complication of bile duct strictures is managed by interventional endoscopic retrograde cholangiography (ERC) but requires retransplantation in the majority of patients. Early portal vein thrombosis is rare ($<1\%$) but may lead to graft loss if not revascularised.

Primary non-functioning graft (PNFG) may be clinically obvious immediately after revascularisation of the allograft. Early signs of liver dysfunction include prolonged coagulation times, elevated liver enzymes (transaminases, cholestasis parameters) without a downward trend, rising lactate, and hypoglycemic episodes. PNFG is a critical situation and requires immediate retransplantation.

Infections occurring during the first month post-LT are usually nosocomial, donor-derived, or due to perioperative complications (Hernandez 2015). Death within the first year after LT is often associated with bacterial infections. Management of infections due to multidrug-resistant gram positive pathogens represents a major therapeutic challenge

in the transplant setting (Radunz 2011).

Overall incidence of fungal infections in LT recipients has declined due to early identification and treatment of high-risk patients. However, overall mortality rate for invasive candidiasis and aspergillosis remains high (Liu 2011).

The clinical symptoms of early T-cell mediated rejection (TCMR) are non-specific, may not be apparent or may manifest as fever, right upper quadrant pain, and malaise. A liver biopsy is indispensable for confirming the diagnosis. High dose corticosteroids (3 days of 500-1000 mg methylprednisolone) are the first-line treatment for moderate and severe TCMR. In selected cases antibody-depleting therapy may be necessary. Mild, moderate and severe TCMR should be treated by an increase in CNI. Diagnosis of acute antibody-mediated rejection (AMR) requires a liver biopsy demonstrating classic histology and C4d⁺ staining (Charlton 2018). Mild AMR should be treated with steroid boluses. Moderate to severe cases can include plasmapheresis and intravenous immunoglobulins with or without anti-B cell agents.

Long-term complications after liver transplantation

Management issues for the long term include opportunistic infections, chronic ductopenic rejection, side effects due to immunosuppression including cardiovascular complications and renal dysfunction, *de novo* malignancies, biliary complications, osteoporosis and disease recurrence.

Opportunistic infections

Opportunistic infections in the medium and long term after LT are primarily viral and fungal in origin. Opportunistic bacterial infections are uncommon after 6 months in patients receiving stable and reduced maintenance doses of immunosuppression with good graft function. There is still a need for prospective interventional trials assessing the potential effects of preventive and therapeutic strategies against bacterial and fungal infection for reducing or delaying the development of chronic allograft dysfunction.

Cytomegalovirus (CMV) infection plays an important role in the LT setting (Mumtaz 2014) (Figure 3). CMV DNA assay is the commonly used laboratory tool to diagnose and monitor CMV infection. Current guidelines recommend antiviral prophylaxis over pre-emptive therapy in preventing

CMV disease in high-risk LT recipients (CMV-seronegative recipients of organs from CMV-seropositive donors [D+/R-], [Kotton 2018]). The period of prophylaxis should be no shorter than 3 months in D+/R- patients. Delayed-onset CMV disease occurs in 15-38% of CMV D+/R- LT patients after prophylactic treatment for 3 months (Eid 2010).

The procedure in the transplant centres is inconsistent for intermediate-risk (R+) patients. If a preemptive strategy is adopted, screening for CMV every 1-2 weeks in the first 3 months post-LT is not entirely achievable in routine clinical practice in most centres. If prophylaxis is carried out in D+/R+ or D-/R+ patients, this should last 3 months. D-/R- patients have the lowest risk of CMV infection and disease.

A controlled clinical trial demonstrated that valganciclovir, an oral prodrug of ganciclovir, is as effective and safe as intravenous (IV) ganciclovir for the prophylaxis of CMV disease in solid organ (including liver) transplant recipients (Paya 2004). In a published study by Kim et al. (2015) LT patients experiencing CMV infection were administered oral valganciclovir (900 mg/day) daily or IV ganciclovir (5 mg/kg twice daily) as antiviral preemptive treatment. A total of 83 patients had preemptive antiviral therapy, of those 61 patients received ganciclovir and 22 patients received valganciclovir. The median time from LT to CMV infection in the IV ganciclovir group was shorter than in the oral valganciclovir group (21 days vs. 30 days, $p = 0.001$). Recurrent CMV infection rates after treatment were 14.8% in the ganciclovir and 4.5% in the valganciclovir group ($p = 0.277$). None of the patients in either group experienced CMV disease. The authors concluded that oral valganciclovir was equally effective as IV ganciclovir in preemptive treatment of CMV infection following LT.

In cases of ganciclovir-resistant CMV disease, therapeutic options include CMV hyperimmune globulins, or in rare cases, alternative antiviral medication) (Eid 2010).

Occurrence of posttransplant lymphoproliferative disease (PTLD) in the first year after solid-organ transplantation is typically related to Epstein-Barr virus (EBV) infection. EBV-seronegativity of the recipient before infection, high EB viral load, intensity of immunosuppression and young age have been reported as risk factors for PTLD (Smets 2002). Outcomes have improved since rituximab has been incorporated into treatment regimens (Kamdar 2011). Therapeutic management options include reduction of immunosuppression, rituximab, combination chemotherapy and adoptive immunotherapy.

Oral reactivation of human herpes simplex virus-1 (HSV-1) after LT is common. Development of varicella-zoster virus (HHV-3) after LT is typically related to intense immunosuppressive therapy and its therapy does not differ from the non-transplant setting.

Human herpesvirus 6 (HHV-6A and HHV-6B) can cause primary

or reactive infection in LT recipients and may often be restricted to the infected organ and asymptomatic but it can also display a variety of clinical syndromes, including fever, hepatitis, and higher rates of graft dysfunction. It may have indirect effects including increased risks of mortality and fibrosis as well as hepatitis C progression. Recipients with inherited chromosomally integrated HHV-6 (ciHHV-6) may have an increased risk of graft rejection and opportunistic infections (Phan 2018). HHV-6 and HHV-7 may have a potential role as co-pathogens in the direct and indirect illnesses caused by CMV. HHV-6 infection can be determined by quantifying viral DNA in plasma or blood, however, biopsy remains the gold standard for diagnosis. Clinically significant tissue-invasive infections can be treated with ganciclovir, foscarnet or cidofovir.

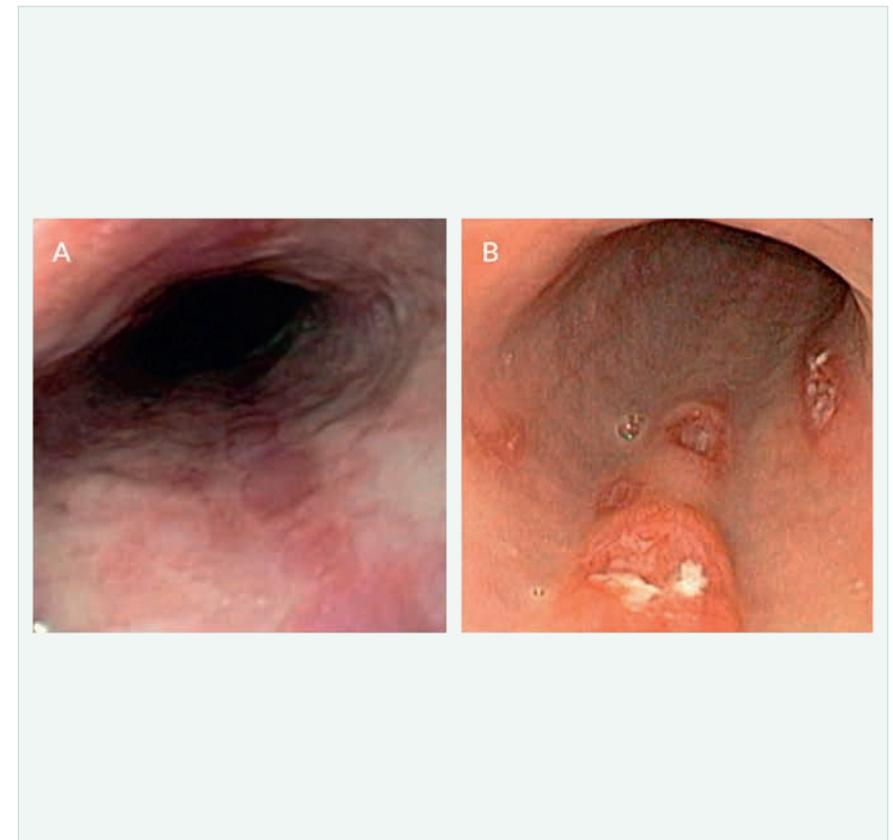


Figure 3. Cytomegalovirus (CMV) infection of the upper gastrointestinal tract. A. Liver-transplanted patient complaining of dysphagia and epigastric discomfort with multiple longitudinal oesophageal ulcers seen at upper endoscopy. B. Endoscopic findings of deep oesophageal ulcerations with fibrinoid necrosis in another immunocompromised patient. In both cases, lesions were caused by CMV infection. Diagnosis depends on a positive mucosal biopsy, which should include specimens from the ulcer margins and ulcer base. Hematoxylin and eosin staining typically reveals "owl's eye" cytoplasmic and intranuclear inclusion bodies.

Hepatitis E

There is often a multifactorial pathogenesis for allograft hepatitis in LT patients. It is advisable to incorporate HEV RNA determination into the differential diagnostic investigation where patients have unexplained elevated liver enzymes or histological signs of allograft hepatitis (Borg 2016). Recently, molecular testing was suggested for HEV in transplant liver biopsies for evaluating patients with elevated transaminases of unknown origin (Protzer 2014).

According to the Guidelines for Hepatitis E & Solid Organ Transplantation (https://bts.org.uk/wp-content/uploads/2017/05/BTS-HEV-Guideline-CONSULTATION_DRAFT.pdf; McPherson 2018) solid organ transplant recipients with liver transaminases above the upper limit of normal or symptoms suggestive of hepatitis E virus (HEV) infection are tested for HEV using an HEV RNA or an antigen assay. Treatment of acute HEV infection with RBV may be indicated in specific cases of acute infection with severe liver dysfunction or extrahepatic manifestations. Chronic disease courses with HEV infections as well as reactivation after apparent cure have been reported in organ transplant patients. In the transplant setting, HEV Guidelines from UK (McPherson 2018) define diagnosis of persistent HEV infection leading to chronic hepatitis when HEV RNA is detectable in blood or stool for more than three months after the onset of relevant symptoms, raised liver enzymes, or from the first positive HEV RNA test.

A German LT cohort comprising 287 adult patients was prospectively tested in a real-life setting using HEV polymerase chain reaction assay, irrespective of their liver enzyme levels. In 4 patients (1.4%) only, chronic HEV infection was diagnosed. These results suggest that general screening of all LT recipients with normal liver enzymes in low-endemic countries does not seem to be justified (Galante 2015).

The risk of HEV infection becoming chronic in immunocompromised (transplanted) patients is high, at around 60-65% (Kamar 2010a, 2011, Legrand-Abravanel 2010, McPherson 2018). Quantification of HEV viral load is useful before initiation of antiviral therapy. In immunosuppressed patients with chronic hepatitis E, anti-HEV antibodies are often undetectable (EASL Clinical Practice Guidelines on hepatitis E virus infection; [https://www.journal-of-hepatology.eu/article/S0168-8278\(18\)30155-7/pdf](https://www.journal-of-hepatology.eu/article/S0168-8278(18)30155-7/pdf)). A baseline quantitative HEV RNA assessment is undertaken on both plasma and stool at the start of treatment. A strong decrease of viral load may predict viral elimination.

A group from the Hannover Transplant Center performed HEV serology tests in 226 LT patients, 129 non-transplanted patients with liver disease, and 108 healthy controls (Pischke 2010). HEV antibodies were detectable in

4% of the transplant group, 3% of the group with liver disease and 1% of the healthy control group. Three patients from the transplant group were HEV RNA positive, two of whom developed HEV viral persistence. Anti-HEV seroconversion was observed no earlier than four months after detection of HEV RNA.

The outcome, progression and individual variables associated with HEV infection becoming chronic were analysed in a retrospective study (Kamar 2011) including data from 17 transplant centres. In the largest cohort to date involving HEV-infected transplanted patients, 74/85 patients were recruited in French centres, three in Germany, five in the Netherlands and one patient each in the UK, Belgium and the US. The vast majority of the patients had received kidney (n=48) or liver (n=27) allografts. Chronification of HEV infection was defined as persistently elevated liver enzymes and positive detection of HEV replication in serum and/or feces over a minimum of six months. 65/85 patients (65.9%) developed a chronic disease. All 59 patients who underwent HEV genotyping had genotype 3. In contrast to the non-immunosuppressed patients, transaminases were usually only moderately elevated (~100-300 IU/l). Anti-HEV IgM was detectable in only 41% and IgG was detectable in 80.8%. 14.3% of the patients developed cirrhosis of the liver by the final follow-up (29.5 months, range 9-117 months). As it is possible that seroconversion may not occur until four months or more after detection of RNA in organ transplant patients, serological testing for HEV IgG and IgM in transplanted patients is of limited use and diagnostic standard is therefore nucleic acid amplification techniques.

Successful RBV monotherapy of chronic hepatitis E has been reported after LT (Kamar 2015, Carmo 2016) as well as in other solid organ transplantations (Kamar 2010b, Mallet 2010, Chaillon 2011).

With regard to PEG-interferon α treatment of HEV infection (Abbas 2014, Kamar 2010c), there is little data available for LT patients and this treatment approach should not be used as first line therapy. HEV RNA testing in plasma and stool at day 7 and monthly after RBV treatment initiation is recommended. A 3-month course of RBV monotherapy seems to be an appropriate treatment duration if stool tests are negative for HEV RNA at month 3 on two occasions (McPherson 2018). If HEV RNA is positive at month 3, RBV is continued until stool tests are negative for HEV RNA on two occasions one month apart or RBV is continued for 6 months. A test of SVR is conducted by testing plasma and stool samples for HEV RNA at three and six months after cessation of antiviral therapy.

Chronic rejection (TCMR and AMR)

Advances in immunosuppressive regimens have greatly reduced the incidence of chronic rejection and allograft failure. Chronic rejection begins within weeks to months or years after LT and accounts for a small proportion of late graft dysfunction (Suhling 2016). It affects about 4% to 8% of patients (Neuberger 1999).

Sub-therapeutic immunosuppression and nonadherence to immunosuppressive therapy also coincides with increased risk of rejection, substantial increases in the rates of graft loss and death. Special attention should be posed on immunosuppression-related physical side effects as a major reason for non-adherence. Multidisciplinary evaluation, in particular by transplant hepatologists and psychologists are warranted to improve adherence before and after LT. Chronic TCMR and AMR may appear indolently and might only become apparent as liver test injury abnormalities (GGT, AP, bilirubin, transaminases). The diagnosis needs to be confirmed by histopathologic examination. Chronic TCMR results in potentially irreversible bile duct and vascular injury. Treatment is difficult. Patients on cyclosporine (CSA) should be switched to tacrolimus (TAC). Diagnosis of chronic AMR includes inflammation with low grade interface activity, fibrosis and C4d⁺ staining. There is currently no defined treatment strategy. Switching the baseline immunosuppression from CSA to TAC and initiating mycophenolate mofetil (MMF) rescue therapy represents a treatment option in these patients (Daly 2002).

Calcineurin inhibitor-induced nephrotoxicity and alternative immunosuppressive protocols

Despite the introduction of new immunosuppressive agents (Table 4), calcineurin inhibitors (CNI) remain the key drugs in most immunosuppressive regimens. Both CSA and TAC inhibit the calcineurin-calmodulin complex and therefore IL-2 production in T lymphocytes. TAC is available as traditional twice-daily immediate-release tacrolimus and once-daily prolonged/extended released formulations. Renal failure, mainly due to CNI nephrotoxicity, is the most common complication following orthotopic LT. The incidence of chronic renal dysfunction characterised by arteriolar hyalinosis resulting in a variety of tubulointerstitial and glomerular lesions has been reported in up to 70% of patients in the long term after LT and varies widely depending on the length of follow-up, the definition of chronic kidney disease and the intensity of immunosuppressive therapy (Beckebaum 2013b). End stage renal disease has been described in 18% of patients during a posttransplant follow-up of 13 years (Gonwa 2001).

Randomised trials have shown that induction therapy maintains immunosuppressive efficacy in steroid-free regimens. For instance, delayed CNI initiation (e.g. to days 4-5 posttransplant) can prevent deterioration of renal function posttransplant, but requires induction with an interleukin-2 antagonist receptor (IL-2RA) agent or rabbit antithymocyte globulin (rATG) to maintain early immunosuppressive efficacy.

A group from Regensburg initiated a single arm pilot study to determine the safety and efficacy of a CNI-free combination therapy (basiliximab induction/MPA and delayed [10 days posttransplant] SRL in patients with impaired renal function (GFR <50 mL/min and/or serum creatinine >1.5 mg/dL) at LT (Schnitzbauer 2015). Twenty-seven patients were included with a median labMELD of 28. Incidence of biopsy proven acute rejection was 18.5%, no steroid-resistant rejections occurred within 1 month. SRL was started on day 10 (range, day 1 to day 48), 44% of patients were switched to CNI treatment by 12 months. Renal function improved significantly ($p = 0.006$). The critical time period for relevant improvement of kidney function seemed to be the first month, independently from SRL administration.

In LT patients with CNI-induced nephrotoxicity, a complete replacement of CNI with conversion to MMF has shown conflicting results with respect to the occurrence of rejection, anywhere from 0% to 60% (Creput 2007, Schmeding 2011, Moreno 2004). MMF inhibits inosine monophosphate dehydrogenase, a critical enzyme in the *de novo* pathway of purine synthesis. Results from previous studies with immunosuppressive regimens including MMF and minimal CNI treatment suggest a significant improvement in renal function in this patient group (Beckebaum 2011, Cicinnati 2007a, Beckebaum 2004a, Cantarovich 2003, Garcia 2003, Raimondo 2003).

De novo immunosuppression with MMF combined with induction therapy and delayed CNI introduction is another approach to reduce CNI-related nephrotoxicity especially in patients with higher MELD score or significant renal dysfunction. In a randomised clinical trial, a daclizumab/MMF/delayed low-dose TAC-based regimen was compared with a standard TAC/MMF regimen (Yoshida 2005). In both study arms, corticosteroids were tapered over time. Statistically significant higher median GFR was found in the delayed CNI group, although acute rejection episodes were not statistically significant different between the groups. Similar results were seen in two retrospective studies in LT patients receiving thymoglobulin induction therapy and a delayed initiation of CNI (Bajjoka 2008, Soliman 2007).

Another approach to maintain renal preservation is replacement of CNI by mTOR inhibitors such as SRL or everolimus (EVL) (Sanchez 2005, Harper 2011, Saliba 2011, Kawahara 2011, Hüsing (a) 2015).

An Italian consensus Transplant panel even recommended routine use of EVL in predefined clinical scenarios, particularly in light of posttransplant

nephrotoxicity (de Simone (a) 2016).

In the multicentre randomised (1:1) controlled PROTECT study (CRAD001HDE10) *de novo* patients were treated with CNI (CSA or TAC) + basiliximab ± steroids for 4-8 weeks after LT and were then randomised to an EVL-based treatment arm or a CNI-based control arm (Fischer 2012). In the EVL-based treatment arm (n=101), a 70% reduction of CNI (± steroids) was carried out over a period of 2 months, followed by treatment with EVL ± steroids. In the control arm (n=102) treatment with CNI (standard dose ± steroids) was continued. Using the MDRD equation, the endpoint could be achieved with a difference in calculated GFR of at least 8 mL/min between the two treatment arms (p=0.02). The incidence of graft rejection, graft loss and death was not significantly different between the two treatment arms. A 24-month extension phase was performed in 81 patients to month 35 post-randomisation. The adjusted mean eGFR benefit from randomisation to month 35 was 9.4 mL/min/1.73 m² with MDRD. The difference in favour of the CNI-free regimen increased gradually over time due to a small progressive decline in eGFR in the CNI group (Sterneck 2014).

Efficacy and safety of a TAC-free and a TAC-reduced regimen were compared with a TAC standard dose (TAC-C) regimen in a multinational, randomised controlled licensing trial (CRAD001H2304) in *de novo* LT recipients (Saliba 2011b). After a 1-month run-in phase on TAC-based immunosuppression (+/-MMF), patients were randomised to an EVL/prednisone/TAC-free group (TAC-WD) including TAC withdrawal at 4 months post-LT, an EVL/prednisone/reduced TAC group (EVL+rTAC) or a standard TAC control group (TAC-C). The primary combined endpoint included biopsy-confirmed acute rejection, allograft loss or death, and the secondary endpoint was renal function at 1 year. The TAC-WD arm was stopped prematurely due to a significantly higher incidence of biopsy-confirmed acute rejections (19.9% [TAC-WD] vs. 4.1% [EVL+rTAC] vs. 10.7% [TAC-C]).

At 1 year, significantly more patients in the TAC-C group had reached the combined primary endpoint compared to the EVL+rTAC group (9.7% vs. 6.7%; p<0.001). Kidney function was significantly better (p<0.001) in the EVL+rTAC arm than in the TAC-C arm. The increased rejection rate in the TAC-WD group at month 4 may be caused by the immunosuppressive strategy used. Unlike the CRAD001HDE10 study, no induction therapy with an anti-IL-2 inhibitor was performed and there was no weaning of CNI over 2 months. Instead, CNI were stopped abruptly.

Lin (2016) conducted a systematic review and meta-analysis of randomised controlled trials (RCT) analysing the effect of EVL on renal function in patients (EVL n=465, control n=428) with baseline GFR >30 mL/min undergoing a CNI minimisation or withdrawal protocol. Based on these results, EVL use with CNI minimisation in LT recipients was

associated with improved renal function at 12 months (95% CI 2.75-17.8) but not associated with an increased risk of biopsy proven acute rejection (RR 0.68, 95% CI 0.31-1.46), graft loss (RR 1.60, 95% CI 0.51-5.00), or mortality (RR 1.34, 95% CI 0.62-2.90). However, it was associated with an increased risk of overall infections (RR 1.45, 95% CI 1.10-1.91).

In the randomized controlled multicenter SILVER trial the per protocol analysis identified LT recipients with early CNI minimization and introduction of SRL within 4 to 6 weeks after LT with significantly superior eGFR and lowest rate of chronic kidney disease (≥ stage 3) from year 1 during a follow-up period of 5-years (Buchholz 2019).

Early institution at one month of EVL in combination with low dose TAC (≤5 ng/mL) for preserving kidney function has also been recommended by the International Liver Transplant Society Consensus guidelines on immunosuppression in LT recipients (Charlton [c] 2019).

In future, there might be further development of cell therapeutic approaches and mesenchymal stem cells to launch tolerogenicity rather than development of new immunosuppressive drugs (Charlton [c] 2019).

Table 4. Clinically used immunosuppressive agents in liver transplantation

Immunosuppressant class	Immunosuppressive agent
Corticosteroids	Prednisone, prednisolone, methylprednisolone
Calcineurin inhibitors	Cyclosporin, tacrolimus
Antimetabolites	Mycophenolate mofetil, azathioprine
mTOR Inhibitors	Sirolimus, everolimus
Polyclonal antibodies	Antithymocyte globulin
Monoclonal anti-CD3 antibodies	Muromonab-CD3 (OKT3)
Chimeric monoclonal antibodies	Anti-IL-2 receptor inhibitor (basiliximab)
Monoclonal anti-CD52 antibodies	Alemtuzumab (campath-1H)

Other side effects of CNI

Besides potential nephrotoxicity, CNI therapy is associated with side effects that include cardiovascular complications, tremor, headache, electrolyte abnormalities, hyperuricaemia, hepatotoxicity, and gastrointestinal symptoms. Neurotoxicity, including tremor, paresthesia, muscle weakness, and seizures, more often occurs in TAC-treated patients; gingival hyperplasia, a rare event, and hirsutism are associated with CSA treatment.

Cardiovascular side effects due to CNI and steroids include hyperlipidaemia, arterial hypertension, and diabetes (Beckebaum 2004b).

The prevalence of new-onset diabetes mellitus after LT has been reported

to occur in 9–21% of patients (John 2002, Konrad 2000). The prevalence of posttransplant diabetes is even higher if cofactors such as hepatitis C are present. In various studies, the diabetogenic potential has been reported to be higher in patients receiving TAC than in those receiving CSA. In contrast, CSA has a more pronounced effect on lipid levels. CSA can act by modulating the activity of the LDL receptor or by inhibiting the bile acid 26-hydroxylase that induces bile acid synthesis from cholesterol.

Numerous ongoing studies aim to determine the most effective immunosuppressive protocols while minimising drug-related side effects. These protocols often combine several drugs with different mechanisms of action and toxicities allowing dose adjustment. There is also a trend towards tailored immunosuppressive regimens following the aetiology of liver disease and comorbidities such as renal dysfunction and cardiovascular disease.

Corticosteroid minimisation/avoidance protocols and additional strategies to reduce metabolic complications

There is ongoing discussion of steroid avoidance due to dyslipidaemia, osteoporosis, development of cataracts, weight gain, hypertension, and a deleterious impact on glucose control. As cardiovascular disease is the second leading cause of death in the late transplant period, steroid-minimised/free regimens may be favoured in particular in patients with high risk of metabolic syndrome.

A recent metaanalysis including 16 studies with 1347 participants showed that glucocorticosteroid avoidance or withdrawal appears to reduce diabetes mellitus and hypertension (Fairfield 2018). In a study, Yoo et al. (2015) evaluated outcomes of 500 consecutive LT recipients who received a steroid-free protocol with rATG induction and a single dose of methylprednisolone given before the first dose of rATG. Mean MELD at transplantation was 22 ± 6 . MMF was initiated postoperatively with delayed TAC initiation at 4.79 ± 13.3 days. TAC was replaced by SRL if serum creatinine remained above 2.0 mg/dL after 1 week. Patients were switched to TAC or SRL monotherapy at 12 weeks. Posttransplant peak creatinine was at 1 month 1.43 ± 0.95 mg/dL and improved to 1.26 ± 0.60 mg/dL ($p < 0.05$) at 2.5 years. Lowest GFR rate was observed at 1 month (65.6 ± 30.0) and improved by 12 months (72.7 ± 28.2 , $p < 0.01$). One-year patient and graft survival were 92.8% and 89.6%, respectively. Rejection occurred in 22.8% of patients, 6.6% of patients had steroid-dependent rejection.

Other research groups have reported encouraging findings with steroid-free protocols including basiliximab induction therapy (Filipponi 2004, Llado 2008, Becker 2008). In a multicentre, 24-week, randomised, open-label,

phase IIIb trial (DIAMOND study) renal function was investigated with once-daily, prolonged-release TAC-based immunosuppression in de novo LT recipients. Patients were administered prolonged-release TAC (initial dose 0.2 mg/kg/day); prolonged-release TAC (0.15–0.175 mg/kg/day) plus basiliximab or prolonged-release TAC (0.2 mg/kg/day delayed until Day 5) plus basiliximab. All patients had comedication with MMF plus a bolus of corticosteroids. Lower dose prolonged-release TAC (0.15–0.175 mg/kg/day) immediately posttransplant in combination with basiliximab and MMF was associated with lower TAC exposure, significantly reduced renal function impairment and biopsy-confirmed acute rejection incidence vs. prolonged-release TAC (0.2 mg/kg/day) administered immediately after LT. Delayed higher-dose prolonged-release TAC exposure significantly reduced renal impairment compared with immediate administration (Trunecka 2015).

A published literature review (Lerut 2009) analysed the actual status of corticosteroid minimisation protocols in LT based on a detailed analysis of 51 peer-reviewed and 6 non-peer-reviewed studies. Results from the majority of studies showed that these protocols have clear metabolic benefits and are safe with respect to graft and patient survival. These results are in line with a recent metaanalysis of 16 studies with 1347 participants demonstrating that metabolic complications such as diabetes and hypertension were statistically significantly less frequent in patients undergoing steroid avoidance or withdrawal protocols vs. steroid-containing immunosuppression (Fairfield 2018).

A healthy diet and regular exercise represent additional effective strategies to avoid or reduce serious cardiovascular complications. In patients with dyslipidaemia, hydrophilic statins such as pravastatin and fluvastatin should be preferred as they are not metabolised by cytochrome P450–3A4.

De novo malignancies

Incidence of malignancies is higher in transplant patients and depends on the length of follow-up, characteristics of the transplant population, choice of immunosuppressive therapy and the era when the LT was performed (Buell 2005, Fung 2001). A cumulative risk has been reported of 10%, 24%, 32% and 42% at 5, 10, 15 and 20 years, respectively, for development of *de novo* cancers after LT (Finkenstedt 2009). The highest risks in the transplant setting are non-melanoma skin cancers, mainly squamous cell carcinoma and basal cell carcinoma (Figure 4). Regular cancer surveillance programmes have been proposed by several groups; however, scientific evidence is lacking and surveillance programmes may vary from centre to centre.

Bhat et al. (2018) investigated potential risk factors for malignancies after

LT analysing data from the Scientific Registry of Transplant Recipients database comprising 108,412 LT recipients. During median follow-up of 6.95 years malignancies during follow-up were 4,483 (41.3%) skin, 1,519 (14.0%) hematologic, and 4,842 (44.7%) solid organ. The 10-year probability of *de novo* malignancy was 11.5% (11.3–11.8%). Multivariable analysis showed that age by decade, male gender, Caucasian race, multiorgan transplant, previous malignancy and alcohol-related, autoimmune-related, and NASH-related liver disease and PSC pre-LT (compared to HCV, $p < 0.001$) were associated with higher risk of post-LT malignancy. There was no correlation between type of immunosuppression and risk of cancer. Patients with replicative EBV infection and immunosuppressive regimens, i.e. ATG, are at a higher risk of developing PTLD. These patients may present with lymphadenopathy and/or fever, weight loss and night sweats, and meticulous examination, serologic and imaging tests are required. Diagnosis and classification of PTLD is currently based on histologic criteria, and a multidisciplinary team is required including hematologists and transplant hepatologists for treatment of PTLD, monitoring of immunosuppressive therapy and preservation of allograft function.

In a prospective single-centre study the relationship between the development of solid organ cancers following LT and the level of CN1 exposure was assessed (Carencio 2015). Data are based on 247 TAC-treated LT recipients who survived at least 1 year posttransplant. Study results showed that 43 (17.4%) patients developed *de novo* solid cancers. Mean TAC concentration during the first year after LT was significantly higher in patients who developed solid malignancies (10.3 ± 2.1 vs. 7.9 ± 1.9 ng/mL, $p < 0.0001$). Independent risk factors in multivariate analysis were tobacco consumption pretransplant (OR=5.42; 95% CI [1.93–15.2], $p = 0.0014$) and mean annual TAC concentration during the first 12 months posttransplant ($p < 0.0001$; OR=2.01; 95% CI [1.57–2.59], $p < 0.0001$). Similar results have been shown in a subgroup of patients exposed to TAC continuously for ≥ 3 years. Premalignant lesions such as actinic keratoses are mostly located on sun-exposed areas. Squamous cell carcinoma and basal cell carcinoma are increased by factors of ~65–200 and ~10, respectively, in organ transplant recipients as compared to the immunocompetent population (Ulrich 2008). An annual routine dermatologic follow-up exam, limitation of sun exposure and protective measures including sunscreens are highly recommended for transplant patients. Due to a higher incidence of colon cancer in patients transplanted for PSC and concomitant inflammatory bowel disease (Hanouneh 2011) an adequate colonoscopic surveillance is required at regular intervals (annual colonoscopy) even in the absence of active disease (Fevry 2011). A trend has recently been reported toward an increased incidence of advanced colon polyps and colon carcinoma in patients transplanted for diseases other than PSC after LT. However, larger studies

are needed to determine whether posttransplant colon cancer surveillance should be performed more frequently than in the non-transplant setting (Rudraraju 2008).

Studies have reported a significantly higher incidence of aerodigestive cancer including lung cancer among patients who underwent LT for alcohol-related liver disease (Vallejo 2005, Jimenez 2005). These patients should undergo a more intensive surveillance protocol for the detection of upper gastrointestinal and oropharyngeal-laryngeal malignancies (Benloch 2004). In cases of positive smoking history surveillance for lung cancer should be implemented. In a retrospective study, conversion from CNI to an mTOR inhibitor (EVL) improved the prognosis of *de novo* malignancies after LT for alcoholic cirrhosis (Thimonier 2014). One- and five-year survival was 77.4% and 35.2% in the EVL cohort vs. 47.2% and 19.4% in the non-EVL cohort, respectively ($p = 0.003$).



Figure 4. Non-melanoma skin scancers and liver transplantation (LT). Organ transplant recipients have an increased risk of development of non-melanoma skin cancers as compared to the non-transplant setting. Premalignant lesions such as actinic keratoses [A] are predominantly located on sun-exposed areas. Squamous cell carcinoma [B,C] is the most frequent skin cancer after LT followed by basal cell carcinoma [D] (Photographs kindly provided by Prof. Dr. Hillen, Transplant Dermatology Outpatient Unit, Department of Dermatology, University Hospital Essen, Germany)

Studies have shown that mTor inhibitors (SRL, EVL) exert antiangiogenic activities that are linked to a decrease in production of vascular endothelial growth factor (VEGF) and to a markedly inhibited response of vascular

endothelial cells to stimulation by VEGF (Guba 2002). Furthermore, the ability of mTor inhibitors to increase the expression of E-cadherin suggests a mechanism for blocking regional tumour growth and for inhibiting metastatic progression. Therefore, we give special consideration for mTOR inhibitor-based immunosuppressive regimens not only in patients transplanted for HCC but also those with *de novo* malignancies after LT. There is evidence from meta-analyses and studies performed mainly in the kidney transplant setting that switching from CNI to mTOR-based immunosuppression is associated with a lower incidence of non-melanoma skin cancers (Euvrard 2012, Caroti 2012, Gu 2012). A multicentre study involving CNI-treated patients with a previous history of at least one squamous cell carcinoma randomly allocated patients to an arm in which CNI was replaced by SRL, or to an arm in which the CNI-based immunosuppression was continued (Euvrard 2012). The squamous cell carcinoma-free survival was significantly longer in the SRL group than in the CNI control group. The appearance of a new squamous cell carcinoma was observed in 14 patients (22%) in the SRL group versus 22 patients (39%) in the control group ($p=0.02$). The authors concluded that SRL obviously has an antitumour effect regarding the reappearance or the new appearance of non-melanoma skin cancers.

Biliary complications

The clinical outcome of patients posttransplant can be significantly affected by biliary complications (Lisotti 2015). Biliary leaks generally present as an early posttransplant complication and occur in 5% to 10% of deceased donor LT (Kapoor 2015) and in 10% to 15% of LDLT (Iida 2010). Biliary leaks are typically treated with placement of a biliary stent to bridge the leak, usually with sphincterotomy. In patients with biliary stones, endoscopic sphincterotomy and stone extraction are the treatment of choice. Biliary stone disease and in particular formation of biliary casts is common in the setting of LT and may occur without or in the setting of strictures due to impaired biliary flow. The exact aetiology of biliary cast disease is unknown but ischaemia and strictures have been described as predisposing factors (Pereira 2018). In a recently published retrospective study complication rates during the first 15 days after endoscopic sphincterotomy were assessed in patients who underwent conventional or precut endoscopic sphincterotomy (Hüsing (b) 2015). A total of 24 complications (15.2%) were reported, including 9 cases (5.7%) of pancreatitis, 6 cases (3.8%) of bleeding, and 1 case (0.6%) of perforation. Complication rates were not significantly different between the two sphincterotomy techniques.

Damage (ischaemia, infectious complications or rejection) of the biliary

tree mucosa can provoke cast which consists of desquamated epithelial cells mixed with bile products within the biliary system and occurs in 3% to 18% of LT patients (Shah 2003).

Biliary strictures are one of the most common complications after LT, with a reported incidence of 5.8-34% (Graziadei 2006). Early anastomotic strictures usually have a technical origin, while strictures appearing later have a multifactorial origin. Non-anastomotic strictures without underlying hepatic artery thrombosis are commonly referred to as ischemic-type biliary lesions (ITBL).

Risk factors for ITBL include preservation-induced injury, prolonged cold and warm ischaemia times, altered bile composition, ABO blood incompatibility and immunologic injury (Verdonk 2007, Buis 2009). Our group found that specific chemokine receptor polymorphisms of the recipient are associated with the development of post-LT biliary strictures (Iacob 2012). Moreover, screening of anti-HLA antibodies might be useful for early identification of at-risk patients who could benefit from closer surveillance and tailored immunosuppressive regimen (Iacob 2012).

ERC or percutaneous transhepatic cholangiography (PTC) have typically been used as the primary approach, leaving surgical intervention for those who are non-responsive to endoscopic interventions or who have diffuse intrahepatic bile duct damage. Radiological methods such as magnetic resonance cholangiopancreatography (MRCP) have been introduced as an additional diagnostic tool for biliary complications. In cases of biliary cast and ischemic cholangiopathy, endoscopic ultrasound (EUS) was found to be diagnostically superior to ERCP and had a significant impact on clinical decision-making. EUS was less reliable when diagnosing anastomotic strictures (Hüsing 2014). EUS can complement ERCP to improve diagnosis of biliary complications after LT and impact on treatment decision.

The long-term efficacy and safety of endoscopic techniques have been evaluated in various transplant centres (Qin 2006, Zoepf 2012, Pascher 2005). Non-anastomotic strictures are commonly associated with a less favourable response to interventional endoscopic therapy in comparison to anastomosis stenosis (Figure 5). An Austrian group found anastomotic strictures in 12.6% of patients transplanted between October 1992 and December 2003 and non-anastomotic strictures in 3.7% during a mean follow-up of 53.7 months after LT (Graziadei 2006). Interventional endoscopic procedures were effective in 77% of patients with anastomosis stenosis, while treatment of non-anastomotic strictures showed long-term effectiveness in 63% of patients. A surgical approach was required in 7.4% of transplant recipients.

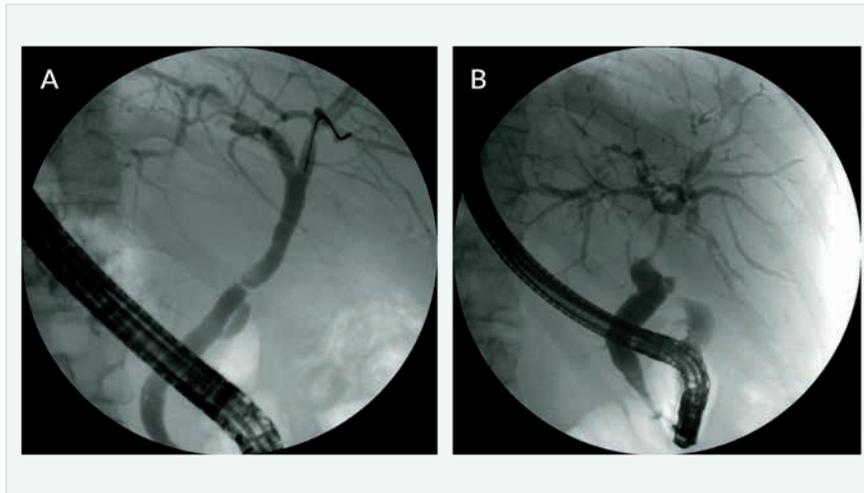


Figure 5. Biliary tract complications after liver transplantation. A. Endoscopic retrograde cholangiography (ERC) showing posttransplant short filiform anastomotic biliary stricture in a 46-year-old patient transplanted for hepatitis C virus (HCV) infection and alcohol-related cirrhosis 6 months earlier. Therapy sessions include dilatation and an increasing number of bile duct endoprosthesis at short intervals of every 2-3 months. Prior to endoscopic therapy an endoscopic sphincterotomy is performed. B. ERC of a 41-year-old patient transplanted for HCV diagnosed with ischemic-type biliary lesions (type 3) with long non-anastomotic stricture extending proximally from the site of the anastomosis and strictures throughout the entire liver.

Results from 75 transplanted patients undergoing ERC for suspected anastomotic strictures were retrospectively analysed (Zoepf 2006). Balloon dilatation alone and combined dilatation and endoprosthesis placement was efficacious in 89% and 87% of cases respectively, but recurrence occurred in 62% and 31% of cases respectively. However, results of these strategies are inconsistent in the literature. In our centre, we use dilatation with or without stenting with endoscopic reassessment in anastomotic strictures. Repeated ERC sessions are commonly performed with increasing endoprosthesis diameter every three months and double or triple parallel stenting in selected cases. Up to 75% of patients are stent-free after 18 months of endoscopic intervention (Tung 1999).

In a prospective case series (n=13) we recently found an excellent safety and effectiveness of paclitaxel-coated balloons in the dilatation of symptomatic anastomotic stenoses (Kabar 2012). A sustained good clinical outcome of the intervention, defined as no further endoscopic intervention for at least 6 months, was achieved in 12/13 patients. Out of these 12 patients, one (n=9), two (n=1) or three (n=2) endoscopic interventions were necessary. The mean bilirubin level fell from 6.8 ± 4.1 mg/dL to 1.4 ± 0.9 mg/dL.

Medical treatment for bile duct strictures consists of ursodeoxycholic acid (UDCA) and additional antibiotic treatment in stricture-induced cholangitis. Complications related to bilioenteric anastomosis require PTC or surgical intervention.

Metabolic bone disease

Liver cirrhosis, heavy alcohol use, smoking, poor nutrition, hypogonadism, cholestatic liver disease, and therapy with corticosteroids are risk factors for the development of osteoporosis in pretransplant patients (Schreiber 2018). In a study assessing both vertebral and nonvertebral (rib, pelvic, and femur) fractures in pretransplant patients with PBC and PSC, 20% and 1.4% of the patients had experienced fracturing and avascular necrosis, respectively (Guichelaar 2007). Screening with bone densitometry using dual-energy x-ray absorptiometry should begin prior to LT (Wibaux 2011).

A further increase in bone turnover has been described after LT going along with bone density decrease within the first 3 to 6 months after transplant. Bone density gradually returns to pretransplant levels thereafter (Singh 2015). Posttransplant bone disease contributes significantly to patients' morbidity and mortality after transplantation and plays a role for their quality of life (Nel 2016). Factors favouring spinal bone gain from 4 to 24 months after transplantation include lower baseline and/or 4-month bone density, premenopausal status, lower cumulative glucocorticoids, no ongoing cholestasis, and higher levels of vitamin D and parathyroid hormone (Guichelaar 2006). CNI administration is a risk factor for osteoporosis after LT (Moreira Kulak 2010).

The risk of osteoporotic vertebral and nonaxial fractures was 14% and 21% at 1 and 2 years posttransplant, decreased with time, and was highest in patients with pretransplant osteopenia and cholestatic liver disease (Singh 2015).

A cumulative incidence of fractures at 1 year and at 8 years posttransplant was reported in 30% and 46% of patients transplanted for PBC and PSC (Guichelaar 2007). Nine percent experienced avascular necrosis after LT. This event was positively correlated with pretransplant and posttransplant lipid metabolism, bone mineral density and fracturing, and posttransplant glucocorticoid administration (Guichelaar 2007).

EASL Clinical Practice Guidelines focusing on Liver Transplantation (<http://dx.doi.org/10.1016/j.jhep.2015.10.006>) recommends bone mineral density screening yearly for patients with pre-existing osteoporosis and osteopenia, every 2-3 years in patients with normal bone mineral density and further screening intervals depending on impairment of bone mineral density and on risk factors. Regular bone mineral density screening may be hampered in some countries as it is not necessarily covered by (statutory) health insurances. There are no specific therapies for posttransplant osteoporosis besides those for non-transplanted patients. General interventions to reduce fracture risk include adequate intake of calcium and vitamin D. Secondary hyperparathyroidism and adverse lifestyle

factors should be addressed and corrected. Bisphosphonates are currently the most effective agents for treatment of posttransplant osteoporosis (Moreira Kulak 2010) (www.dv-osteologie.org). A meta-analysis and systematic review of randomised controlled trials demonstrated that bisphosphonate therapy in the first 12 months post-LT is associated with reduced accelerated bone loss and improved bone mineral density at the lumbar spine (Kasturi 2010).

Recurrent diseases after liver transplantation

Disease recurrence may occur in patients transplanted for viral hepatitis, tumour disease, autoimmune or cholestatic or alcohol-related liver diseases.

Recurrence of hepatitis B in the allograft

HBV recurrence using combined prophylactic regimens is less than 5%. However, recurrence rates differ among various studies as most of them are small, with varying proportions of patients with active viral replication at LT and varying follow-up periods after LT. Combined use of hepatitis B immunoglobulin (HBIG) and nucleos(t)ide analogs (NUC) has emerged as treatment of choice in transplanted HBV recipients (Figure 6) (Cai 2011) and its efficacy has been investigated extensively. There is a high variability (dose, duration and method of HBIG administration) in the prophylactic protocols. According to the German guidelines (Cornberg 2011) patients receive 10,000 IU HBIG IV in the anhepatic phase followed by 2000 IU during the first posttransplant week. For long-term HBIG prophylaxis, trough anti-HBs levels at or above 100 IU/L should be maintained.

The European Commission granted a marketing authorisation valid throughout the European Union for subcutaneous (SC) HBIG in 2009, and it has been launched in the last years in many European countries. SC HBIG application has advantages over intramuscular (IM) and IV administration (Yahyazadeh 2011, Beckebaum 2012, Beckebaum 2013c). It can be performed by patients at home, which is an important factor in improving patients' flexibility and mobility in daily life, lowering the frequency of physician consultations and avoiding AEs attributable to high peak and low trough serum anti-HBs levels compared with IV administration (Yahyazadeh 2011, Beckebaum 2012, Beckebaum 2013c).

De Simone et al. (b) (2016) demonstrated that early introduction of SC HBIG administration by week 3 posttransplantation, combined with HBV virostatic prophylaxis, is safe and effective for prevention of HBV reinfection.

Recent data from a retrospective study including 371 adults transplanted for HBV-related disease at 20 European centers and treated with IV HBIG (n=299), SC HBIG (n=236), and other HBIG preparations for 12 months ± NUC therapy were analyzed (Beckebaum 2018). The majority (93.5%) received NUC therapy. Recurrence was 16/371 (4.3%) (annual rate 0.65%); 5/16 patients with recurrence had discontinued HBIG and 7/16 had low anti-HBs titer (<100 IU/L). The recurrence rate in HBIG-treated patients was 1 per 2069 months. Risk of HBV recurrence in patients who discontinued HBIG was increased by 5.2-fold as compared to those on SC HBIG therapy.

Economic issues have led to a conduct of studies investigating whether NUC therapy instead of combined long-term NUC/HBIG is sufficient for antiviral prophylaxis (Cholongitas 2014, Teperman 2013, Naoumov 2001, Buti 2007, Lo 2005, Angus 2007, Knighton 2012, Gane 2007, Stravitz 2012, Wesdorp 2012, Fung 2011).

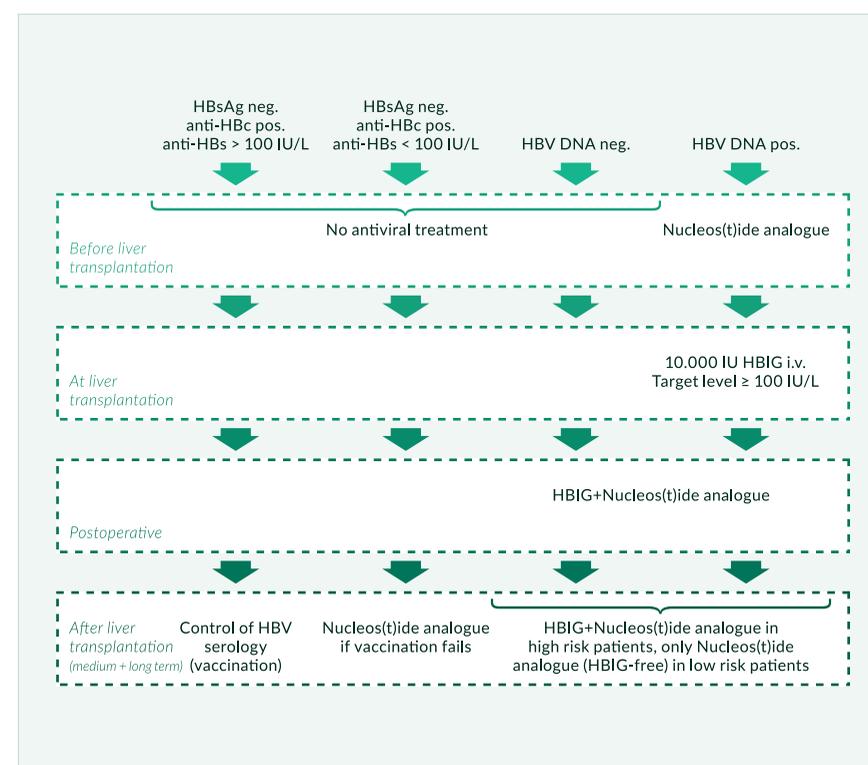


Figure 6. Prophylaxis of HBV recurrence after liver transplantation (LT). Postoperative combined use of nucleos(t)ide analog(s) and hepatitis B immunoglobulin (HBIG) is still the gold standard for prophylaxis of HBV reinfection after LT. HBIG therapy can be withdrawn in the medium and long term after LT in low-risk patients. Those who are anti-hepatitis B core (anti-HBc)-positive and without detectable anti-hepatitis B surface (anti-HBs) titres or with anti-HBs titres <100 IU/L should be vaccinated. In case of no or little response (anti-HBs <100 IU/L) to vaccination, lamivudine (LAM) monotherapy can be initiated. In patients who have protective anti-HBs titres of >100 IU/L, antiviral therapy is not necessary but long-term monitoring of HBV serology including anti-HBs titres is required. Neg., negative; pos., positive

In a published study from Hong Kong, HBIG-free monoprophylaxis with ETV was evaluated. Only 26% of patients had undetectable HBV DNA at the time of LT. HBsAg loss occurred in 91% within 24 months posttransplant but 13% had reappearance of HBsAg within a follow-up period of 36 months and 22.5% were HBsAg positive at the time of their last follow-up visit (Fung 2011).

The efficacy of a switch after at least 12 months of HBIG/LAM to combination therapy with an oral nucleoside and nucleotide analogue was investigated (Saab 2011). Estimated HBV reinfection rate was 1.7% at 1 year after HBIG withdrawal.

A prospective, multicentre study in which 20 HBV patients received 800 IU HBIG (IM) in the anhepatic phase and for another 7 days after transplant surgery was published (Gane 2013). Patients with genotypic detection of LAM resistance and creatinine levels ≥ 1.8 mg/dL were excluded. ADV was administered as add-on therapy to existing LAM treatment. Previously untreated patients received combined ADV plus LAM treatment, which was continued after transplantation. Serum HBsAg and anti-HBs were measured monthly in the first 3 months, then every 3 months. HBV DNA determination was only performed annually and at the end of the follow-up observation period. HBV recurrence was defined as the reappearance of HBsAg or detection of HBV DNA. The median follow-up was 57 months (range 27–83 months). At transplantation 68% of patients had demonstrable virus replication and 26% had viral replication $>4 \log_{10}$ IU/mL. After the end of the study, another 28 HBV patients received a liver allograft. The patients ($n=18$) who had HBV DNA $<3 \log_{10}$ IU/mL at transplantation were given no posttransplant HBIG therapy at all. The median follow-up was 22 months (range 10–58 months). Looking at both cohorts it was shown that there was a loss of HBsAg in 47/48 patients within 8 weeks posttransplantation and in one patient within 6 months after transplantation. In one patient with recurrence of HCC, there was a transient reappearance of HBsAg in the follow-up period.

In a randomised, prospective, controlled phase 2 trial, patients ($n=40$) received emtricitabine, TDF and HBIG for 24 weeks (Teperman 2013). Subsequently all patients who were negative for HBsAg and HBV DNA (<400 copies/mL) were randomly allocated to continue with all three drugs or to an arm with emtricitabine and TDF but without HBIG. The median period of time from LT was 3.4 years (range 1.9–5.6 years). During an observation period of 72 weeks, no HBV recurrence in terms of HBsAg or HBV DNA detection was observed in any of the patients.

Most HBV prophylactic posttransplant studies to date are limited, small and with short follow-up periods. EASL Clinical Practice Guidelines on the Management of Hepatitis B Virus Infection (2017) recommend combined hepatitis B immunoglobulin (HBIG) and NUC for prevention of recurrent

HBV infection after LT.

As a life-long therapy, this accounts in particular for patients with a high risk for HBV recurrence (HBV DNA positive at the time of LT, HBeAg positive, HBV underlying HCC, and HDV or HIV coinfection). EASL Clinical Practice Guidelines determine that patients with a low risk of recurrence can discontinue HBIG and proceed with indefinite nucleos(t)ide analogue monoprophylaxis.

According to updated AASLD Hepatitis B Guidance (Terrault et al. 2018) prophylaxis with or without HBIG for 5–7 days and NUCs posttransplant followed by long-term potent NUC therapy in low risk patients is an appropriate approach. ETV or TDF, an ester prodrug of tenofovir (TFV) or TAF, a phosphonate prodrug of TFV, with more favourable renal and bone safety than TDF are preferred antiviral drugs because of their low rate of resistance with long-term use. Combination antiviral therapy and HBIG is recommended by Terrault et al. (2018) for those with high risk of recurrent disease posttransplant (HDV- and HIV-coinfected patients and nonadherent patients).

For HBsAg negative LT recipients receiving HBsAg negative, anti-HBc-positive allografts, the reported risk of HBV transmission varies with the HBV immune status of the recipient. Those who have detectable anti-HBs titres have a significant lower risk as compared to those without detectable anti-HBc or anti-HBs titre. EASL Clinical Practice HBV Guidelines (2017) recommend LAM as prophylactic approach; whereas AASLD Hepatitis B Guidance (Terrault et al. 2018) positively emphasises highly potent ETV, TDF or TAF for long-term prophylactic use in this scenario.

There is no rationale for continuing HBIG therapy in case of viral breakthrough with detectable HBV DNA. The choice of antiviral therapy in patients with HBV recurrence depends on the current antiviral medication, the viral load, and the resistance profile. Antiviral drug resistance can easily be established by genotypic assays that identify specific mutations known to be associated with decreased susceptibility to particular drugs.

Recurrence of hepatitis C in the allograft

HCV infection always recurs in the allograft in patients with detectable serum HCV RNA. The severity of HCV reinfection can be determined by liver biopsy. Transient elastography (TE) and acoustic radiation force impulse (ARFI) play a substantial complementary role for measurement of fibrosis in HCV and non-HCV transplant recipients (Cross 2011, Beckebaum 2010).

Antiviral treatment initiated after LT may be favourable after postoperative convalescence (approximately 3 months after LT). Patients with elevated liver enzymes and hepatic inflammation, and/or the risk of

rapid fibrosis progression should be treated earlier. Moreover, fibrosing cholestatic hepatitis (FCH) represents an urgent treatment indication. Studies based on smaller patient cohorts demonstrated excellent results in patients with FCH treated with SOF/LDV+RBV for 12 or 24 weeks (Charlton (a) 2015, Manns 2016). Treatment of severe recurrence after primary LT may therefore reduce the need for retransplantation.

Preemptive DAA therapy after LT can not be recommended on a routine basis except in patients with FCH. Initiation of DAA therapy between 3-6 months after LT is encouraged.

There is a known association between HCV infection and chronic kidney disease. HCV-infected patients are 4.5 times as likely to develop some types of renal disease and 1.4 times as likely to develop renal insufficiency. Therefore, some studies recently focused on the impact of DAA on renal function after LT. Data from a recently published study have demonstrated that LT recipients with HCV, achieving SVR, have a significantly lower risk of a decline in renal function (Satapathy 2018).

In the SOLAR-I and SOLAR-II, phase 2 studies, patients with advanced liver disease infected with HCV genotype 1 or 4 and CPT class B or C cirrhosis or after LT were treated for 12 or 24 weeks with a combination of SOF+LDV and RBV (Charlton (b) 2015, Manns 2016). In the SOLAR-I study (Charlton (b) 2015), SVR12 was achieved in 86-89% of patients. A post hoc analysis using data from this study showed that early on-treatment HCV RNA quantification (week 2 and 4) is of limited use in patients with advanced liver disease and/or LT and does not predict SVR12 (Welzel 2016). Results of the SOLAR-II study (Manns 2016) showed among transplanted patients with genotype 1, SVR12 in n=42/45 (93%) patients without cirrhosis (12 weeks treatment); 44/44 (100%) patients without cirrhosis (24 weeks treatment); 30/30 (100%) CTP-A patients (12 weeks treatment); 27/28 (96%) of CTP-A patients (24 weeks treatment); 19/20 (95%) CTP-B patients (12 weeks treatment); 20/20 (100%) of CTP-B patients (24 weeks treatment); one/two (50%) CTP-C patients (12 weeks treatment); and four/five (80%) CTP-C patients (24 weeks treatment). No concerns were raised with respect to safety profile. Seven deaths were reported that were not considered to be related to treatment.

The safety and efficacy of SOF has been investigated but not been established so far in patients with severe renal impairment. The main SOF metabolite (GS-331007) is eliminated primarily via renal clearance. Pharmacokinetic studies in HCV negative patients with renal dysfunction show a significant increase in serum levels of SOF and the metabolite GS-331007 as compared to patients with normal renal function (Gane 2014). Based on available data SOF may be used in the setting of mild-moderate renal impairment, but should not be used for severe renal impairment (GFR less than 30 mL/min) or in patients on hemodialysis.

Grazoprevir+elbasvir are renally excreted and there is no need for dose adjustments in chronic kidney disease. The largest study in the renally impaired patient population was the C-SURFER study (Roth 2015). In this placebo-controlled trial, patients (n=224) were randomised to immediate treatment with grazoprevir/elbasvir or to deferred treatment including placebo for 12 weeks, then grazoprevir/elbasvir at follow-up week 4. The cohort included patients with GFR<30 mL/min and those on hemodialysis. SVR was 99% (95% CI 95.3-100.0; 115/116), with one relapse 12 weeks after end of treatment. This regimen had a low rate of adverse events and seems to be a promising approach in genotype 1 HCV patients with stage 4-5 chronic kidney disease.

In the MAGELLAN-2 study, 80 liver and 20 kidney transplant recipients on a stable immunosuppressive regimen were included (Agarwal 2017; Reau 2018). Prednisone/prednisolone was permitted at ≤10 mg/day and CSA at ≤100 mg/day at the time of screening. SOF/VEL for 12 weeks was highly effective in LT recipients with recurrent, chronic HCV infection. Three patients did not achieve SVR, one with one early discontinuation and 2 relapses. All patients (n=4) with baseline Y93H resistance-associated substitutions (RASS) (3 GT 3 and 1 GT 1b) achieved SVR12. No changes in immunosuppression were needed for rejection or suspected drug-drug interactions.

Available data on treatment of HCV recurrence with the new DAA showed SVR rates similar to the non-transplant setting also in other transplant studies (ALLY-1 phase-III study [Poordad 2015], phase II CORAL-I study [Kwo 2014], the TARGET-cohort [Sulkowski 2016]).

According to EASL Recommendations on Treatment of Hepatitis C (2018) patients with posttransplant HCV recurrence with non-cirrhotic changes of the allograft, with Child-Pugh A, B, C cirrhosis can be treated with SOF and LDV (genotypes 1, 4, 5 or 6), or with SOF and VEL (all genotypes). Treatment regimen of patients with posttransplant recurrence of HCV genotype 2 or 3, without cirrhosis or with Child-Pugh A comprises SOF and VEL for 12 weeks. Pre-treatment adjustment of immunosuppressive dose is not necessary. Daily weight-based RBV (1,000 or 1,200 mg in patients <75 kg or ≥75 kg, respectively or initial dose of 600 mg daily, with subsequent dose increase, if tolerated) is recommended in Child-Pugh B and C cirrhosis.

As in the non-transplant setting, PI should not be used in transplanted patients with Child B and C cirrhosis. Patients with decompensated cirrhosis and contraindications or intolerance to RBV, should be treated with SOF and LDV (genotypes 1, 4, 5 or 6) or the fixed-dose combination of SOF and VEL (all genotypes) for 24 weeks.

Because of frequent drug-drug interactions and the need for immunosuppressant drug dose adjustments, treatment regimens including a PI are suboptimal for HCV treatment post-LT. However, in LT recipients

with impaired kidney function, the combination of glecaprevir and pibrentasvir for 12 weeks is an alternative to SOF-based regimens. Non-cirrhotic patients and Child-Pugh A cirrhotic patients with posttransplant recurrence and impaired renal function (eGFR <30 mL/min/1.73 m²) can be treated (irrespective of genotype) with glecaprevir and pibrentasvir for 12 weeks. This treatment regimen requires monitoring of immunosuppressant drug levels and potential dose adjustments.

Recurrence of cholestatic liver disease and autoimmune hepatitis

Data on the frequency of recurrent cholestatic and AIH-related liver disease vary in the literature depending on the follow-up period and criteria chosen for definition of disease recurrence which may be more aggressive than the original disease in some transplant patients (Carbone 2014). The posttransplant prognosis for PBC patients is excellent, with an approximately 80% 5-year survival reported by most large centres (Carbone 2011, Silveira 2010). It has been reported that HLA-A, -B, and -DR mismatches between the donor and the recipient decrease the risk of disease recurrence in PBC patients (Morioka 2007a, Hashimoto 2001). A published study with long term follow-up data reported recurrent PBC in one-third of patients at 11-13 years posttransplant (Charatcharoenwittaya 2007). This study and various other studies reporting recurrent PBC are depicted in Table 5.

Table 5. Recurrence rates in patients transplanted for autoimmune-related or cholestatic liver disease

	Reference	Patients, n	Follow-up after liver transplantation	Recurrence rate
AIH	Duclos-Vallée 2003	17	>120 months	41%
AIH	Prados 1998	27	mean 44 months	33%
AIH	Molmenti 2002	55	median 29 months	20%
AIH	Campsen 2008	66	median 81 months	36%
AIH	Vogel 2004	28	mean 100 months	32%
PBC	Charatcharoenwittaya 2007	154	mean 130 months	34%
PBC	Jakob 2006	100	up to 17 years	16%
PBC	Liermann-Garcia 2001	400	mean 56 months	17%
PBC	Montano-Loza 2010	108	mean 88 months	26%
PBC	Hytiroglou 2008	100	mean 44 months	16%
PSC	Cholongitas 2008	69	median 110 months	13%
PSC	Alabraba 2009	230	median 55 months	24%
PSC	Vera 2002	152	median 36 months	37%
PSC	Graziadei 1999	150	mean 54 months	20%
PSC	Goss 1997	127	mean 36 months	9%

Diagnosis of PBC in the transplanted liver is usually more challenging than diagnosis in the native liver. Anti-mitochondrial antibodies (AMA) often persist, and elevated cholestatic enzymes may be due to other causes of bile duct damage such as ischemic cholangiopathy or chronic ductopenic rejection. Recurrent PBC is a histological diagnosis, typically appearing as granulomatous cholangitis or duct lesions. The frequency of recurrence will be considerably underestimated if a liver biopsy is carried out only when clinical features are apparent.

In a Japanese multicentre study, recipient aged 61 years or older, HLA mismatches of four or more (maximum of six), graft:recipient weight ratio less than 0.8, and husband donor were reported as negative predictors of patient survival in PBC patients after LDLT (Egawa 2016). Some investigators have found that CSA-based immunosuppressive therapy is associated with lower PBC recurrence rates as compared to TAC-based immunosuppression (Wong 1993, Montano-Loza 2010). However, long-term survival has been shown to be not significantly different between CSA- and TAC-treated patients (Silveira 2010). Recent data show that younger age at the time of PBC diagnosis or at LT, TAC use, and biochemical markers of cholestasis after LT are risk factors for PBC recurrence by the Global PBC Study Group (Montana-Loza 2019).

In the Mayo Clinic transplant cohort, 50% of recurrent PBC patients receiving UDCA showed normalisation of serum alkaline phosphatase and alanine aminotransferase levels over a 36-month period compared to 22% of untreated patients (Charatcharoenwittaya 2007). Although no significant differences in the rate of histological progression was detected between the treated and untreated subgroups, the proportion of individuals with histological progression was significantly lower in those that showed improvement of biochemical parameters regardless of treatment.

A published multicentre study showed that preventive treatment with UDCA reduces the risk of PBC recurrence after LT (Bosch 2015). The 5, 10, and 15-year rates of recurrence were 11%, 21%, and 40%, respectively, under UDCA treatment, and 32%, 53%, and 70%, respectively, without preventive UDCA. However, neither preventive UDCA nor recurrence had a significant impact on survival. Recently published German Guidelines for autoimmune related liver diseases recommend use of UDCA in patients with recurrent PBC (Strassburg 2017). EASL Clinical Practise Guidelines on Liver Transplantation (2016) do not recommend so far prophylactic use of UDCA in patients transplanted for PBC and PSC (<https://dx.doi.org/10.1016/j.jhep.2015.10.006>).

Obeticholic acid (OCA) is a promising new therapy that has been shown to substantially improve the long-term outcomes of PBC patients with inadequate response or intolerance to UDCA in the non-transplant setting. However, data are awaited to examine the effects of OCA on clinical outcome

in patients with recurrent PBC and the need for an alternative treatment option other than UDCA. Since bile salts are responsible for the secondary toxic consequences, bile salt and nuclear hormone directed therapies may improve secondary toxic injury and are under current investigation. However, so far, these drugs are not available yet.

The reported recurrence rates for PSC after LT range between 9% and 37% (Cholongitas 2008, Alabraba 2009, Vera 2002, Graziadei 1999, Goss 1997). Biliary complications and diagnosis of recurrent PSC can be easily managed in patients with duct-to-duct biliary reconstruction. While Roux-en-Y hepaticojejunostomy was previously the common anastomotic technique for LT in patients with PSC, duct-to-duct reconstruction is currently recommended if there is no evidence of pathological changes of the common bile duct.

Recently published German Guidelines for Autoimmune Related Liver Diseases state that UDCA can be used for patients transplanted for PSC as randomised controlled studies on the efficacy of UDCA in patients transplanted for PSC are not available (Strassburg 2017). UDCA does not seem to have an influence on PSC recurrence rates. Preclinical studies in the non-transplant setting suggest that FXR- and PPAR-agonists, inhibitors of the apical sodium-dependent bile salt transporter (ASBT-inhibitors) and the C23 UDCA derivative nor-UDCA are promising agents for the treatment of PSC. However, data from studies targeting new therapeutic approaches in LT patients with recurrent PSC are not available. Various risk factors for PSC recurrence have been identified including the presence of cholangiocarcinoma prior to LT; presence of certain human leukocyte antigen (HLA) such as HLA-DRB1*08, HLA DR52 in the recipient or donor; male recipient, a recipient-donor gender mismatch; recipient age, an intact colon in the recipient prior to LT, the presence of ulcerative colitis and early cholestasis after LT; use of extended donor criteria grafts; acute cellular rejection, steroid-resistant acute cellular rejection or use of OKT3; maintenance of steroid therapy for ulcerative colitis for more than 3 months; and CMV infection in the recipient (Faisal 2015, Montano-Loza 2016). An increased risk of recurrence has been reported in recipients of grafts from first-degree living related donors in two small single centre series from Japan (Tamura 2007, Haga 2007).

Recurrent PSC is diagnosed by histology and/or imaging of the biliary tree and exclusion of other causes of non-anastomotic biliary strictures. Histopathological findings in PSC include fibrous cholangitis, fibro-obliterative lesions, ductopenia, and biliary fibrosis.

It has been described that recurrence of PBC and AIH does not significantly impact long term outcome including overall survival whereas recurrent PSC has been associated with a higher retransplantation rate (Tanaka 2020).

A British LT group found significantly better recurrence-free survival rates in patients who underwent colectomy before or during LT and in those with non-extended donor criteria allografts (Alabraba 2009).

Interestingly, despite immunosuppression, a significantly higher corticosteroid requirement was reported in the transplant compared to the non-transplant setting, with 20% of PSC patients with concomitant PSC becoming corticosteroid dependent after LT (Ho 2005). A recent study reported that maintenance steroids (>3 months) for ulcerative colitis post-LT were a risk factor for recurrent PSC (Cholongitas 2008). A Scandinavian group studied the risk of colorectal neoplasia among 439 PSC patients, 80% of whom had chronic inflammatory bowel disease prior to LT and 3% of whom had developed *de novo* chronic inflammatory bowel disease (Jørgensen 2012). The median history of chronic inflammatory bowel disease was 15 years (range 0–50 years) and the follow-up period posttransplantation was 5 years (range 0–20 years). A fourth of the PSC patients who additionally had bowel involvement developed colorectal neoplasias. This frequency was twice as high postoperatively than before LT. Patients receiving TAC and MMF had a significantly higher risk of chronic inflammatory bowel disease-associated active inflammation than patients taking CSA and azathioprine (Jørgensen 2013). Moreover, in a recently published Swedish study (Lindström 2018) TAC was reported as an independent risk factor for PSC recurrence. However, due to conflicting results in literature, impact of immunosuppression on PSC recurrence needs further investigation.

AIH recurrence has been reported in about one-third of patients within a posttransplant follow-up period of ≥ 5 years (Mendes 2011, Tripathi 2009, Campsen 2008, Vogel 2004). Transplantation centres commonly maintain AIH patients on prednisone after LT to reduce rejection and recurrence rates. However, there is limited evidence for this approach (Stirnemann 2019) and impact of type and dosing of immunosuppressive drugs on outcome needs further investigation. Survival rates post-LT are approximately 90% and 70% at 1 and 5 years (Montano-Loza 2016). A long-term follow-up study (>10 years) by a French group found AIH recurrence in 41% of the patients. The authors recommended regular liver biopsies, because histological signs precede abnormal biochemical liver values in about one-fourth of patients (Duclos-Vallee 2003). The diagnosis of recurrent AIH may include histological features, the presence of autoantibodies, and increased gamma globulins. Histological signs of recurrence include interface hepatitis, lymphoplasmacytic infiltration, and/or lobular involvement. The majority of published studies did not confirm a posttransplant prognostic role of antibodies in patients undergoing LT for AIH. Conflicting data exist regarding the presence of specific HLA antigens that predispose patients to AIH recurrence after LT (Gonzalez-Koch 2001, Molmenti 2002).

Recurrent AIH must be distinguished from *de novo* AIH, which is a

clinical entity resembling AIH and develops in LT recipients transplanted for other liver disorders. It was originally described in children after LT. The incidence of *de novo* AIH is variable because multiple descriptions have been used in case series. The Banff working group on liver allograft pathology has recently suggested that the nomenclature '*de novo* AIH' should be replaced by the terminology 'plasma-cell rich rejection' (Montano Loza 2016, Demetris 2016).

Outcome and recurrence in patients transplanted for hepatic malignancies

The results of early studies of LT for HCC were disappointing. More than 60% of patients developed tumour recurrence within the first two years posttransplant (Ringe 1989). Currently, there are recurrence rates of 10-15% in patients fulfilling the Milan criteria (Zavaglia 2005). In analyses of predictors of survival histological grade of differentiation, macroscopic vascular invasion and satellitosis were identified as independent predictors of survival and tumour recurrence (Zavaglia 2005, Hoyos 2015). Others identified MELD score >22, AFP >400 ng/mL and age >60 years as negative predictors for survival in HCC (Sotiropoulos 2008b, Jelic 2010). Several retrospective cohort studies are published in literature which demonstrated statistically significant differences in survival and recurrence between different RECIST criteria after LT (Morris 2016). AFP independently predicts tumour recurrence and correlates with vascular invasion and differentiation (Duvoux 2016). A French group of researchers developed a selection model called the AFP score. This score allows patients with HCC not meeting Milan criteria but scored 2 or lower, with AFP levels less than 100 ng/mL and a low 5-year risk of recurrence to be transplanted with excellent results (Duvoux 2016). In another study, Notarpaolo (2016) tested this AFP score in a population of non-French patients transplanted for viral hepatitis underlying HCC. The authors concluded that in this specific population, the AFP model better selects patients with HCC as compared to Milan criteria and that the AFP score can also be implemented in countries with an important burden of HCC occurring on post-hepatitic cirrhosis.

For patients having an indication for LT despite exceeding the Milan criteria, the use of marginal grafts or performance of LDLT has been considered as a reasonable option.

Expansion beyond the Milan criteria to University of California San Francisco (UCSF) criteria (single tumour <6.5 cm; two to three tumours, none >4.5 cm or total diameter <8 cm, no vascular invasion) or even more liberal criteria (no portal invasion, no extrahepatic disease) have been discussed widely (Sotiropoulos 2007, Silva 2011, Jelic 2010). Centers such as the San Francisco Transplant Group as well as the UCLA Transplant Group have demonstrated 5-year survival rates of 50-80% after LT for tumours beyond

the Milan criteria but within UCSF criteria (Duffy 2007, Yao 2007). In a recently published study (Victor 2020) from the Houston transplant group, 220 HCC patients were transplanted, 138 inside Milan, 23 inside UCSF, and 59 beyond UCSF criteria. Interestingly, patient survival was similar at 1, 3, or 5 years despite pathologic tumor size.

The 'up to seven' criteria (7 being the sum of the size and number of tumours for any given HCC) was suggested as an approach to include additional HCC patients as transplant candidates. However, acceptance of a more liberal organ allocation policy would result in a further increase of HCC patients on the waiting list and in denying the use of these organs to other non-HCC patients.

The existence of several scoring systems in this era of LT shows on the one hand the widely held conviction of the transplant community that the well-established Milan criteria are too restrictive, not allowing many HCC patients the LT opportunity; on the other hand, this situation reflects some limitations of the existing pretransplant radiological evaluation (Sotiropoulos 2009). Multiple reports in the radiology literature address nodule detection in cirrhotic livers by means of CT, MRI, or ultrasonography. Many of them conclude that contrast-enhanced MRI is the most sensitive technique for detecting liver nodules (Teefey 2003, Tokunaga 2012). MRI has been shown to depict only 39 of 118 HCC in cirrhosis, for an overall sensitivity of 33% (Krinsky 2002). Detection of small tumours was inadequate, with only 11 of 21 lesions (52%) between 1 and 2 cm and 3 of 72 lesions (4%) <1 cm correctly classified. The sensitivity in the series from Essen was similarly poor, 0% for tumours <1 cm and 21% for tumours between 1 and 2 cm (Sotiropoulos 2005). Similar findings have been reported (Bhartia 2003) with the conclusion that the identification rate of tumours <1 cm is still limited. The presence of microvascular invasion and, in some cases, macrovascular invasion of segmental branches can usually be determined by pathologic inspection of the explanted liver. This, together with inaccurate tumour detection, leads to upgrading of the tumour stage or the classification according to the different sorts of criteria in the posttransplant period, compared to assumed stages by radiological evaluation. More important, however, is the fact that some patients might not be given the opportunity to undergo LT on the basis of inaccurate radiological and clinical preoperative staging.

Mazzaferro et al. (2018) found that patients with HCC achieve a 70% chance of HCC-specific survival 5 years after LT, if AFP level are <200 ng/mL and the sum of number and tumour size (in centimeters) do not exceed 7. The authors created a model comprising level of AFP, tumour size, and tumour number, to determine the risk of death from HCC-related factors after LT and to define selection criteria for LT in HCC patients. For this purpose they provided an online calculator to predict 5-year survival and

risk of HCC-related death.

Expansion of criteria in the LDLT setting is even more challenging due to the donor risk and the risk of selection of tumours with unfavourable biology following the concept of fast-tracking (Hiatt 2005). Novel molecular biology techniques, such as genotyping for HCC, may become relevant for determining recurrence-free survival and improving patient selection, but these biomarkers can not yet be used for clinical decision making.

A potential survival benefit was reported in studies and a meta-analysis of controlled clinical trials with SRL-based immunosuppression in patients transplanted for HCC (Kneteman 2004, Zimmerman 2008, Toso 2007, Liang 2011). These results are in line with a retrospective analysis based on the Scientific Registry of US Transplant Recipients, which included 2491 HCC LT recipients and 12,167 recipients with non-HCC diagnoses. Moreover, the SILVER Study, a large prospective RCT, comparing SRL-containing versus SRL-free immunosuppression showed a benefit in recurrence-free survival and overall survival in the SRL group in the first 3 to 5 years, in particular in low risk patients, but did not improve long-term recurrence-free survival beyond 5 years (Geissler 2016).

Sorafenib (SOR) is currently used for HCC recurrence after LT when patients are not suitable for surgical/locoregional treatments. In an Italian study (Invernizzi 2019) treatment response was obtained in 16% and stable disease in 50% in those treated with SOR (74% were on mTOR inhibitors). Median time to radiological progression was 6 months. Baseline predictors of overall survival were SOR+mTOR inhibitors, previous curative treatments and AFP>100 ng/ml.

Although initial post-LT survival rates were poor in patients with unresectable hilar CCA outcomes, after introduction of the Mayo Clinic protocol, outcomes have been more promising. Neoadjuvant chemoradiation and subsequent LT has shown promising results for patients with localised, unresectable hilar cholangiocellular carcinoma (CCC) (Welling 2014, Masuoka 2011). In a published US study, the outcome of 38 patients who underwent LT was compared to that of 19 patients who underwent combined radical bile duct resection with partial hepatectomy (Hong 2011). The tumour was located in the intrahepatic bile duct in 37 patients and in the hilar bile duct in 20 patients. Results demonstrated that LT combined with neoadjuvant and adjuvant therapies is superior to partial hepatectomy with adjuvant therapy. Challenges of LT attributable to neoadjuvant therapy include tissue injury from radiation therapy and vascular complications including HAT. Predictors of response to the neoadjuvant protocol prior to LT need to be determined (Heimbach 2008). Increasing age, high pretransplant tumour marker, residual tumour size in the explant >2 cm, tumour grade, previous cholecystectomy and perineural invasion were identified as predictors of recurrence following LT (Knight 2007).

Machairas et al. (2020) conducted a systematic review investigating long-term outcomes of patients (n=698) with hilar CCC undergoing LT. A total of 13 studies were included in this systematic review. The majority (74.4%) received neoadjuvant therapy (combined chemotherapy and radiation). One-, 3- and 5-year overall survival rates ranged between 58%-92%, 31%-80% and 20%-74%, respectively. Recurrence rates ranged widely between 16% and 61%, and perioperative mortality ranged between 0% and 25.5%. Results revealed that LT could provide acceptable long-term outcomes in the setting of neoadjuvant therapy using strict patient selection criteria.

Metastatic lesions originating from neuroendocrine tumours (NET) may be hormone-producing (peptide hormones or amines) or may present as nonfunctional tumours (Frilling 2006). They are characterised by slow growth and frequent metastasis to the liver, and their spread may be limited to the liver for protracted periods of time. Most studies in patients transplanted for NET are limited and usually restricted to small numbers of patients. An analysis based on the UNOS database including patients transplanted for NET between October 1988 and January 2008 showed that long-term survival of NET patients was similar to that of patients with HCC. Excellent results can be obtained in highly selected patients and a waiting time for LT longer than 2 months (Gedaly 2011). Long-term results from prospective studies are needed to further define selection criteria for patients with NET for LT, to identify predictors for disease recurrence, and to determine the influence of the primary tumour site on patient posttransplant survival.

Recurrent alcohol abuse after liver transplantation for alcoholic liver disease

Recent trials have shown that uEtG or hair-EtG determinations are reliable markers for detection of alcohol relapse after LT (Staufer K 2011). Reported rates of returning to drinking after LT for ALD vary in the literature. Studies revealed a mean incidence of relapse in one-third of patients ranging from 10% to 50% in up to 5 years of follow-up (EASL CPG Management of alcohol-related liver disease [2018]). Approximately 10% to 15% of patients with recurrent ALD resume heavy drinking with damage of the new liver (Marroni CA 2015, Marroni 2018).

Marot et al. (2018) performed a systematic review and metaanalysis in patients with AH. Pooled estimated risk for alcohol relapse was 0.22. This risk was not statistically significant different between AH and AC with 6 months of abstinence. Pooled estimated rate for 6 month survival was 0.85 and similar between both groups.

Predictors of recurrence include positive family history of substance use, pretransplant abstinence, failed rehabilitation attempts, history of prior alcohol-related legal issues, history of substance abuse (other than

alcohol), smoking, lack of social support, lack of familiar support, denial of drug-related problems and addiction, length and intensity of alcoholic liver disease and psychiatric comorbidities (Perney 2005, Dew 2008).

Patient and graft survival is excellent in those maintaining alcohol abstinence after LT. A recent study (Parrish 2019) considering SRTR data from patients (n=53,788) transplanted between 2014 and 2017 showed that patients with ALD and HCV had superior graft survival rates (90.7% at 1 year, 78.9% at 3 years and 90.0% at 1 year, 79% at 3 years, respectively) as compared to those with nonalcoholic steatohepatitis (NASH) (87.5% at 1 year, 77.9% at 3 years).

The American Consortium of Early Liver Transplantation for Alcoholic Hepatitis analysed outcome of early LT for patients without mandatory period of sobriety with severe alcoholic hepatitis. Data derived from 12 centres from 8 UNOS regions (Lee (c) 2018). The authors reported a cumulative incidence of any alcohol use (slips or sustained alcohol use) of 25% at 1 year (95% CI, 18%-34%) and of 34% at 3 years (95% CI, 25%-44%) after LT. The cumulative incidence of sustained alcohol use was 10% at 1 year (95% CI, 6%-18%) and 17% at 3 years (95% CI, 10%-27%) after LT. Patients overall survival after 1 year (94%) and 3 years (84%) was not significantly worse compared to patients undergoing LT for other indications but sustained drinking after LT was associated with increased mortality (hazard ratio, 4.59; P=.01). A significant decrease of the medium- and long-term survival in severe chronic alcohol consumption after LT has also been shown in previous studies (Pfitzmann 2007).

For LT recipients with a history of ALD (and positive smoking history), a more intensive surveillance protocol including annual skin and ear nose throat (ENT) examinations as well as upper endoscopy (every 2–3 years) and abdominal ultrasound should be considered. Modifiable factors such as life style habits including cigarette smoking, physical inactivity, and obesity should be avoided. A systemic evaluation including malnutrition, vitamin and trace element deficiency, and osteoporosis is recommended.

According to results from the European Liver Transplant Registry (ELTR), mortality and graft failure were more often related to de novo tumors, cardiovascular and social factors in alcoholic LT patients as compared to patients transplanted for other etiologies (Burra 2010). LT recipients with a prior diagnosis of ALD might benefit from immunosuppressive regimens that minimize CN1 exposure and favor mTOR-containing regimens. However, prospective studies are needed to gain more insight into this issue.

Recurrent non-alcoholic fatty liver disease

The increasing incidence of obesity and the metabolic syndrome throughout developed countries results in an increasing proportion of

patients transplanted for NAFLD (Darwid Murash 2015). Younossi et al. (2016) constructed a steady-state prevalence model to quantify the economic and clinical burden of NAFLD in the United States and Europe. Data were validated using a computerised disease model. In the United States, over 64 million people are projected to have NAFLD, with an annual direct medical burden of approximately \$103 billion (\$1,613 per patient). In Germany, France, Italy, and United Kingdom, the authors estimated ~52 million people with NAFLD with an annual cost of approximately €35 billion (from €354 to €1,163 per patient). Life style interventions are of utmost importance and overweight patients who achieve significant reductions in body weight through physical activity and low caloric diet can decrease liver fat, visceral and subcutaneous adipose tissue (Copaci 2015). Treatment of NAFLD will likely involve a holistic, multidisciplinary and personalised approach (Malhotra 2015).

Patients transplanted for NAFLD had similar outcomes compared with patients transplanted for other indications (Burra 2014). Reported NAFLD recurrence rates after LT vary in the literature, ranging between 20 and 40%. The components of metabolic syndrome are often exacerbated following LT by factors such as immunosuppression requiring an aggressive management of cardiovascular complications after transplantation.

The transplant group from Stockholm (Tokodai 2019) conducting a retrospective study identified recipient age and 1-year BMI in multivariate analysis as independent risk factors for post-LT fatty liver disease development. Weight gain after LT is significantly greater in patients with older age (>50 years) and in those transplanted for chronic compared with fulminant liver failure. Thus, at least for steroid-free regimens, weight gain seems to be unrelated to any specific immunosuppressive drug. The greatest weight gain has been observed after the first 6 months posttransplant. Physical activity in LT recipients should be proposed as part of their therapeutic regimens. It also appears to improve health-related quality of life after LT, thus regular exercise programmes and a healthy diet may be incorporated to avoid cardiovascular morbidity and mortality and NAFLD recurrence (Cotter 2020).

There are continuous efforts on finding novel agents to help prevent and to slow down the progression of NAFLD (Younossi ZM 2019, Tang 2019). The importance of the gut microbiome in mediating hepatocyte inflammation and intestinal permeability may also offer future treatment options.

Pregnancy after liver transplantation

Adequate preconception counseling is crucial to provide optimal conditions for pregnancy and to modify immunosuppressive therapy if necessary to minimise risks for both the mother and the fetus. Female LT patients of reproductive age should preferentially use contraception during the first 12 months after transplantation. Fetal loss, prematurity, and low birth weight have been reported in women who have undergone transplantation, and maternal risks include hypertension, preeclampsia, gestational diabetes, and graft dysfunction. The rate of caesarean section is considerably higher in post-LT patients. Steroids, CNIs have not been reported to be teratogenic and should be maintained during pregnancy; whereas mycophenolate mofetil has shown to cause malformations in animal models and should be avoided. mTOR inhibitors may affect spermatogenesis in male recipients. More studies should be designed to investigate the role of immunosuppression on sexual dysfunction. In a retrospective recently published study, Zaffar et al. (2018) considered 41 pregnancies in 28 transplanted women. Mean transplant-to-pregnancy interval was 8.5±5.1 years. Immunosuppressive therapy consisted of TAC±azathioprine (n=26), CSA (n=4) and prednisone with other immunosuppressive drugs (n=11). During pregnancy the following adverse events have been reported: hypertension (n=10), impairment of renal function (n=6), gestational diabetes (n=4), impairment of allograft function (n=2), and blood transfusion requiring anaemia (n=1). Two miscarriages, three stillbirths and one neonatal death occurred. Moreover, five small-for-gestational-age infants, one minor congenital anomaly and premature delivery in fourteen infants (38.9%) have been reported.

Although there is an increased risk for pregnancy-related complications as compared to the general population an appropriate multidisciplinary care, stable graft function at pregnancy onset and adherence to immunosuppressive regimens are a good prerequisite for a successful pregnancy and delivery after LT.

Experiences with liver transplantation in inherited metabolic liver diseases in adult patients

LT is regarded as an effective treatment strategy for patients with Wilson's Disease, which presents as deterioration of cirrhosis not responsive to treatment, as acute-on-chronic disease or fulminant hepatic failure (Moini 2010). LT reverses the abnormalities of copper metabolism

by converting the copper kinetics from a homozygous to a heterozygous phenotype, thus providing an adequate increase of ceruloplasmin levels and a decrease of urinary copper excretion posttransplant. The King's College Hospital reported excellent long-term results after LT in patients who have undergone LT for Wilson's Disease since 1994 with 5-year patient and graft survival rates of 87.5% (Sutcliffe 2003). There are several reports in the literature indicating a reversal of neurological symptoms after LT (Martin 2008). However, the course of neurological symptoms remains unpredictable and it is still a matter of debate whether LT should be considered in patients with severe neurological impairment (Pabón 2008).

AAT deficiency is a common genetic reason for paediatric LT, but a rare indication in adults. The Z allele is most commonly responsible for severe deficiency and disease. LT corrects the liver disease and provides complete replacement of serum AAT activity. 567 AAT recipients who underwent LT between 1995 and 2004 were retrospectively investigated (Kemmer 2008). Results based on UNOS data revealed 1-, 3-, and 5-year patient survival rates of 89%, 85%, and 83%, respectively.

In haemochromatosis, iron depletion therapy prior to LT may be associated with a better outcome after LT and is strongly recommended (Weiss 2007). It has been reported that the survival of patients who undergo LT for hereditary haemochromatosis is markedly lower in comparison to other indications (Dar 2009, Brandhagen 2001). Reduced posttransplant survival in patients with haemochromatosis has been attributed to cardiac problems and increased infectious complications. Findings derived from the UNOS database revealed 1- and 5-year survival rates of 75% and 64% in patients with iron overload, as compared to 83% and 70% in those without iron overload (Brandhagen 2001). More recent results from patients with haemochromatosis (n=217) transplanted between 1997 and 2006 revealed excellent 1- (86.1%), 3- (80.8%), and 5-year (77.3%) patient survival rates, which were not different from those transplanted for other liver diseases (Yu 2007).

LT halts production of mutated transthyretin (TTR) and therefore represents an accepted treatment for hereditary transthyretin (ATTR) amyloidosis, a systemic amyloidosis mainly affecting the peripheral nervous system and heart (Rocha 2016). Okumura et al. (2016) recently assessed 29 non-transplant and 36 transplant FAP V30M patients using an FAP clinical scoring system. They found that LT had beneficial effects on FAP clinical manifestations in these patients. However, the effects of transplantation on the clinical manifestations of FAP have not been systematically investigated and future studies are urgently warranted.

Outcome after liver transplantation for acute hepatic failure

About half of acute hepatic failure (AHF) patients undergo LT. ALF accounts for 5-12% of LT activity worldwide.

Of patients listed for transplantation, approximately one third will recover spontaneously without the need for grafting; thus, in as many as 20% of ALF patients LT is required (Lee 2012). Transplantation should be considered in those patients fulfilling Clichy or Kings College criteria (EASL CCPG on the Management of Acute (Fulminant) Liver Failure (2017); <http://www.easl.eu/medias/cpg/ALF/English-report.pdf>). Drug-induced liver injury due to acetaminophen overdose is the most common cause of LT for acute liver failure in developed countries (Craig 2010, Au 2011). Other etiologies comprise idiosyncratic drugs (such as isoniazid/rifampicin, coumarins, acetaminophen, ecstasy, tricyclic antidepressants), Budd-Chiari syndrome, Wilson's Disease, hepatitis A, B and E infection or autoimmune disease.

Early postoperative complications in patients transplanted for AHF include sepsis, multisystem organ failure, and primary graft failure. Serum creatinine concentrations above 200 µmol/L pretransplant, non-white race of the recipient, donor body mass index >35 kg/m² and recipient age >50 years have been suggested as risk factors for posttransplant mortality (Wigg 2005). Others reported that extended donor criteria rates and severe cerebral edema were associated with worse outcome (Chan 2009). The Edinburgh LT centre investigated the impact of perioperative renal dysfunction on posttransplant renal outcomes in AHF patients. They found that older age, female gender, hypertension, CSA and non-acetaminophen-induced AHF but not the severity of perioperative renal injury were predictive for the development of chronic kidney injury (Leithead 2011).

The results in patients transplanted for AHF have improved within the last decade due to the establishment of prognostic models, improved intensive care management and the option for LDLT which has a limited role in the US and Europe but plays a major role in Asia (Lo 2008). AHF was the indication for LDLT in more than 10% of the cohort reported by two Asian groups (Morioka 2007b, Lo 2004).

It has been reported that survival in patients with AHF is inferior to that of recipients with non-acute indications for LT in the first year but comparable in the long-term (Chan 2009, Wigg 2005). The US Acute Liver Failure Study Group found that two-year outcomes in initial survivors of AHF are generally good but that non-acetaminophen patients have a significantly lower survival, which may be related to pre-existing medical comorbidities (Fontana 2014).

Conclusion

LT is challenging due to a shortage of organs and a prolonged waiting-list time. The large disparity between the number of available deceased donor organs and recipients awaiting LT has created an ongoing debate regarding the appropriate selection criteria. A variety of approaches have been implemented to expand the organ donor pool including national efforts to expand deceased donor donation, split organ donations including LDLT, increased use of more elderly and obese donors and greater utilisation of expanded criteria donors. The rationale of allocation systems utilising the MELD score is to prioritise patients with severe liver dysfunction ("the sickest first"). This results in decreased waiting list mortality from 20 to 10% in the Eurotransplant region but also in a reduction of 1-year posttransplant survival by approximately 10%. A potential modification of the MELD allocation system or development of an improved prognostic scoring system is urgently warranted to optimise organ allocation in the future.

Due to the availability of antiviral drugs, the survival of patients undergoing LT for HBV infection has dramatically improved and has become comparable to or even better than the survival of patients with non-virus-related liver diseases. Protocols have been published in literature implementing withdrawal of HBIG or HBIG-free regimens, using only oral antivirals, in particular in patients at low risk of recurrence.

The availability of DAA all-oral combinations constitutes a substantial improvement in HCV therapy and in particular in patients formerly difficult-to-treat such as cirrhotic patients and in managing HCV infection after LT. However, HCV treatment pretransplant may result in lowering the priority of LT (MELD score reduction) without clinical improvement, thereby delaying potentially curative transplantation. Thus, robust predictors of improvement in hepatic function and quality of life are currently being investigated to identify patients with decompensated cirrhosis who benefit from DAA therapy prior to LT. Liver and renal impairment should be taken into account before treatment initiation. SVR rates in LT patients are comparable with nontransplant patients and can be achieved with excellent tolerability. SVR has also been shown to reduce the risk of renal impairment and cardiovascular-related morbidity. Some important issues still remain, such as the evaluation of safety of these new DAAs in patients with decompensated cirrhosis, the role of RBV in all-oral combinations and drug-drug interaction profiles, in particular after LT.

Expansion of the donor pool by including HCV positive organs in the DAA era could substantially decrease waiting times and mortality rates for patients listed for LT. Mounting data demonstrate the safety of using organs from HCV-infected donors with subsequent treatment of HCV in

the recipient. However, use of HCV positive donors in HCV negative LT recipients may currently be restricted to urgent situations and necessitates a robust informed consent process.

Data about the frequency of disease recurrence in cholestatic and autoimmune liver diseases vary in the literature. Diagnosis of disease relapse in cholestatic and autoimmune liver disease is more challenging than in the non-transplant setting. Most studies report excellent medium-term and long-term results despite limited therapeutic options for disease recurrence.

LT in HCC patients provides excellent outcomes and low recurrence rates following the Milan criteria. Expansion of transplantation criteria beyond the Milan criteria has been discussed at length. The acceptance of a more liberal organ allocation policy may result in a further increase of the proportion of patients transplanted for HCC and denying the use of these organs to other patients for whom better results may be achieved. Recent developments in genomic and proteomic approaches may allow the identification of new biomarkers for prediction of HCC recurrence.

ALD is the leading indication for LT in European and US transplant centers. Early LT without fulfilling the 6-month abstinence rule should be restricted to those with severe disease who are not responding to medical therapy, have been subjected to a careful selection process and have a favourable addiction and psychosocial profile. German regulations require 6 months of alcohol sobriety in patients with ALD, however, in exceptional cases patients can get access to the waitlist through an audit process requested by the corresponding transplant center and organized by Eurotransplant.

There should be psychosocial evaluation of the patient with ALD prior to LT considering possible risk factors for recurrence. Implementation of prognostic instruments for prediction of alcohol relapse are recommended. ALD patients on the waiting list should be monitored for alcohol use by regular clinical interviews and laboratory tests to confirm abstinence. However, standardization and unified policy of the selection process may be helpful. Prospective studies are urgently needed to resolve the controversies that still surround the criteria for selection of those patients for LT.

The management of cardiovascular, renal, coagulopathic, cerebral and infectious complications in patients with AHF is clinically challenging. Prognostic models are helpful but not entirely accurate in predicting those who will require LT. Due to advances in intensive care medicine and surgical techniques, outcomes for patients with AHF have progressively improved over the last 2 decades.

CNI, at least at low doses, with or without other immunosuppressive drugs, have been so far the cornerstone of immunosuppressive regimens in a substantial proportion of LT patients. Much attention has been directed

to reducing CNI-associated long-term complications. Cardiovascular comorbidities due to metabolic complications such as diabetes mellitus, dyslipidaemia, obesity, and arterial hypertension account for 30-70% of long-term morbidity. Current trends of immunosuppressive strategies include CNI-sparing or CNI-free protocols including MMF- and/or mTOR-based immunosuppressive regimens and corticosteroid-avoidance protocols. CNI delay with induction therapy for bridging the early postoperative phase should be considered especially in patients with high MELD scores. Finally, “individually tailored immunosuppressive” protocols may optimise drug efficacy, minimise drug toxicity and improve transplant outcome.

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20. End-stage liver disease, HIV and liver transplantation

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Introduction

The introduction of effective combined antiretroviral therapy (cART) changed HIV into a chronic disease with significant reductions in AIDS-related deaths and large increases in life expectancy (ART-CC 2008, Barre-Sinoussi 2013). This has, however, been accompanied by a steady increase in liver-related morbidity and mortality due to coinfection with chronic hepatitis B (HBV) and hepatitis C (HCV) (Joshi 2011, Ioannou 2013). As a consequence, end-stage liver disease (ESLD) has become one of the main causes of death among people living with HIV who are coinfecting with HCV or HBV (Smith 2014, Weber 2006, Weber 2013).

Hopefully, the burden of morbidity and mortality due to HCV coinfection will decrease with the uptake of the recently-introduced direct acting antivirals (DAAs). Meanwhile, the medical management of liver-related complications is essential, and liver transplantation (LT) remains the only therapeutic option for appropriate HIV positive candidates with end-stage liver disease (ESLD).

The aim of the present review is to give an overview presenting epidemiological data on ESLD and liver-related mortality in the setting of HIV and discussing the role of liver transplantation in this population.

End-stage liver disease in HIV positive patients

Magnitude of the problem and natural history

Of the approximately 35 million people living with HIV globally, between two and four million are chronically infected with HBV (Alter 2006) and around seven million have chronic HCV (Soriano 2010).

The prevalence of HCV and/or HBV coinfection varies considerably depending on the mode of HIV transmission and the geographical region (Peters 2014, Taylor 2012, Chew 2016, Klein 2016). Overall, the prevalence

of HCV coinfection is 25% to 30% (Kim 2013, Peters 2014, Rockstroh 2005, Chew 2016) and of HBV coinfection around 5% to 20% (Konopnicki 2005, Soriano 2013). However, a number of reports have revealed changes in the epidemiological pattern (Ioannou 2013, Kim 2013, Taylor 2012). In Spain there was a significant decrease in prevalence of HCV/HIV coinfection, from 25.3% in 2004–2005 to 8.2% in 2010–2011 (Serrano-Villar 2015). This trend was consistently observed in all risk groups: PWID 92.4% to 81.4%; MSM, 4.7% to 2.6%; heterosexual men, 13.0% to 8.9%; and heterosexual women, 14.5% to 4.0%. Moreover, a decrease in prevalence from 35% to 25% in people with HCV/HIV coinfection was also observed in the US (Ioannou 2013).

It is well known that HIV has a deleterious effect on the natural history of HCV infection. HIV infection leads to higher HCV viraemia, decreased responsiveness to HCV therapy with pegylated interferon (PEG-IFN) and ribavirin (RBV), accelerated rates of fibrosis, increased risk of developing decompensated cirrhosis and death, and a significant risk of developing hepatocellular carcinoma (HCC) (Chen 2014, Garcia-Samaniego 2001, Graham 2001, Gunthard 2014, Konerman 2014, Mohsen 2003, Poynard 2003, Sherman 2015, Rotman 2009, Operskalski 2011, Mastroianni 2014, Chew 2016, Klein 2016).

The mechanisms associated with accelerated fibrosis progression rates among people with HCV/HIV coinfection are not well understood, but multiple hypotheses have been proposed. These include a direct viral effect of HIV on hepatocytes and/or the stellate cells, microbial translocation and many immunologic alterations such as diminished HCV-specific T cell responses, immune activation, increased hepatocyte apoptosis and immunologic dysregulation, that promote hepatic fibrosis (Rotman 2009, Operskalski 2011, Lin 2013, Mastroianni 2014, Chen 2014, Mastroianni 2014, Sherman 2015, Chew 2016).

The prevalence of cirrhosis in people with HCV/HIV coinfection is 21% and 49% at 20 and 30 years following the acquisition of HCV infection, respectively (Thein 2008). The risk of cirrhosis development is two-fold higher in patients with HCV/HIV coinfection patients to HCV mono-infection (Thein 2008). Additionally, patients with HCV/HIV coinfection who are on cART have a two-fold higher risk of fibrosis progression, if they have uncontrolled HIV replication (Cooper 2015). Indeed, a higher grade of fibrosis is associated with an increased rate of hepatic decompensation (Chen 2014, Limketkai 2013, Macias 2014, Berenguer 2015, Lo Re 2014, Macias 2013, Chen 2009, Branch 2012). To the contrary, those patients with HCV/HIV coinfection who achieve sustained virologic response of HCV infection with PEG-IFN and RBV have a higher probability of hepatic fibrosis regression (Casado 2013, Lissen 2006), as well as a lower risk of developing hepatic decompensation events and death (Berenguer 2012, Labarga 2015,

Mira 2013, Berenguer 2014). It is expected that HCV-related morbidity and mortality will further decrease with the introduction of the interferon-free DAA regimens (Rockstroh 2015). The advent of these new agents has dramatically improved the treatment options of patients with coinfection, with SVR rates similar to those obtained in HCV mono-infection (EACS 2019, Shafran 2015, Sherman 2015, Arends 2015). Therefore, patients with HCV/HIV coinfection should be treated similar to HCV mono-infection (Sherman 2015, EACS 2019, Shafran 2015, Arends 2015, Karageorgopoulos 2015).

The effect of HCV on the progression of HIV is not well defined. Some studies however have observed a negative impact (Miller 2005, Grint 2014, Hua 2013).

First, patients with HCV/HIV coinfection with active HCV replication have a significantly higher cART discontinuation rates due to toxicity than those who do not have HCV replication or who are not HCV-infected (Grint 2014). Second, patients with HCV/HIV coinfection patients who initiate cART develop virologic failure earlier than patients with HIV mono-infection (Hua 2013). Third, the CD4+ cell increase seems impaired in HCV/HIV coinfection compared to HIV mono-infection (Hua 2013, Miller 2005), and CD8 downregulation would be hampered by HCV/HIV coinfection (Zaegel-Faucher 2015).

In the early cART era, increased liver related morbidity and mortality was observed in numerous studies. From 1996 to 2009, the prevalence of decompensated cirrhosis increased from 2% to 6% in patients with HCV/HIV coinfection (Ioannou 2013). ESLD accounts for approximately 10% of deaths among people with HIV infection (Farahani 2017).

A French prospective multicentre study that followed 21,000 HIV positive patients (4,000 of whom were coinfecting with HCV or HBV) reported that ESLD accounted for 23.7% of non-AIDS-related deaths (Rosenthal 2007). In this population, ESLD was fatal in 1.5% of patients in 1995, 6.6% in 1997, 14.3% in 2001, and 12.6% in 2003. In addition, 92.6% of patients who died from ESLD had chronic HCV. Another prospective study comprising 11 cohorts from Europe, the United States (US) and Australia included 23,500 HIV positive patients (22.5% were HCV positive) recorded 1,250 deaths (Weber 2006). Deaths related to AIDS were the most frequent (31.1%), while liver disease was the most frequent non-AIDS related cause of death (14.5%). Moreover, HCV was shown to be an independent predictor of liver-related death. It is worth noting that the overall and cause-specific mortality in HCV/HIV coinfection remained stable over the last years whereas a decrease in mortality was observed in HIV mono-infection (Berenguer 2012).

Clinical features of HIV-coinfected patients with ESLD

The clinical pattern of the different complications related to cirrhosis shows some differences in cART-treated HCV/HIV coinfection compared to HCV mono-infection (Lo Re 2014). Firstly, people with HCV/HIV coinfection receiving cART have a significantly higher rate of decompensation and mortality (33% versus 15%) than patients with HCV mono-infection (Lo Re 2014).

Ascites is the most frequent event of hepatic decompensation ranging from 36% to 83% in HCV/HIV coinfection (Pineda 2005, Merchante 2006, Pineda 2009, Anderson 2013, Ioannou 2013, Lo Re 2014). The development of spontaneous bacterial peritonitis (SBP) is similar in both groups. SBP in HCV/HIV coinfection had a high incidence of *Streptococcus pneumoniae* exceeded only by *Escherichia coli* (Shaw 2006). In addition, variceal haemorrhage seems less common in HCV/HIV coinfection (Lo Re 2014, Pineda 2005).

Prognosis after hepatic decompensation

The mortality rate for patients with HCV/HIV coinfection with decompensated and compensated cirrhosis was 27/100 person-years and 4/100 person-years, respectively (López-Diéguez 2011). This high mortality impacts the survival rates after hepatic decompensation of these patients, which range from 50% to 66% in the first year (Merchante 2006, Murillas 2009, Pineda 2005, López-Diéguez 2011), 30% to 43% at three years (Merchante 2006, López-Diéguez 2011) and 25% to 30% at five years (Merchante 2006, Murillas 2009, Pineda 2005, López-Diéguez 2011). These survival rates are significantly lower than those observed in patients with HCV mono-infection. The median survival time after the hepatic decompensation is around 13–19 months in HCV/HIV coinfection (Merchante 2006, Murillas 2009, Pineda 2005), while in patients with HCV mono-infection it is 48 months (Pineda 2005).

Several risk factors for hepatic decompensation have been identified: advanced fibrosis stage at presentation (Macias 2014, Lo Re 2014), more advanced liver cirrhosis (Child-Turcotte-Pugh scale -CTP- > 5 points) (Pineda 2009), low CD4 cell count (< 300 cells/mm³), lack of past treatment against HCV (Pineda 2009), anaemia at baseline, and diabetes mellitus (Lo Re 2014). Factors independently associated with mortality in this population are the degree of hepatic fibrosis (Macias 2014), the severity of liver disease measured by the Model of End Stage Liver Disease (MELD) scale (Pineda 2005, Murillas 2009), a higher score on the CTP (Pineda 2005, Merchante 2006, López-Diéguez 2011) and CD4 cell count below 100 cells/

mm³ (Merchante 2006, López-Diéguez 2011). Treatment with cART has a protective role in HCV/HIV coinfection slowing the progression of hepatic fibrosis (Cooper 2014, Anderson 2014, Merchante 2006, López-Diéguez 2011, Murillas 2009, Thorpe 2011). As a result, likelihood of liver decompensation is decreased by 30% by successful cART (Anderson 2014) and the probability of death after the first hepatic decompensation by 40% (Merchante 2006). By contrast, discontinuation of cART (López-Diéguez 2011) or a detectable serum HIV viral load may facilitate progression of fibrosis (Cooper 2014) and increase by more than three times the probability of death in these patients (Murillas 2009, Ingle 2014).

Mortality during the evaluation process for liver transplantation

High mortality rates among patients with HCV/HIV coinfection with ESLD waiting for LT have also been reported in observational studies. Indeed, episodes of decompensation are frequent among patients on LT waiting lists (Warren-Gash 2017). Overall mortality during the evaluation period prior to waiting list entry ranges from 25% (Maida 2005) to 43% (Ragni 2005, Tan-Tam 2014). In addition, once patients are enlisted, mortality on the waiting list may vary from 14% (Subramanian 2010, Tan-Tam 2014, Martel-Laferrrière 2015) to 67% (Murillas 2009). Waiting list mortality was 14% in patients with HIV infection (n=167) and 11% in the control group without HIV (n=792) (p=0.30), with MELD score being the only variable independently associated with death (Subramanian 2010).

For these reasons, physicians attending HIC/HCV coinfection patients with cirrhosis should closely follow and evaluate them for LT after the first clinical decompensation or upon the development of HCC. Both prevention and effective treatment of these complications might improve the likelihood of survival until LT (EACS 2019, Martel-Laferrrière 2013, Tsochatzis 2012).

Management of complications of cirrhosis

The management of complications of cirrhosis (portal hypertension, ascites, gastrointestinal bleeding, encephalopathy, SBP, HCC, and hepatorenal syndrome) is basically the same as for the HIV negative population and is reviewed elsewhere (Forner 2018, Gines 2012, Jalan 2014, EACS 2019, Martel-Laferrrière 2013, Liou 2014, Spengler 2011, Harrison 2016, EASL 2018).

Cirrhotic HIV positive patients should receive cART because it has been shown to be beneficial by reducing complications and mortality (Cooper 2014, Anderson 2014, Merchante 2006, López-Diéguez 2011, Murillas 2009,

Thorpe 2011). Some antiretroviral drugs (ARVs) should be adjusted according to liver function and the use of others is not recommended in cases of cirrhosis (e.g., stavudine, didanosine, zidovudine – are all now very rarely used) (EACS 2019, Martin-Laferrrière 2014, University of Liverpool 2020).

Lifestyle factors and drugs that may accelerate the progression of liver disease, such as hepatotoxic drugs (e.g., didanosine, nonsteroidal anti-inflammatory drugs), alcohol, tobacco, cannabis should be assessed and discontinuation is strongly recommended wherever possible. In some studies, smoking has been linked to more severe fibrosis in patients with chronic HCV (Tsochatzis 2009) and may also increase necroinflammation, irrespective of alcohol consumption (Hezode 2003). Alcohol consumption was higher in patients with HCV/HIV coinfection who died from ESLD (92%) (Rosenthal, 2007, Cooper 2005). Study results assessing the effect of smoking cannabis on liver fibrosis are controversial (Brunet 2013, Hezode 2008, Ishida 2008, Liu 2014).

Vaccination status should be evaluated in the assessment period prior to transplantation and vaccines should be updated according to national schedules (Blumberg 2019)

HCV/HBV management

Several treatments for HBV and HCV infection are currently available. Indications for HCV treatment are identical to those in patients with HCV mono-infection (EASL 2018, EACS 2019, Sherman 2015, AASLD-IDSA 2019).

The main objective of antiviral HCV treatment is to achieve SVR at the time of LT in order to minimise the risk of HCV recurrence posttransplant. HCV eradication reduces the rate of decompensation and might diminish the risk of HCC (EACS 2019).

Treatment of chronic HCV with PEG-IFN+RBV is contraindicated in patients with decompensated liver disease. Safety regarding this regimen in HCV/HIV coinfection is a concern (Mauss 2004). Hepatic decompensation was observed in HCV/HIV coinfecting patients with advanced cirrhosis, and its incidence was 10.4% (14/134). Six of these 14 patients (43%) died as a result of hepatic decompensation. Antiretroviral treatment with didanosine identified as a risk factor. In contrast, no hepatic decompensation was noted in patients with HCV/HIV coinfection without cirrhosis.

The introduction of DAAs has also changed the standard of care for patients with advanced liver cirrhosis, resulting in substantial improvement in HCV cure rates (Campos-Varela 2015, EACS 2019, EASL 2018, Sherman 2015, AASLD-IDSA 2019). IFN-free regimens with fewer side effects, high efficacy and shorter treatment durations are now standard treatment options for difficult-to-treat patients (*see chapters 15 and 19*). In

fact, IFN-free regimens are the only sensible option in HCV/HIV-coinfecting patients due to their virological efficacy, ease of use, safety and tolerability. In addition, adherence to DAAs in patients with HCV/HIV coinfection is high and comparable to that in HCV mono-infection (Townsend 2016). HCV/HIV coinfecting patients with ESLD waiting for LT can be treated before transplantation, although the benefit for these patients is not clearly established (EASL 2018). Furthermore, the widespread use of DAAs and its high therapeutic efficacy in high income countries can impact on the number and type of indications of LT, decreasing notably ESLD due to HCV infection (Figure 1). All this implies that in the future the indications of transplantation for decompensated cirrhosis by HCV will be substantially reduced.

Drug-drug interactions between DAAs and ARVs should be assessed before initiating therapy Karageorgopoulos 2014, Kiser 2013, Sherman 2015, El-Sherif 2015, MacBrayne 2016, EACS 2019). As this is an extremely rapidly evolving area, consultation of up-to-date databases on drug interactions is mandatory. The interactions of the antivirals used for the treatment of HCV can be found on some well established websites (University of Liverpool 2020, Toronto General Hospital's Hepatitis C Drug Information Web site 2017, EACS 2019).

HIV has a negative impact on the progression chronic HBV by increasing HBV replication, reducing the rate of spontaneous clearance of HBeAg and increasing the risk of developing cirrhosis (Thio 2009). Since ongoing HBV replication is a contraindication for LT and only patients without HBV viraemia are accepted for LT, treatment of HBV should be a priority. HIV positive patients with chronic HBV can be treated with lamivudine (or emtricitabine) and tenofovir-DF (TDF) as part of their antiretroviral therapy (Saag 2018, EACS 2019). Due to its high stability against the development of HBV resistance TDF is the standard treatment for HBV in coinfecting patients. Entecavir is an alternative to TDF in addition to fully suppressive cART in selected cases (Saag 2018, EACS 2019). In cohort studies, after five years of continuous treatment, HBeAg seroconversion was achieved in 21% of patients with HIV/HBV coinfection treated with lamivudine, 50% in the group on TDF and in 57% in those receiving TDF/emtricitabine (Saag 2018, Kosi 2012). Moreover, most patients with HIV/HBV coinfection achieved complete suppression of HBV replication with TDF-based HBV therapy despite high baseline viraemia (*see Chapter 14*).

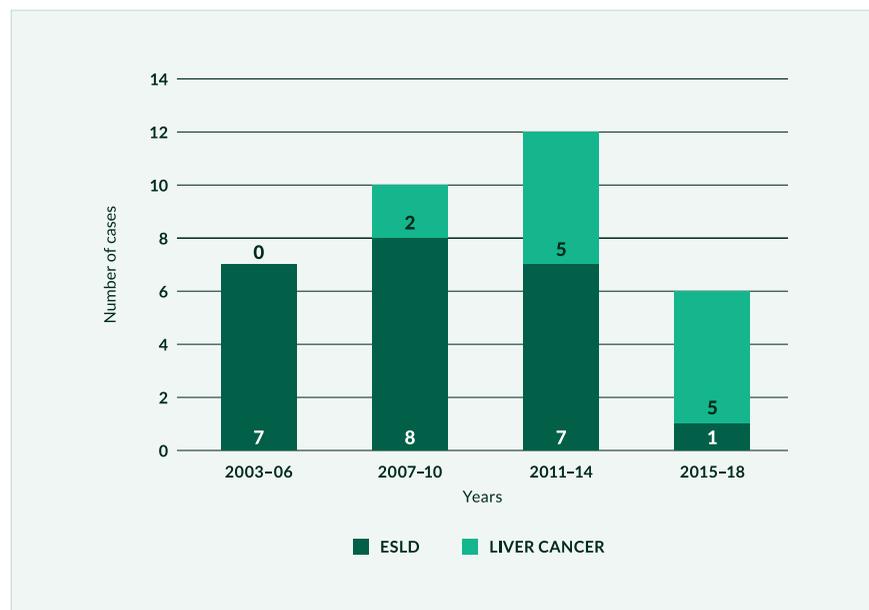


Figure 1. Indications of LT in HIV-infected patients at Hospital Clinic of Barcelona (N=35)

Combined antiretroviral therapy (cART)

Effective cART has been associated with improved clinical outcomes in patients with advanced liver fibrosis and chronic HBV and HCV (Anderson 2013, Limketkai 2012, Lopez-Dieguez 2011, Pineda 2007, Thorpe 2011). In contrast, permanent discontinuation of cART was associated with an increased risk of fibrosis progression (Thorpe 2011), a higher risk of first hepatic decompensation and poorer survival rate (Lopez-Dieguez 2011).

cART should be carefully planned in persons with HIV and ESLD. In general, cART should follow the current guidelines (Saag 2018, EACS 2019). However, some ARVs may be contraindicated in cirrhotic patients (e.g., didanosine, nevirapine), and in advanced liver cirrhosis dosing should be adjusted according to the degree of hepatic impairment in particular for HIV protease inhibitors (University of Liverpool 2020, Wyles 2005). In addition, liver function must be closely monitored for signs of hepatotoxicity (Sherman 2015). Due to their pharmacokinetic characteristics, integrase inhibitors offer advantages in these patients. Raltegravir (RAL) has demonstrated adequate serum levels, without dose adjustment and was well tolerated in patients with decompensated liver cirrhosis stage C on the CPT scale (Barau 2014, Hernández-Novoa 2014). Dolutegravir has a higher barrier to resistance than RAL and also has the advantage of once-daily administration (Blumberg 2019).

Therapeutic drug monitoring may be useful for efavirenz and

protease inhibitors. In addition, atazanavir (and the no-longer used indinavir) can increase unconjugated bilirubin levels by inhibiting UDP-glucuronyltransferase. As total bilirubin is a component of both the Child-Turcotte-Pugh and MELD scores both drugs can affect the score.

Other important pharmacokinetic/pharmacodynamic interactions may exist between ARVs and HCV drugs. cART should be selected or modified to suit the HCV treatment. Fatal lactic acidosis and acute pancreatitis have been described with the concomitant use of ribavirin and didanosine. Zidovudine and stavudine should also be avoided in patients treated with ribavirin due to an increased risk of hematological and neurological toxicities, respectively (Saag 2018, Sherman 2015).

The use of cobicistat-based regimens, efavirenz, etravirine, nevirapine, ritonavir, and any HIV protease inhibitor, boosted or not by ritonavir, is not recommended in HIV positive patients receiving simeprevir (EACS 2019, Sherman 2015). Indeed, simeprevir can only be used with the following ARV drugs: raltegravir, rilpivirine, maraviroc, enfuvirtide, tenofovir, emtricitabine, lamivudine, and abacavir (AASLD-IDSA 2019, Sherman 2015, EASL 2018). The daily dose of daclatasvir should be adjusted in patients receiving atazanavir or efavirenz. On the contrary, no drug-drug interaction has been reported between sofosbuvir and ARVs.

Finally, given the speed with which new ARV and HCV drugs will debut, new interactions may be relevant and physicians should regularly consult updated databases on drug-drug interactions (University of Liverpool 2020).

Hepatocellular carcinoma in HIV positive patients

HCC prevalence (Ioannou 2013) and incidence (Merchante 2013, Sahasrabudde 2012) have steadily increased among individuals with HIV/AIDS over the past decades. In addition, the contribution of HCC to liver-related mortality in patients with HCV/HIV coinfection has significantly increased from 5% in 1995 to 25% in 2005 (Rosenthal 2009) and 40% in 2010 (Rosenthal 2015).

HCC occurs at a younger age in patients with HIV (Dika 2016); some studies have suggested that HCC might have a faster and worse outcome in HCV/HIV coinfection than in HCV mono-infection (Berretta 2011, Puoti 2004, Vibert 2011). However, other reports have failed to demonstrate lower survival rates in HCV/HIV coinfection (Brau 2007, Lim 2012). The comparison between studies may be misleading because of limited sample size, differences in study design and patient characteristics.

Although HIV positive patients with HBV or HCV coinfection should

be systematically screened for HCC (Dika 2016) there is evidence that HCC screening is far from optimal in this population (Jain 2007, Beauchamp 2013, Hearn 2015). The proportion of patients with HIV/HBV coinfection undergoing HCC screening was significantly lower than observed in patients with HBV monoinfection (36% vs 81%) (Hearn 2015). These results are similar to those observed in a previous study (Jain 2007) in which abdominal ultrasound was performed in only 36% (130/357) of HIV/HBV coinfecting patients during a four year period (1999–2003). More recently, a Canadian study showed that over a third of patients with HCV/HIV coinfection with cirrhosis were not screened for the presence of HCC by ultrasound (Beauchamp 2013).

Survival can be improved if HCC is diagnosed in the setting of a screening programme (Berretta 2011). However, so far no data from larger studies on cost-effectiveness of screening for HCC in cirrhotic patients with HIV/HBV coinfection are available (Joshi 2011, Gelu-Simeon 2014). These data do exist for HCV and HBV monoinfections.

Of note, HCC as indication for LT in HIV/HCV coinfecting patients has shown an increase in recent years (Figure 1). The widespread use of DAAs and the consequent decrease of ESLD due to HCV infection may be the underlying factors of this fact.

Evaluation process for liver transplantation in HIV positive patients

The involvement of a multidisciplinary team with expertise in the different areas is vital when assessing HIV positive patients who are coinfecting for LT (Miro 2007, Joshi 2011, Blumberg 2013). These teams should consist of members from the LT unit (from the medical and surgical areas), infectious disease specialists, and experts in the field of mental health and addictions and social workers (Miró 2007). The evaluation process to determine if an HIV positive patient is a suitable candidate usually lasts between seven and ten months (Martel-Laferrière 2015). HIV positive patients have a significantly lower probability of being listed than those who are HIV negative (18% versus 42%, respectively). The most common reason for not listing HIV positive patients is a lack of sufficient severity of liver disease (23%) (Martel-Laferrière 2015). The presence of HCC and a higher score on the MELD scale in HIV positive patients being evaluated for LT are factors independently associated with listing (Martel-Laferrière 2015).

Donor evaluation

Finally, the pre-LT donor evaluation should follow the same criteria as for the general population (Miro 2007).

The use of deceased HCV positive donors for HCV/HIV co-infected recipients showed inferior results in the NIH trial (Terrault 2012) of LT in HIV-infected individuals. However, it should be reassessed in future in both LT and KT in HIV-infected recipients due to the good preliminary results in KT in HIV-uninfected individuals, where pre- and post-transplant HCV treatment with DAAs was safe and prevented chronic HCV-infection in HCV D+/R- kidney transplant recipients (Miro 2019).

In the United States (and also in Spain), federal law banned HIV D+/R+ transplantation in 1984, but it was revised with the HIV Organ Policy Equity (HOPE) Act in 2013 (Fishman 2016, Blumberg 2019), allowing HIV D+/R+ transplants in research trials. Multicenter national trials investigating the practice of HIV D+/R+ deceased-donor kidney and liver transplantation (NCT02602262, NCT03500315) are underway. In other countries, HIV D+/R+ liver transplant experience is limited to a few case reports without unusual complications. These could be suitable approaches to mitigate the current organ shortage for this population (Richterman 2015, Miro 2019).

Liver transplant (LT) in HIV positive patients

HIV infection *per se* is not a contraindication for LT (Miro 2007, Blumberg 2019, Miro 2014). Indeed, LT is the only therapeutic option for appropriate HIV positive candidates with ESLD. The evaluation of LT candidates with HIV infection prior to being listed should be based on three main criteria: A) the degree of liver disease, B) the status of HIV infection, and, C) other criteria (psychiatric and drug use evaluation).

Liver disease criteria

The criteria are basically the same as for the HIV negative population. Briefly, they are acute liver failure, ascites with other factors associated with poor outcome such as CPT >7 points or MELD score >12 points, refractory ascites, hepatorenal syndrome, malnourishment or history of SBP, encephalopathy in patients with poor liver function (CPT >7 points), variceal bleeding that is difficult to manage with standard therapy and/or associated with poor liver function, hepatopulmonary syndrome and the development of HCC fitting the Milan criteria (one lesion ≤5 cm or no more than three tumour nodules ≤3 cm, in the absence of macroscopic vascular

invasion or extrahepatic disease).

A new indication for LT in HIV positive patients was described in a French study (Tateo 2008). Three patients underwent LT due to nodular regenerative hyperplasia. LT is the only therapeutic option in cases of severe portal hypertension caused by nodular regenerative hyperplasia, and disease does not seem to recur after LT (Sultanik 2013).

HIV criteria

Most LT groups from Europe and North America use similar HIV criteria. These are summarised in Table 1 (Blumberg 2019, Morabito 2016, Miro 2005, O'Grady 2005).

Table 1. HIV criteria for liver transplantation in HIV positive patients in Europe and the US

	Spain Miro 2005	France Duclos- Vallee 2008	Italy Morabito 2016	UK O'Grady 2005	US Blumberg 2019
Previous AIDS-defining events					
Accepted opportunistic infections (OIs)	Some*	Some*	None in the previous year	None after cART-induced immune reconstitution	Most**
Neoplasms	No	Not defined	No		No**
CD4 cell count/mm³					
No previous OIs	>100	>100***	>100	>200 or >100 if portal hypertension	>100
Previous OIs	>200	>100***	>200		>200
Plasma HIV-1 RNA viral load <50 copies/mL on ART****	Yes	Yes	Yes	Yes	Yes

*Patients with previous tuberculosis, *Pneumocystis jirovecii* pneumonia, or oesophageal candidiasis can be evaluated for LT.

**Only progressive multifocal leukoencephalopathy, cryptosporidiosis, multidrug systemic fungal infections, lymphoma, and visceral Kaposi's sarcoma are exclusion criteria.

***Patients with CD4 < 100 cells/mm³ were not excluded in France (case by case evaluation).

****If HIV plasma viral load is detectable, post-LT suppression with cART should be expected in all patients.

Clinical criteria

Some authors are in favour of waving exclusion criteria for some OIs that can be effectively treated and prevented, such as tuberculosis, candidiasis, and *Pneumocystis jirovecii* pneumonia (Neff 2004, Radecke 2005, Roland 2004). In fact, the US NIH has updated the inclusion criteria and only untreatable diseases continue to be exclusion criteria for LT (e.g., progressive multifocal leukoencephalopathy, chronic cryptosporidiosis, multidrug-resistant systemic fungal infections, primary CNS lymphoma, and visceral Kaposi's sarcoma) (Blumberg 2019).

Immunological criteria

All groups agree that the CD4+ T lymphocyte count should be above 100 cells/mm³ for LT (Neff 2004, Roland 2004). This figure is lower than that for kidney transplantation (CD4 >200 cells/mm³), because patients with cirrhosis often have lymphopenia due to hypersplenism, which leads to a lower absolute CD4 cell count, despite high CD4 cell percentages and good virologic control of HIV. In Spain, Italy, and the US, the CD4 cell count must be greater than 200 cells/mm³ in patients with previous OIs (Blumberg 2019, Morabito 2016, Miro 2005).

In Italy (Grossi 2012) and the UK (O'Grady 2005), the CD4 cut-off is 200 cells/mm³, unless patients have decompensated cirrhosis or portal hypertension; in this situation, the CD4 cell count threshold is 100 cells/mm³.

Virologic criteria

The essential criterion for LT is that the patient must have the option of effective, safe and long-lasting cART during the posttransplant period (Fung 2004, Neff 2004). The best situation is stable cART before transplantation with undetectable HIV viral load by ultrasensitive techniques (<50 copies/mL). Currently, cART is recommended for all HIV positive adults (Saag 2018). However, in the limited number of patients in which the benefit of initiating cART is not clear (e.g., elite controllers), it is unknown whether and when (pretransplant or posttransplant) it would be beneficial to initiate cART in order to reach an undetectable HIV plasma viral load. The proportion of HIV positive patients are not admitted on the LT waiting list for reasons related to their HIV (e.g., history of OIs or uncontrolled HIV infection) may vary between 6% and 10% (Martel-Laferrière 2015, Gelu-Simeon 2015).

Other criteria

To be included on the LT waiting list, HIV positive people must have had a favourable psychiatric evaluation. Psychiatric problems are the reasons for contraindication against LT in 3% of HIV positive LT candidates (Gelu-Simeon 2015).

Patients who use recreational or injecting drugs should not be placed on the waiting list. In Spain, patients must undergo a two-year period without using heroin and cocaine (Miro 2005), and six months with no consumption of other drugs (e.g., cannabis, alcohol). A recent paper reported that 13% of those HIV positive LT candidates were not enlisted due to active drinking (Gelu-Simeon 2015).

Patients who are on stable methadone maintenance are accepted for transplantation and can continue opioid maintenance after transplantation (Jiao 2010). Finally, as with other transplant candidate, HIV positive patients must have an appropriate degree of social stability in order to ensure adequate care in the posttransplant period. Around 20% of HIV positive patients who are not enlisted for LT due to psychosocial reasons such as lack of family/social support or toxic consumption (Martel-Laferrière 2015, Gelu-Simeon 2015).

Outcome of LT in HIV positive patients

Overall, mid- and long-term survival rates of HIV positive LT recipients are comparable to HIV negative patients, except for HCV/HIV coinfection. Survival rates in patients with HCV/HIV coinfection is lower compared to HCV monoinfected LT recipients (Table 2) (see below) (Coffin 2010, Duclos-Vallee 2008, Miro 2012, Terrault 2012, Locke 2016). However, with the high HCV eradication rates currently seen with DAAs, life expectancy in HCV-HIV LT recipients should be the same as in HIV negative recipients.

Most patients-maintained HIV viral suppression with good immunological status after LT (Miro 2015). Moreover, case reports of HIV positive LT recipients receiving organs from HIV positive deceased donors have been promising (Calmy 2016, Hathorn 2016). Studies under the HOPE Act (Fishman 2016) will provide more robust evidence in the coming years.

Complications after LT in HIV positive patients

After LT, patients and medical staff responsible for their care face a complex clinical setting (Miro 2007, Miro 2015). Patients should continue cART while immunosuppressive agents and antibiotic prophylaxis for OIs

are also needed. Additionally, some patients will receive treatment for posttransplant complications such as de novo diabetes mellitus or arterial hypertension. Patients who are receiving methadone treatment can restart after LT. As a general rule, HIV positive patients should follow the same recommendations of care as any other LT recipient (Lucey 2013).

People living with HIV have not shown an increased risk of post-operative complications or a higher incidence of OIs or tumours than HIV negative patients (Harbell 2013, Miro 2015, Samuel 2008).

Infectious complications

Posttransplant infections are a major cause of morbidity and mortality in LT recipients who are HIV positive (Miro 2015). However, incidence and aetiology of infections in HIV positive patients during the early posttransplant period are similar to those reported in HIV negative patients (Miro 2015). A high rate of severe non-opportunistic (43%) and opportunistic (11%) infections in a cohort of 84 patients with HCV/HIV coinfection who underwent LT has been reported (Moreno 2012): bacterial infections occurred in 38 patients (45%), CMV infections in 21 (25%), uncomplicated herpes virus infections in 13 (15%), and fungal infections in 16 patients (19%, 7 invasive cases). A pretransplant MELD score >15, history of category C AIDS-defining events and non-tacrolimus-based immunosuppressive regimens were factors independently associated with severe infections.

A French study found that 37% (40/109) of HIV positive LT recipients developed at least one infection during the first year after transplantation (Teicher 2015). Most were respiratory bacterial infections (45%) followed by those affecting the biliary tract (20%). Three patients developed CMV disease (colitis, pneumonia and hepatitis) and four developed an opportunistic infection (two oesophageal candidiasis, one lymph node tuberculosis and one atypical mycobacterial infection). The mortality associated with infections was 21% (9/43). A MELD score > 17 points at the time of LT was associated with a two-fold higher risk of developing severe infections posttransplantation (Teicher 2015).

Other complications

A high incidence of posttransplant hepatic artery thrombosis (HAT) (12%, 3/24) was observed in one small cohort (Cherian 2012), while in HIV negative people, HAT is reported in about 4.4% (Bekker 2009). However, this finding was not confirmed in two other cohorts: the first including 32 patients (none of whom presented with HAT) (Gastaca 2012) and the second

including 125 HIV positive liver recipients which reported six (5%) cases of HAT (Harbell 2013). Larger studies are needed in order to obtain more robust data on this relevant complication.

Table 2. Liver transplantation in HIV positive patients: nationwide cohorts in the late cART era (2003 to 2015)

Country	Time period	Number and type of patients	Survival rates (years)					p-value
			1	2	3	5	10	
France (Duclos-Valeè 2008)	1999–2005	HIV+/HCV+ (n=44)	–	73%	–	51%	–	4
		HIV-/HCV+ (n=35)	–	91%	–	81%	–	
Spain (Miro 2012)	2002–2006	HIV+/HCV+ (n=84)	88%	71%	62%	54%	–	8
		HIV-/HCV+ (n=252)	90%	81%	76%	71%	–	
US (Terrault 2012)	2003–2010	HIV+/HCV+ (n=89)	76%		60%		–	1
		HIV-/HCV+ (n=235)	92%		79%		–	
US (Coffin 2010)	2001–2007	HIV+/HBV+ (n=22)	85%		85%	85%	–	0.09
		HIV-/HBV+ (n=20)	100%		100%	100%	–	
US (Locke 2016)	2002–2011	HIV+ (n=149)	77%		62%	56%	39%	1
		HIV- (n=1490)	88%		79%	72%	57%	

The risk of recurrent or de novo malignancy after solid organ transplantation in HIV positive patients is low (Nissen 2012). After a median follow-up of 2.8 years posttransplant, 12 out of 125 (9.6%) liver recipients developed 14 malignancies: 11 de novo malignancies (nine skin cancer, one Kaposi's sarcoma and one lymphoma) and three recurrences of pre-LT malignancy: two HCC and one cholangiocarcinoma (Nissen 2012).

Aseptic osteonecrosis in three (12.5%) out of 24 patients who underwent LT has been reported (Cocchi 2012). The incidence of this complication should be analysed in future research.

Table 3. Posttransplant opportunistic infections (OI) in HIV positive patients who underwent liver transplantation

	Spain (Moreno 2012)	France (Teicher 2015)	US (Terrault 2012)
Number of patients	84	109	125
Follow-up (months)	24	46	32
Number (%) of patients with at least one OI	9 (11)	7 (6)	6 (5)
Type of OI			
Tuberculosis	2	1	0
Pneumocystis jirovecii pneumonia	1	0	1
Esophageal candidiasis	2	2	3
Other invasive fungal infections*	3	0	0
CMV disease	2	3	0
Other OI	0	1 [†]	1 [‡]
Neoplasms			
Kaposi's sarcoma	0	NR	1
Non-Hodgkin lymphoma	0	NR	0

NR: Not reported; *mucormycosis (2) and aspergillosis (1)

[†] atypical mycobacterium; [‡] bronchial candidiasis

Pharmacokinetic interactions in the posttransplant period

Clinical management in the posttransplant period is complex, and handling pharmacokinetic interactions is challenging (Primeggia 2013).

Efavirenz is an inducer of CYP3A4, while ritonavir-boosted HIV protease inhibitors (PIs) are CYP3A4 inhibitors. Ritonavir and a new selective CYP3A inhibitor without intrinsic anti-HIV activity, cobicistat, have a potent inhibitory effect (Deeks 2013). This fact has a considerable impact on patient management (Frassetto 2013). Subjects taking concomitant ritonavir- or cobicistat-boosted ARVs (e.g., PIs, elvitegravir) will require rapid and significant dose adjustments of both calcineurin inhibitors and mTOR inhibitors (Deeks 2013, Frassetto 2013). In the presence of HIV PIs, the increase in the ciclosporin exposure could be two- to four-fold (AUC) and for tacrolimus more than ten-fold. With a combination of an HIV PI plus efavirenz, the interaction is complex and needs to be closely monitored. Nevirapine has no significant effect on calcineurin inhibitor pharmacokinetics (Frassetto 2013).

Raltegravir (RAL), which is mainly metabolised by uridine diphosphate glucuronyltransferase, is not a substrate of CYP450 and can safely be used in HIV positive LT recipients (Barau 2014, Tricot 2009). A study enrolling 13 HIV positive solid organ transplantation recipients (eight liver and five

kidney) on RAL, reported a lack of significant interaction between RAL and calcineurin inhibitors (Tricot 2009). These findings were later confirmed in a cohort of 16 HIV positive solid organ transplant recipients (Mirò 2013). Therefore, the combination of two nucleos(t)ide reverse transcriptase inhibitors (TDF/emtricitabine or abacavir/lamivudine) plus RAL is probably the cART regimen of choice in transplant recipients. The introduction of dolutegravir (and probably bictegravir), which shares the same metabolic pathway and has shown superior virological efficacy over RAL, will be another option to safely treat LT recipients (Cahn 2013, Castellino 2013, Waki 2011). Furthermore, previous reports have mentioned the hypothetical anti-rejection and antifibrotic properties of the CCR5 inhibitor maraviroc (Haim-Boukobza 2013, Macias 2012). These findings remain preliminary and the results of larger studies in humans are necessary to confirm these interesting effects.

In addition, telaprevir and boceprevir (HCV PIs that are no longer used) increase the drug levels of ciclosporin and tacrolimus to a magnitude similar to that seen with HIV protease inhibitors. Management of drug-drug interactions is a challenging issue and is even more complex given the higher incidence of chronic kidney disease observed in these patients (Bahirwani 2014).

Finally, since this is an extremely rapidly evolving area, consultation of up-to-date databases on drug interactions is mandatory. The interactions of the antivirals used for the treatment of HCV can be found on the following websites:

- <https://www.hep-druginteractions.org>
 - <https://app.hivclinic.ca>
 - <https://www.hivmedicationguide.com>
- as well as in the product labels.

Immunosuppression and rejection in HIV positive LT recipients

The optimal immunosuppressive regimen in HIV positive LT recipients is currently not known. However, HIV positive coinfecting patients undergoing LT usually receive the same immunosuppressive regimens used in LT recipients without HIV (Miro 2007, Miro 2015). In general, the most commonly used immunosuppressive regimen combines a calcineurin inhibitor with corticosteroids. Findings from the two major LT cohorts (Miro 2012, Terrault 2012) confirm that individuals with HCV/HIV coinfection are more likely to have acute rejection than those with HCV mono-infection. A 38% acute rejection rate was reported in HIV coinfection compared to 20% in HIV negative patients ($p < 0.001$) (Miro 2012).

This higher rate of acute rejection may be due to difficulties in achieving adequate serum levels of immunosuppressant agents due to drug-drug interactions between ARVs and calcineurin inhibitors. In addition, a higher rate of misinterpretation of acute rejection (mainly versus recurrent HCV infection) cannot be ruled out in HCV/HIV coinfection (Terrault 2012).

HCV recurrence after LT

For those patients with replicating HCV, the recurrence of HCV is universal after LT, regardless of HIV status. The impact of this fact on the post-LT outcome of these patients has dramatically change since the advent of DAAs.

Pre-DAAs era

Recurrence of HCV in patients with HCV/HIV coinfection was more severe and occurs earlier (Antonini 2011, Castells 2006) than in HCV mono-infected patients in the era of PEG-IFN plus RBV due to the low rate of SVR, impacting on mid-term survival of HIV positive LT recipients (de Vera 2006).

The three major nationwide cohorts of LT recipients with HCV/HIV coinfection (France, Spain and the US) showed, uniformly, that post-LT survival rates are lower than those of HCV mono-infected patients (Table 2) (Duclos-Valeè 2008, Miro 2012, Terrault 2012). Survival rates vary from 76% to 88% in the first year, 60% to 62% at three years, and 51% to 54% at five years in patients with HCV/HIV coinfection. On the other hand, HCV mono-infected patients have survival rates of 90% to 92%, 70% to 76% and 71% to 81% in the first, third- and fifth-year post-LT, respectively. HIV was independently associated with mortality (Miro 2012, Terrault 2012). Other risk factors for death were HCV genotype 1 and a higher donor risk index (DRI) (Miro 2012). By contrast, the absence of HCV replication was associated with a significantly lower risk of death at five years (HR 0.23) (Mirò 2012).

Several studies have explored the effectiveness of treatment of HCV recurrence after LT with PEG-IFN plus RBV (Castells 2015, Duclos-Vallee 2011, Terrault 2014). They consistently found a very low SVR rate. HIV infection, donor age >60 years, HCV genotype 1 or 4 and severe histological hepatitis were identified as risk factors for virologic failure (Castells 2015). The main results are summarised in Table 4. The US (Terrault 2014) and Spanish (Castells 2015) cohort studies showed an SVR of only 10% in patients with genotype 1. In the Spanish study, a 59% rate of SVR was obtained in patients with genotypes 2/3 compared with only 7% in patients with genotype 1/4.

DAA era

At the beginning of the era of DAAs, HIV positive patients were not included in most studies in the setting of LT (Campos-Varela 2015) and data on the efficacy of these drugs in this clinical scenario was derived from case reports (Antonini 2015, Borentain 2014). However, current evidence confirms the high efficacy of DAAs in the setting of LT recipients with HCV/HIV coinfection (Campos-Varela 2016, Londoño 2016, Grant 2016, Faggioli 2016, Castells 2017, Manzardo 2018,) (Table 4). Most of these patients were treated with sofosbuvir plus ledipasvir with/without ribavirin. Indeed, SVR at 12 weeks is similar in LT recipients both with and without HIV infection (Manzardo 2018). The conclusion from these studies is that IFN-free regimens for post LT HCV recurrence in HIV positive individuals were highly effective and well tolerated, with results comparable to HCV monoinfection. In fact, current guidelines recommend DAAs regimens as the only options in HCV/HIV-coinfected patients after LT because of their virological efficacy, safety and tolerability (EASL 2018). Therefore, the post-transplant HCV recurrence and its fearful consequences seen until a few years ago in the PEG-IFN plus RBV era have disappeared with DAA treatment due to the high rate of virus eradication.

Table 4. Summary of studies evaluating the efficacy of treatment of HCV reinfection in LT

Author + Year of Publication	HCV/HIV-coinfected patients		HCV-monoinfected patients (Control Group)	
	N	SVR n (%)	n	SVR n (%)
IFN-based regimens				
Duclos-Vallée 2011	36	4 (11)		
Terrault 2014	37	5 (14)	-	-
Castells 2015	78	16 (21)	176	64 (36%)
Total	151	25 (17)	176	64 (36%)
IFN-free regimens				
Grant 2016	8	7 (87.5)		
Castells 2017*	6	6 (100)	16	16 (100)
Londoño 2016*	11	11 (100)		
Campos-Varela 2016	20	16 (89)		
Manzardo 2018	47	44 (94)	148	141 (95)
Total	75	67 (89)	164	157 (96)

*Some of these patients may be included in the Manzardo study and were, therefore, not considered for the overall response rate estimation.

HBV recurrence after LT

Cohorts of patients with HIV/HBV coinfection are not as large as those with HCV/HIV coinfection. The outcome of LT is much better, as effective control of HBV replication with anti-HBV hyperimmune globulin and HBV polymerase inhibitors is almost always possible (Coffin 2010, Tateo 2009). Probably due to the low incidence of HBV recurrence, survival rates in the short and medium term in HIV/HBV coinfection LT recipients are similar to those observed in HBV monoinfected LT recipients. A French study that included 13 patients with HIV/HBV coinfection revealed 100% graft and patient survival after a mean follow-up of 32 months (Tateo, 2009). Consistent with these findings, a US study enrolling 22 patients with HIV/HBV coinfection and 20 HBV monoinfected patients reported a cumulative patient and graft survival at three years of 85% in the HIV/HBV-coinfected patients and 100% in the HBV-monoinfected group ($p=0.08$).

Hepatocellular carcinoma

Preliminary data from case series showed satisfactory outcomes in people with HIV coinfection undergoing LT for HCC (Di Benedetto 2006, Di Benedetto 2008). In 2011, a French study (Vibert 2011) observed a trend towards a higher drop-out rate HIV positive patients with hepatitis B or C compared to HIV negative controls (5/21, 23% versus 7/64, 10%, respectively; $p=0.08$). From the time of enlisting, the survival rates at one and three years were 81% and 55% in the HIV positive group versus 91% and 82% in the HIV negative group ($p=0.005$). Moreover, the rate of HCC recurrence was two times higher in the HIV positive group than in the control group (30% versus 15%) (Vibert 2011). In contrast, an Italian study (Di Benedetto 2013) enrolling 30 HIV positive and 125 HIV negative LT recipients with HCC, observed that the proportion of HCC recurrence was two-fold higher in patients without HIV infection (2/30, 7% versus 18/125, 14%, respectively, $p=0.15$). Moreover, survival rates at one and three years after LT were similar (77% and 65% versus 86% and 70%, respectively). These two studies have two significant limitations: small sample sizes and limited follow-up periods.

A Spanish report (Agüero 2016) compared the outcome of 74 HIV negative patients undergoing LT for HCC with those of 222 LT recipients without HIV infection. There were no statistical differences regarding the baseline characteristics of tumours in both groups. Survival rates at one, three, and five years for HIV positive versus HIV negative patients were 88% versus 90%, 78% versus 78%, and 67% versus 73% ($p=0.779$), respectively. HCV infection (HR 7.90) and maximum nodule diameter >3 cm in the explanted

liver (HR 1.72) were independently associated with mortality in the whole series. HCC recurrence occurred in 12 HIV positive patients (16%) and 32 HIV negative patients (14%), with a probability of 4% versus 5% at one year, 18% versus 12% at three years, and 20% versus 19% at five years. Microscopic vascular invasion (HR 3.40) was the only factor independently associated with HCC recurrence. HIV infection had no impact on recurrence of HCC or survival after LT. These results support the indication of LT in HIV positive patients with HCC.

In addition, a Spanish study (Agüero 2017) showed that the incidence and the histopathological features of incidental HCC in HCV infected LT recipients being HIV positive or HIV negative were similar. Post-LT survival was, however, lower in HIV positive patients, probably because of a more aggressive HCV recurrence.

Liver retransplantation

Currently, in patients without HIV infection, liver retransplantation (re-LT) accounts for approximately 10% of all liver transplants (Pfitzmann 2007, Reese 2009). Overall post-retransplant patient survival rate is between 15% and 20% lower than the primary LT survival rate (Carrion 2010). This lower survival is of concern due to the significant shortage of available organs.

In HIV positive patients, the frequency of re-LT is similar to the observed LT recipients without HIV infection (6%) (Gastaca 2012, Agüero 2016). Overall survival rates at one and three years after re-LT for HIV positive (n=14) and HIV negative (n=157) patients were 50% versus 72% and 42% versus 64%, respectively (p=0.16).

A prospective international study which enrolled 37 HIV positive patients undergoing re-LT found similar results (Agüero 2016). Five-year survival probability in patients with a positive HCV RNA (n=22) at re-LT was 30% compared to 80% in patients with negative HCV RNA (n=10) (p=0.025). HCV recurrence was the main cause of death (7/22 cases, 32%). Therefore, the indication for re-LT in people with HCV/HIV coinfection with active HCV replication at time of re-LT should be reassessed in the setting of the widespread use of new DAAs.

Conclusions

ESLD is an increasingly frequent clinical scenario in the setting of HIV coinfection with either HCV or HBV.

Early diagnosis of ESLD complications is particularly important and should be actively monitored and treated. In general terms, the management

of ESLD in HIV positive patients should be the same as in those who are HIV negative.

Physicians caring for ESLD patients should follow them prospectively and promptly evaluate them for LT after the first clinical decompensation of liver disease.

LT is a life-saving procedure in this population and is safe and effective in patients with HBV infection. However, the recurrence of HCV infection in HIV positive patients can affect both graft and patient survival in the medium and long term. However due to the availability of effective and interferon free DAA regimen this scenario is currently undergoing a rapid change.

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21. Metabolic liver diseases: haemochromatosis

Claus Niederau

Definition and classification of iron overload diseases

Hereditary haemochromatosis is classified into 4 subtypes (Table 1). Type 1 is the well-known form of iron overload due to an autosomal recessive genetic metabolic malfunction; the homozygous C282Y mutation of the HFE gene on chromosome 6 accounts for more than 90% of clinical phenotypes in populations of Caucasian origin (Feder 1996). This mutation leads to an inadequately high intestinal iron absorption that after decades may cause iron overload and damage to various organs (Figure 1). Types 2a and 2b of genetic haemochromatosis are juvenile forms of iron overload that lead to a severe outcome prior to age 30, with cardiomyopathy and hypogonadism. The corresponding mutations are located in the hemojuvelin and hepcidin genes, respectively (Roetto 1999). Type 3 has mainly been described in Italian families and refers to a mutation in the transferrin receptor 2 gene (Girelli 2002). Clinical consequences of type 3 haemochromatosis are similar to type 1. Types 2 and 3 are autosomal recessive traits. The mutations of the autosomal dominant type 4 haemochromatosis are located in the gene coding for the basolateral iron transporter ferroportin 1 (Njajou 2001). In contrast to the other types, iron is accumulated in type 4 mainly in macrophages; ferritin values are markedly elevated although transferrin saturation is only slightly higher.

Secondary haemochromatosis is usually caused by multiple blood transfusions in hemolytic anaemias such as thalassaemia, sickle cell anaemia and myelodysplasia syndrome. Iron first accumulates in RES macrophages and is later transferred to parenchymal cells. With frequent blood transfusions, iron may accumulate faster than with genetic haemochromatosis; iron overload often leads to severe cardiomyopathy and liver cirrhosis, limiting effective prognosis. Therapy consists of iron chelators because phlebotomies cannot be done due to the underlying anaemia. This review will focus on type 1 HFE haemochromatosis, the most prevalent genetic form in Germany. Most consequences of iron overload are similar, whatever the cause. Thus, the pathophysiology of tissue and organ damage by iron excess is discussed in detail only for HFE haemochromatosis.

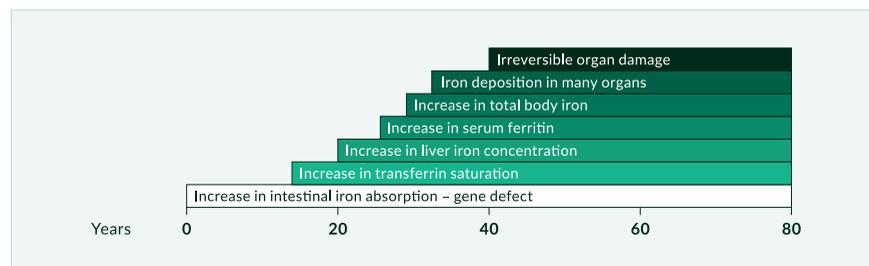


Figure 1. Scheme of natural history of type 1 genetic haemochromatosis

Table 1. Classification of haemochromatosis

I) Genetic haemochromatosis				
Types	Gene defect on	Affected gene	Inheritance	High prevalence
Type 2a	Chromosome 1	Hemojuvelin	Autosomal recessive	Juvenile form
Type 2b	Chromosome 19	Hepcidin	Autosomal recessive	Juvenile form
Type 3	Chromosome 7	Transferrin receptor 2	Autosomal recessive	Italy
Type 4	Chromosome 2	Ferroportin 1	Autosomal dominant	Italy
Neonatal	Unknown	Unknown	Unknown	Very rare
Others	Unknown	Unknown	Unknown	Of non-Caucasian origin
II) Secondary haemochromatosis				
a) Chronic anaemias (thalassaemia, sickle cell disease, MDS, other rare hemolytic anaemias)				
b) Multiple blood transfusions in general				
c) Long-term oral intake of high amounts of iron (diet-related or intravenous)				
III) Non-classified, ill-defined iron overload syndromes				
a) iron overload in Bantu Africans				
b) iron overload in aceruloplasminaemia				

Type 1 HFE haemochromatosis

History

The association between liver cirrhosis, pigment deposits in the liver, and diabetes mellitus was recognised over a century ago (Trousseau 1865, Troisier 1871, Hanot and Schachmann 1886). The term haemochromatosis was first introduced in the 19th century (Recklinghausen 1889), but was not generally accepted until used as the title of a classic monograph (Sheldon 1935). The controversy over whether haemochromatosis was merely a form of alcoholic liver cirrhosis (MacDonald 1960) or a genetic error of iron metabolism (Sheldon 1935, Crosby 1966) lasted almost a century until the

association between special HLA haplotypes and haemochromatosis which recognised the genetic nature of the disease was described (Simon 1975). The mode of inheritance was identified as an autosomal recessive disorder (Simon 1977). Finally, the major mutation on the HFE gene associated with clinical manifestations was identified (Feder 1996).

Epidemiology

Type 1 haemochromatosis is probably the most prevalent genetic metabolic error in Caucasian populations (Adams 2005). The prevalence of C282Y homozygotes is approximately 0.5% in central Europe and in the Caucasian population of North America; the prevalence of C282Y and H63D heterozygotes approaches 40% in similar populations (Adams 2005). Phenotypic expression also depends on several non-genetic factors such as the amount of dietary iron and blood loss (Figure 2). For example, due to menses, females develop clinical consequences of iron overload 5–8 times less frequently and 10–20 years later than males. It is now widely accepted that not all C282Y homozygous men will develop the full clinical manifestation of haemochromatosis. It also remains unclear how many men will show clinical disease during their lifetime and what factors determine that phenotype.

As mentioned previously, the homozygous C282Y mutation accounts for more than 90% of the clinical phenotype in Caucasian populations (Feder 1996, Adams 2005) (Table 2). A point mutation at H63D is also frequently identified in the HFE gene as well as other less frequent mutations. None of these gene alterations or polymorphisms, found in up to 40% of Caucasians, correlates with the phenotype. A subject with a C282Y variation on one allele and a H63D variation on the other is called a “compound heterozygote” (Table 2). Only a small percentage of such compound heterozygotes are at risk for clinical consequences of iron overload (Gallego 2015). A recent meta-analysis showed a positive association between compound heterozygosity for C282Y/H63D and the risk of NAFLD and HCC, but not liver cirrhosis (Ye et al. 2016). C282Y and H63D heterozygotes are at no risk of iron overload (Table 2). In non-Caucasian populations other genes may be involved in causing iron overload.

Aetiology and pathogenesis

Intestinal iron absorption and iron losses are finely balanced under physiological conditions. Approximately 10% of the total daily intake of iron (10–20 mg) is absorbed by the small intestine (1–2 mg). However, subjects

with the homozygous C282Y mutation may absorb up to 20% of iron intake; i.e., up to 2–4 mg/day. Thus, homozygotes have an excessive iron intake of approximately 1 mg/day. It may therefore take several decades until iron stores approach 10 g, above which organ damage is considered to start. Many patients at the clinical end stage of haemochromatosis, including liver cirrhosis and diabetes mellitus, have total body iron stores of 20–30 g. Intestinal iron absorption is downregulated when iron stores increase in these patients, as it is in patients with genetic haemochromatosis. This downregulation, however, occurs on an increased level when compared to subjects without the HFE gene mutation. Correspondingly, intestinal iron absorption is massively increased in patients with haemochromatosis when iron stores have been depleted by phlebotomy. It is important to continue phlebotomies after iron depletion in order to prevent reaccumulation (see Table 4). These regulatory processes however do not explain how HFE gene mutations cause the increase in intestinal iron absorption since the HFE gene product is neither an iron transporter nor an iron reductase or oxidase. However, carriers and regulators of cellular iron uptake and release been identified (Pietrangelo 2002, Fleming 2002, Townsend 2002, Fletcher 2002).

Some of these carriers also interact with the HFE gene product in the regulation of intestinal iron absorption (Pietrangelo 2002, Fleming 2002, Townsend 2002, Fletcher 2002) and the Nramp2 protein is the luminal iron carrier. Luminal iron reductase has also been identified as the Dcytb protein (duodenal cytochrome B) (Pietrangelo 2002, Fleming 2002, Townsend 2002, Fletcher 2002). The basolateral iron transporter ferroportin 1 (also named Ireg1 or MTP1) has also been identified (Donovan 2000, Abboud 2000) as well as the basolateral iron oxidase hepcidin (Vulpe 1999). Mutations in some of these proteins are responsible for the more rare types 2–4 of genetic haemochromatosis, although none of these genes is altered in type 1 haemochromatosis. Two other proteins have been shown to act as important iron regulating proteins, transferrin receptor 2 and hepcidin (Pietrangelo 2002, Fletcher 2002, Fleming 2005). Mutations in the transferrin receptor 2 gene may lead to the rare type 3 haemochromatosis, and mutations in the ferroportin 1 gene to type 4 haemochromatosis. More recent studies also indicate that hepcidin may be the most important regulator of iron metabolism, involved in iron deficiency and overload. Hepcidin has been shown to downregulate the basolateral iron carrier ferroportin. It has also been demonstrated that hepcidin itself is upregulated by HFE. Thus, an HFE mutation may reduce the upregulation of hepcidin that then does not downregulate ferroportin; the corresponding increase in ferroportin expression finally causes the increase in intestinal iron uptake (DeDomenico 2007). There may be further interactions between HFE, transferrin receptor 2, Nramp2, Dcytb, ferroportin, hepcidin and hepcidin, all of which are currently being studied.

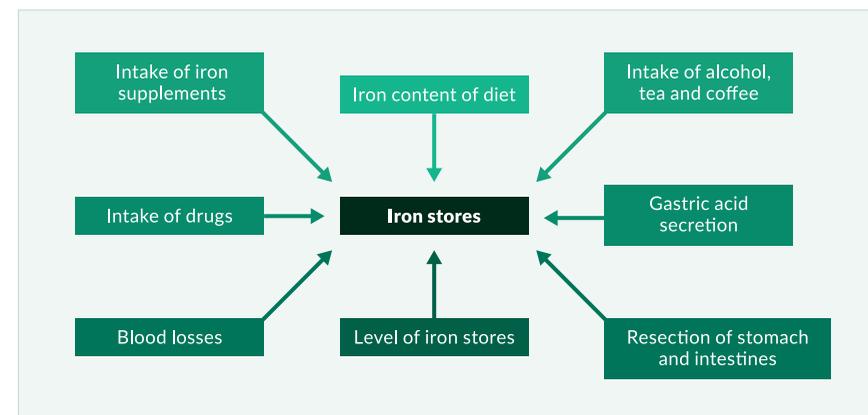


Figure 2. Non-genetic factors that may influence iron absorption

Table 2. Genotype/phenotype correlation in haemochromatosis

Mutations/polymorphisms	Prevalence in Caucasian populations	Risk of advanced clinical phenotype
C282Y/C282Y	85–95%	low if ferritin is <1000 ng/mL
H63D/C282Y	3–8%	very low
C282Y/wild type	-	none
H63D/wild type	-	none
Others	1%	unknown

Diagnosis

Laboratory tests. Any increase in serum iron should start with the exclusion of haemochromatosis so as not to overlook early disease. Normal serum iron, however, does not exclude haemochromatosis, and increased serum iron often occurs in the absence of haemochromatosis. Serum iron values are highly variable and should not be used either for diagnosis or for screening of haemochromatosis. The determination of transferrin saturation is a better indicator of iron overload than serum iron. The increase in transferrin saturation usually precedes the ferritin increase (Figure 1). Transferrin saturation is more sensitive and specific for detection of haemochromatosis when compared to serum ferritin. For screening, a threshold of 50% for transferrin saturation may be optimal under fasting conditions. Ferritin on the other hand is a good indicator of largely increased iron stores and reliably indicates iron deficiency. It has less value for early detection of haemochromatosis.

In haemochromatosis a slightly increased serum ferritin (300–500 ng/mL) is usually accompanied by transferrin saturations exceeding 80–90%. Unfortunately, serum ferritin is also increased, often in the presence of

infections and malignancies, and thus has a low specificity for indicating haemochromatosis (Niederer 1998). Ferritin increases not due to genetic haemochromatosis are usually associated with normal or only slightly elevated transferrin saturation. Therefore, transferrin saturation should be measured in order to correctly interpret ferritin increases.

Liver biopsy and determination of liver iron concentration.

Although simultaneous increases of both serum ferritin and transferrin saturation strongly indicate a risk for haemochromatosis, diagnosis needs to be confirmed by genetic testing or by liver biopsy with a determination of iron content in the liver. Hepatic iron concentration also increases with time in subjects with an HFE gene mutation. In order to obtain the “hepatic iron index”, divide the liver iron concentrations by the patient’s age. (Summers 1990). The semi-quantitative estimation of liver iron stores by the Berlin blue colour is less sensitive and specific than the chemical quantification of liver iron concentration. In case of a homozygous C282Y gene test, liver biopsy is not required for the diagnosis of genetic haemochromatosis (Figure 3).

There may, however, be other reasons to perform a liver biopsy in iron overload: (1) subjects with biochemical or clinical evidence of iron overload in the absence of the homozygous C282Y mutation should have a liver biopsy to substantiate iron overload; (2) in C282Y homozygotes the risk for liver fibrosis and cirrhosis increases at ferritin values >1000 ng/mL (Loreal 1992); in those patients liver biopsy is recommended because the presence of liver cirrhosis markedly increases later hepatocellular carcinoma (HCC) risk and thus warrants HCC screening.

Deferoxamine testing and ferrokinetic measurements.

Determination of urinary excretion of iron after administration of deferoxamine allows some estimation of total body iron stores. The deferoxamine test, however, often only shows pathological results when serum ferritin and transferrin saturation are markedly increased and does not allow diagnosis of early disease. Ferrokinetic measurements today are only done for scientific research or in difficult diagnostic situations.

Computed tomography (CT), magnetic resonance imaging (MRI) and biomagnetometry. CT density measurements of the liver allow a semi-quantitative estimation of iron concentration in the liver. This method however is associated with radiation and therefore not allowed in many countries where alternative methods are available. MRI, on the other hand, allows a reliable measurement of liver iron content, provided that special software is used and the equipment is calibrated for such measurement. In clinical practice most MRI do not fulfil these criteria. Biomagnetometry allows the most accurate non-invasive measurement of liver iron concentration. However, this equipment is expensive and only allows measurement of iron concentration. Consequently, biomagnetometry is done only at a few centres worldwide and is primarily used for scientific

studies and not in daily clinical practice. With the availability of reliable and inexpensive genetic testing, CT and MRI biomagnetometry is not needed for most patients.

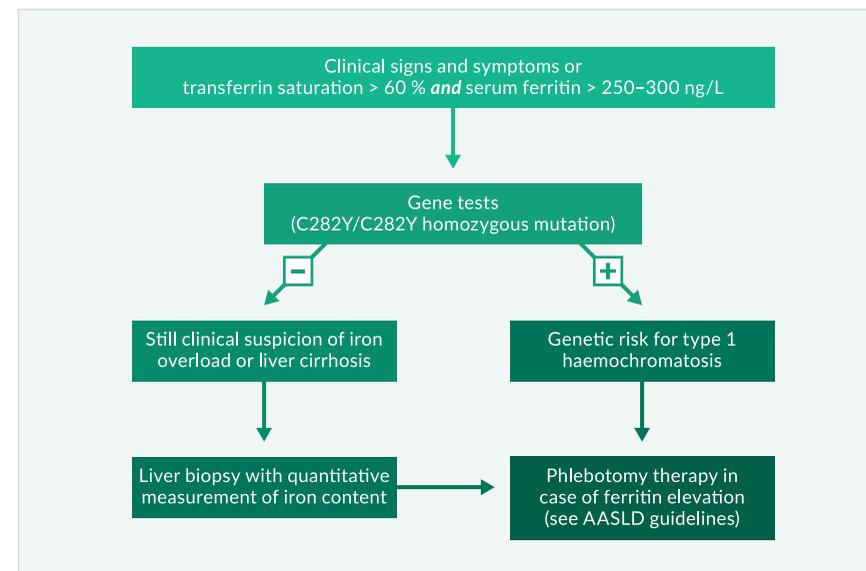


Figure 3. Diagnosis and treatment algorithm for type 1 haemochromatosis

Genetic tests. As outlined previously, in Caucasian populations the homozygous C282Y mutation accounts for more than 90% of patients with the clinical phenotype of type I haemochromatosis (Adams 2005, Erhardt 1999). Approximately 5% of patients with the clinical phenotype are C282Y/H63D compound heterozygotes; the prevalence of C282Y or H63D heterozygosity in patients with the clinical phenotype of haemochromatosis is considerably lower than in the general population. Thus, a subject who is heterozygous for C282Y or H63D *per se* has no risk of iron overload. In subjects homozygous for C282Y, both serum ferritin and transferrin saturation are frequently increased; however, only male subjects have an increased risk for liver disease when compared to subjects without HFE gene alterations in a recent large screening study. It is unknown how many C282Y homozygotes will later develop clinical signs and symptoms due to iron overload. It is increasingly evident that only a minority of C282Y homozygotes progress to end stage iron overload with liver cirrhosis and diabetes mellitus. In subjects who are not C282Y homozygotes but have laboratory, histological or clinical evidence of iron overload, further genes may be analysed for mutations such as hemojuvelin, transferrin receptor 2, ferroportin 1 and hepcidin.

Early diagnosis and screening

The prevalence of C282Y homozygotes is 0.5% in Caucasians (Adams 2005, Erhardt 1999). Clinical manifestations however are variable and depend on non-genetic factors such as dietary iron intake and blood loss. Until 1980, most patients with haemochromatosis were detected with late irreversible complications such as liver cirrhosis and diabetes mellitus. With a better understanding of the disease, the broad use of ferritin and transferrin saturation measurements and the availability of a reliable genetic test, diagnostic efforts have concentrated on the detection of early disease before liver cirrhosis and diabetes mellitus. Several studies have shown that iron removal by phlebotomy is associated with normal life expectancy in patients diagnosed early (Niederau 1985, Niederau 1996, Fargion 1992) (Figure 4). Several other studies have focused on screening procedures in order to diagnose more subjects with early disease (Edwards 1988). These studies include populations with special risks, family members, as well as the general population (Table 3) (see Niederau 2002). It has been shown that an increasing number of patients are now diagnosed early and that this trend increases survival (Figure 5).

A large number of studies have shown that screening is useful for detection of asymptomatic C282Y homozygotes by using transferrin saturation and serum ferritin as well a genetic test for the C282Y mutation (Edwards 1988, Phatak 1998, Niederau 1998). A broad screening of the general population however is as yet not recommended by WHO and CDC mainly because it is unknown how many of the asymptomatic C282Y homozygotes will later develop clinical disease (see US Preventive Services Task Force 2007). The largest screening study analysed HFE gene mutations in almost 100,000 subjects in North America. In Caucasians, C282Y homozygosity was found in 0.44%, a value similar to many previous studies in other populations with a similar background. Asian or Black people in contrast almost never have an HFE gene mutation (Adams 2005). Among the Caucasian C282Y homozygotes only males had a significant increase in liver disease when compared to subjects without an HFE gene variation (Adams 2005). Only further prospective follow-up studies will determine how many asymptomatic C282Y homozygotes will develop clinical consequences of iron overload.

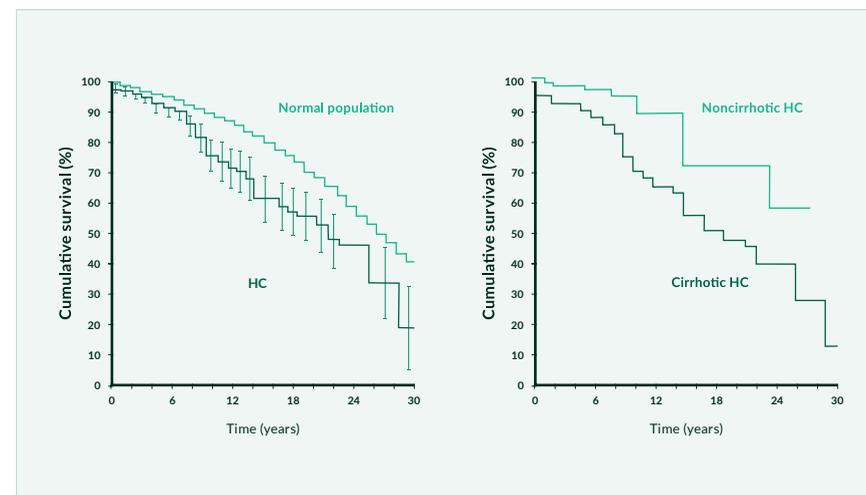


Figure 4. Survival of 251 patients with genetic haemochromatosis (with and without cirrhosis) in comparison with a matched general population. Modified from Niederau 1996

Table 3. Methods for early diagnosis of haemochromatosis

1. Screening in the general population not recommended
Screening of HFE gene alterations is not recommended in the general population because it remains unknown how many of the C282Y homozygotes will develop clinical manifestations. Such screening would be meaningful only in Caucasian populations.
2. Family screening
Genetic testing can reliably determine who, among the first-degree relatives of a haemochromatotic patient, is a heterozygote or homozygote. Heterozygotes are healthy and do not need follow-up. C282Y homozygotes should be followed and treated by phlebotomy if ferritin increases >300 ng/mL in men and >200 ng/mL in women.
3. Haemochromatosis should be excluded in patients with
<ul style="list-style-type: none"> • newly diagnosed diabetes mellitus • chronic liver disease of unknown aetiology • elevation of iron, transferrin saturation or serum ferritin • cardiomyopathy of unknown aetiology • arthropathy of unknown aetiology • loss of potency/libido and amenorrhea of unknown aetiology
4. Every liver biopsy needs to be checked for iron deposits

It is also unknown at which ferritin values phlebotomy treatment should be initiated in asymptomatic C282Y homozygotes (Table 4). The values recommended by the AASLD are based more on the judgment of experts than on solid data. The only solid data show that the risk for liver fibrosis and cirrhosis increases above the threshold of 1000 ng/mL for serum ferritin (Loreal 1996). The value of screening family members is obvious when a first-degree relative has clinical haemochromatosis. Such family screening is easy to do with the genetic test. Heterozygous family members are not at risk for haemochromatosis unless they have other risk factors.

The clinical phenotype of haemochromatosis is seen in 1–2% of patients with newly diagnosed diabetes mellitus and in 3–15% of patients with liver cirrhosis (Niederau 1999). These latter patients should be screened for iron overload although such screening obviously does not aim at a very early diagnosis. Nevertheless, cirrhotic and diabetic patients with haemochromatosis can benefit significantly from phlebotomy therapy. Little is known about the prevalence of haemochromatosis in patients with arthropathy or cardiomyopathy of unclear aetiology. Several smaller studies indicate that arthropathy may be a rather early clinical sign of iron overload, whereas cardiomyopathy usually occurs in late stage iron overload.

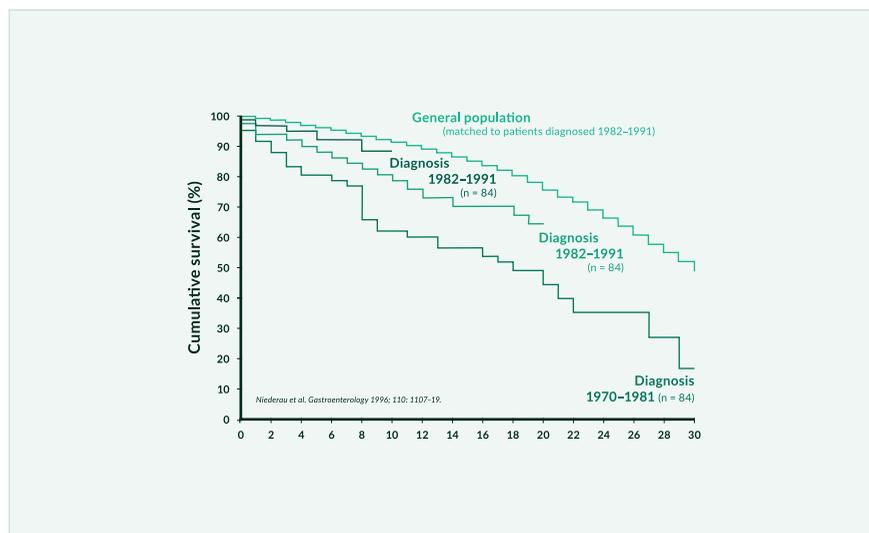


Figure 5. Cumulative survival in 251 patients with genetic haemochromatosis according to the time of diagnosis. Modified from Niederau 1996

Table 4. Iron overload therapy

1. Phlebotomy	
a) In symptomatic genetic haemochromatosis	
<ul style="list-style-type: none"> • Aims: complete iron depletion in 12-24 months; • Treatment: 1-2 phlebotomies of 500 mL each week until serum ferritin is in the range of 50-100 ng/mL; • Long-term therapy with 4-8 phlebotomies per year to keep ferritin between 50-100 ng/mL and thus prevent reaccumulation of iron 	
b) In asymptomatic C282Y homozygotes therapy should be initiated above these ferritin values:	
• Subjects <18 years	>200 ng/mL
• Men	>300 ng/mL
• Women (not pregnant)	>200 ng/mL
• Women (pregnant)	>500 ng/mL

2. Therapy with iron chelators in secondary haemochromatosis and anaemia

- Aims: removal of iron overload by increase of iron excretion in faeces and urine
- In case of further blood transfusions at high frequency at stabilisation of iron balance and reduction of further iron accumulation
- Treatment: until recently, 25-50 mg deferoxamine/kg as sc infusion for 10-12 h daily; today, deferoxamine is largely replaced by the oral chelator deferasirox – 20 mg/kg deferasirox once daily to prevent iron accumulation up to 800 mL erythrocytes concentrates/month
- Long-term treatment necessary
- Normalisation of ferritin and liver iron concentration is often not possible

3. Diet

- Recommended: avoidance of food with very high iron content (e.g., liver) and iron-supplemented food;
- A further strict iron-depleted diet is very difficult to adhere to and not recommended
- A single phlebotomy of 500 mL blood is as effective for iron removal as a very rigid iron-restricted diet for a full year

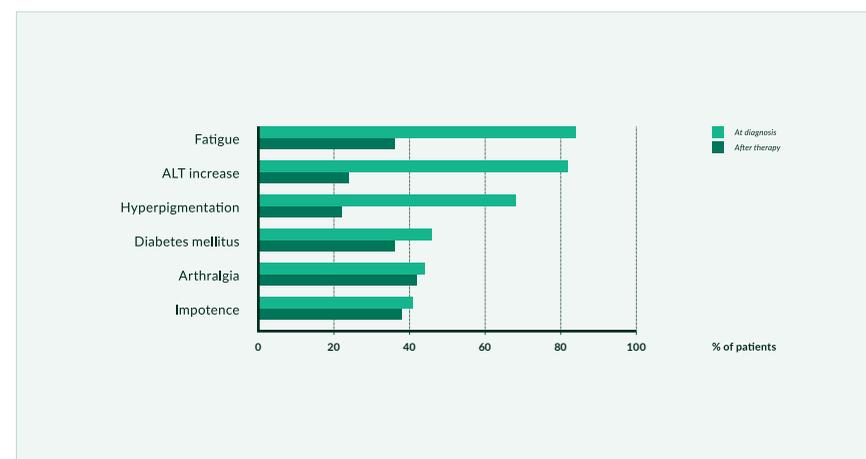


Figure 6. Signs and symptoms in 185 patients with genetic haemochromatosis prior to and after iron removal. Modified from Niederau 1996

Complications of iron overload

Liver cirrhosis, diabetes mellitus and increased skin pigmentation are the classical trio of genetic haemochromatosis. Cardiomyopathy, cardiac arrhythmias and impotence are also typical complications of advanced iron overload. Arthropathy in contrast may be an early sign of haemochromatosis, which may help with diagnosis in the precirrhotic stage (Niederau 1996).

Liver disease. The liver is the organ that is affected by genetic iron overload most early and heavily. At early stages excess iron stores are mainly found in periportal parenchymal cells as ferritin and hemosiderin. When

iron excess further increases, there is development of perilobular fibrosis and iron stores are also found in bile ducts and Kupffer cells. Septal fibrosis eventually progresses towards complete cirrhosis. The stage of fibrosis is closely associated with the degree of excess of iron. In many affected symptomatic patients with type 1 haemochromatosis there are some signs of liver disease at the time of diagnosis (Niederau 1985, Niederau 1996). Many nonspecific symptoms such as abdominal discomfort and fatigue may also be due to liver involvement. In asymptomatic patients diagnosed by a screening procedure, signs of liver disease are infrequent. Complications due to cirrhosis such as ascites, jaundice and portal hypertension are seen only rarely and only in cases of advanced severe iron overload (Niederau 1985, Niederau 1996). The risk for liver cirrhosis increases at ferritin values >1000 ng/mL (Loreal 1996). Similar to insulin-dependent diabetes, liver cirrhosis cannot be reversed by removal of iron (Niederau 1996). However, less advanced stages like hepatic fibrosis and abnormalities in liver enzymes and function respond well to iron removal (Niederau 1996) (Figure 5). Survival is significantly reduced in the presence of liver cirrhosis whereas patients diagnosed in the precirrhotic stage have a normal life expectancy when treated by phlebotomy (Niederau 1996) (Figure 4).

Association of haemochromatosis with other liver diseases. Some studies indicate that C282Y heterozygosity may aggravate the progression of concomitant liver diseases such as porphyria cutanea tarda, chronic hepatitis C, alcoholic hepatitis and non-alcoholic steatohepatitis (NASH). In these latter patients one might find slightly elevated liver iron concentrations and serum ferritin levels when they are C282Y heterozygotes (for review see Erhardt 2003). Most studies however have shown that these associations are of only minor importance in the clinical course of the disease. Phlebotomy has so far only been proven meaningful in porphyria cutanea tarda because it can ameliorate the cutaneous manifestations.

Liver carcinoma. Liver carcinoma develops in approximately 30% of patients with haemochromatosis and cirrhosis independent of iron depletion (Niederau 1996). The interval between complete iron depletion and reported diagnosis of liver cancer is approximately nine years in large cohorts in German patients (Niederau 1985, Niederau 1996). The risk of liver cancer is increased 100–200-fold in patients with haemochromatosis when compared to the general population (Figure 6). Among liver cancers there are hepatocellular carcinoma (HCC) as well as cholangiocellular carcinoma. Most liver cancers develop in patients with cirrhosis. Thus, cancer screening by ultrasound and AFP (twice a year) is only recommended for cirrhotic patients. Patients who develop liver cancer usually have the largest amount of iron accumulation among various subgroups (Niederau 1996, Niederau 1999).

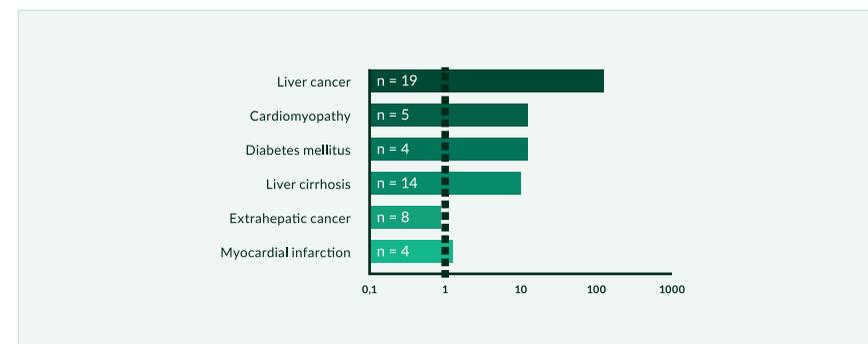


Figure 7. Relative mortality risk of 251 patients with genetic haemochromatosis in comparison to the general population. Modified from Niederau 1996

Diabetes mellitus. The prevalence of diabetes in hereditary haemochromatosis ranges from 20–50% (Niederau 1996, Adams 1991). The prevalence and stage of diabetes is related to the degree of iron deposition in the pancreas. Patients with diabetes have a twofold higher mobilisable iron content than non-diabetics (Yaouanq 1995). Investigations into the prevalence of unrecognised genetic haemochromatosis in diabetic patients show some variation in Europe vs. elsewhere; i.e., screening revealed a prevalence of 5–8 per 1000 unrecognised cases in Europe (Singh 1992) and 9.6 per 1000 in Australia (Phelps 1989). Diabetes mellitus and impaired glucose tolerance are frequent features in several chronic liver diseases (Creutzfeldt 1970, Blei 1982). This author's study (Niederau 1984) showed hyperinsulinaemia and hence insulin resistance without impaired glucose tolerance in noncirrhotic haemochromatosis. The increase in circulating insulin concentrations is likely to be due to a decrease in diminished hepatic extraction of insulin. With the progression of iron overload and destruction of beta cells, insulin secretion becomes impaired (Dymock 1972, Bierens de Haan 1973). In end-stage haemochromatosis, insulin deficiency is associated with severe reduction in the mass of beta cells (Rahier 1987). Insulin resistance observed in early iron overload may be partially reversible after phlebotomy therapy (Niederau 1985, Niederau 1996) whereas insulin-dependent diabetes is irreversible (Niederau 1996). Survival is significantly reduced in patients with diabetes mellitus at diagnosis compared to patients without diabetes (Niederau 1996). Survival of non-diabetic patients is virtually identical to that of a matched normal population.

Heart disease. Cardiomyopathy and cardiac arrhythmias are specific complications of haemochromatosis caused by iron deposition in the heart (Buja and Roberts 1971, Short 1981). Clinical or electrocardiographic signs of heart disease can be found in 20–35% of patients with HFE haemochromatosis (Niederau 1985). Arrhythmias usually respond well to iron removal (Short 1981, Niederau 1996). In type 1 haemochromatosis cardiomyopathy is rare and usually associated with advanced iron

overload and an older patient population. However, particularly in young patients who present with cardiac disease due to haemochromatosis, cardiomyopathy is a frequent cause of death (Finch 1966, Short 1981). It has also become clear that young patients with severe cardiomyopathy may be affected by juvenile type 2 haemochromatosis; these patients may show severe iron overload, hypogonadism, cardiomyopathy, liver cirrhosis, and amenorrhea by ages 15–24. The type 2-associated cardiomyopathy is often irreversible despite initiation of phlebotomy or chelation therapy and may require an immediate transplant of the heart and potentially of the liver as well (von Herbay 1996, Jensen 1993).

Arthropathy. Joint changes in genetic haemochromatosis may occur in two different ways (Schuhmacher 1964, Dymock 1970, Niederau 1985, Niederau 1996). The most prevalent changes are seen in the metacarpophalangeal joints II and III, in the form of cystic and sclerotic changes, cartilage damage and a narrowing of the intraarticular space. Sometimes other joints of the hands and the feet are affected. Large joints, i.e., of the knees and hips, may be affected in the form of chondrocalcinosis. The pathogenesis of joint changes in haemochromatosis remains unclear. Arthropathy is one of the few complications not associated with the degree of iron overload. It has been speculated that iron may inhibit pyrophosphatase and may thereby lead to a crystallisation of calcium pyrophosphates. Alternatively, iron may have direct toxic effects on the joints. Arthropathy may be an early sign of haemochromatosis and may help to make the diagnosis at a precirrhotic stage (Niederau 1996). Haemochromatosis should therefore be considered in all patients with an arthropathy of unknown aetiology.

Endocrine abnormalities. In contrast to the early onset of arthropathic changes, endocrine abnormalities are a late consequence of iron overload. Sexual impotence and loss of libido may occur in up to 40% of male patients (Niederau 1985). The endocrine abnormalities in haemochromatosis are mainly, if not exclusively, due to pituitary failure. This is in contrast to alcoholic cirrhosis where testicular failure is predominant (Kley 1985a, Kley 1985b). In contrast to alcoholic cirrhosis, where oestrogen levels are usually increased, oestrogen levels were found decreased in haemochromatosis (Kley 1985a). Most endocrine changes are late and irreversible complications of genetic haemochromatosis and do not respond well to phlebotomy treatment (Niederau 1996). Iron overload only infrequently affects other endocrine organs such as the thyroid and adrenal glands. Severe hypogonadism with amenorrhea in young women and impotence in young men is today thought to be due to type 2 haemochromatosis.

Skin. Increased skin pigmentation is mainly seen in areas exposed to sunlight. A large part of the darkening of pigmentation is thought to be due to an increase in melanin and not due to iron excess itself. The increase in skin pigmentation is reversible on iron removal (i.e., phlebotomy).

Other potential complications. Iron overload has been speculated to aggravate atherosclerosis; however, the evidence for that is rather weak (for review see Niederau 2000). There have also been reports that extrahepatic malignancies may be increased in HFE haemochromatosis (Amman 1980, Fracanzani 2001) while other studies have not found extrahepatic associations (Bain 1984, Niederau 1996, Elmberg 2003). It is not clear whether HFE gene mutations are involved in the pathogenesis of porphyria cutanea tarda since the prevalence of both risk factors vary greatly in different parts of the world; associations between HFE gene mutations and porphyria have often been described in southern Europe but not in northern Europe (Toll 2006).

Polymorphisms beyond C282Y homozygosity. Recent studies have suggested that the C282Y and H63D polymorphisms in the HFE gene are associated with a selection advantage. This selection may also explain the high frequency of up to 40% of these polymorphisms seen in Celtic populations (Adams 2005). These polymorphisms are almost exclusively found in people with Celtic descent. A French study recently showed that these polymorphisms are seen in 27% of the French general population (Hermine 2015). Interestingly, 80% of French winners of WM, EM and Olympic sport events had one of these polymorphisms (Hermine 2015).

Along this line, a recent Swiss study showed that C282Y homozygotes are several centimetres taller than the reference population (Cippa 2013), although these homozygotes are usually considered not to be healthy. Indeed the greater height and physical fitness of the Celts have already been mentioned by Julius Caesar in his work „De Bello Gallico“ (Caesar 50 a.c.).

Thus, subjects with heterozygous HFE polymorphisms are usually “very healthy” people without a major risk for iron overload and associated organ damage. Only in the presence of other hepatotoxic factors such as hepatitis C or fatty liver disease HFE heterozygotes may have an increased risk to develop liver fibrosis (Erhardt 2003).

Therapy

Phlebotomy treatment. Phlebotomy treatment is the standard of care for removing iron in genetic haemochromatosis. One phlebotomy session removes approximately 250 mg iron from the body. Since patients with the classical clinical phenotype may have an excess of 10–30 g iron, it may take 12–24 months to remove the iron overload when phlebotomies of 500 mL blood are done weekly (Table 4). Phlebotomy treatment is generally well tolerated and hemoglobin usually does not drop below 12 g/dL. Several studies have shown that liver iron is completely removed at such low ferritin values; thus the effect of therapy can be checked by ferritin

measurements and a control liver biopsy is not necessary. After complete removal of excess iron the intervals of phlebotomies may be increased to once every 2–3 months; serum ferritin should be kept in the lower normal range, between 50–100 ng/mL. Phlebotomy should not be interrupted for longer intervals; there is a risk of reaccumulation of iron due to the genetic autosomal recessive metabolic malfunction.

Erythrocytapheresis. Three prospective, randomised studies have compared the advantages and disadvantages of erythrocytapheresis compared to phlebotomy in patients with hereditary HFE haemochromatosis (Rombout-Sestrienkova 2012, Sundic 2013, Rombout-Sestrienkova 2016). Erythrocytapheresis can theoretically remove up to three times more red blood cells per single procedure when compared with regular phlebotomy and thus may have a clinical and economic benefit.

In one of these studies serum ferritin levels initially declined more rapidly in the apheresis group; however, time to normalisation of the ferritin level was equal in both groups (Sundic 2013). The cumulative costs for materials and technician times until achievement of the desired ferritin levels were three-fold higher in the apheresis group (Sundic 2013).

In the other study, after adjustments for initial serum ferritin and body weight, the number of therapeutic procedures was lower for erythrocytapheresis when compared with regular phlebotomy (0.43; 95% CI, 0.35–0.52; $p < 0.001$) (Rombout-Sestrienkova 2012). Cost analysis however showed no significant difference in treatment costs between the two procedures (Rombout-Sestrienkova 2012).

The third study evaluated the effectiveness of erythrocytapheresis over phlebotomy for maintenance therapy in patients with HFE haemochromatosis (Rombout-Sestrienkova 2016). The two treatment-arms, randomised, crossover clinical trial involved 46 patients who were treated for one year with either erythrocytapheresis or phlebotomy to keep the ferritin level < 50 ng/mL. After one year, patients were switched to the other treatment modality. The mean number of treatment procedures per treatment year was significantly higher using phlebotomy versus erythrocytapheresis (3.3 vs. 1.9; $p < 0.01$). There was no significant difference between arms in overall health assessed by SF-36 and EQ-5D, respectively. The mean costs of one treatment year however were 235 € for phlebotomy versus 511 € for erythrocytapheresis.

In summary, regular phlebotomy remains the gold standard for removing excess iron in hereditary haemochromatosis type 1. It has few side-effects and is more cost-effective than erythrocytapheresis.

Monitoring of phlebotomy treatment. Phlebotomy treatment is usually monitored by repetitive measurements of serum ferritin. According to ESAL and AASLD guidelines, phlebotomies should be done at frequent intervals until serum ferritin is reduced to low normal values of about 50–100

ng/mL (Bacon 2011, EASL 2010). Thereafter, the interval of phlebotomies can be prolonged to assure that serum ferritin remains at 50–100 ng/mL. It is known that the liver and other organs do not contain excess iron when ferritin is in that range. On the other hand, it is also known that transferrin saturation may still be increased up to 70% at such ferritin levels in C282Y homozygotes. Recent studies have shown that serum concentrations of Non-Transferrin-Bound Iron (NTBI) and Labile Plasma-Iron (LPI) may increase sharply beyond a transferrin saturation of 70–80% (Cabantchik 2014). Such increases in NTBI and LPI may be associated with oxidative stress and risks for cell damage (Hershko 1978, Le Lan 2005, Pootrakul 2004, Hod 2011, Brissot 2012, Cabantchik 2014). Therefore, there is a current debate whether transferrin saturation should be used for monitoring long-term phlebotomy and transferrin saturation should aim to be kept below 50% (Cabantchik 2014, de Swart 2015). This would mean that a considerable number of patients would be at the risk to become iron deficient – which should be avoided according to EASL and AASLD guidelines (Bacon 2011, EASL 2010). It is also known that the usual ferritin monitoring assures a normal life expectancy in patients diagnosed without liver cirrhosis (Niedermaier 1985, Niedermaier 1996). Thus, as yet the monitoring of phlebotomy treatment should be based on serum ferritin which should be kept at 50–100 ng/mL (Bacon 2011, EASL 2010).

Iron removal by chelators. Deferoxamine therapy for genetic haemochromatosis is not recommended because phlebotomy is more effective with less side effects and lower cost.

A phase 2/3 study proved the safety and effectiveness of the new oral iron chelator deferasirox in genetic HFE haemochromatosis (Phatak 2010). However, deferasirox is only currently approved for secondary haemochromatosis.

Diet. A diet low in iron is not recommended for patients with genetic haemochromatosis. One phlebotomy of 500 mL blood removes approximately 250 mg iron. A difficult-to-follow iron-restricted diet for a complete year would have the effect of a single phlebotomy. It is therefore recommended that patients simply do not eat excessive amounts of food with very high iron content (such as liver) and that they do not eat food to which iron has been added (Table 4).

Liver transplantation. Advanced liver cirrhosis and carcinoma may be indications for a liver transplant in haemochromatosis (Kowdley 1995, Brandhagen 2000). The prognosis of patients who have a liver transplant for haemochromatosis is markedly worse than that for patients with other liver diseases; a considerable number of patients with haemochromatosis die after transplant from infectious complications or heart failure (Brandhagen 2000). Liver transplantation does not heal the original genetic defect.

Prognosis

Untreated haemochromatosis often has a bad prognosis in the presence of liver cirrhosis and diabetes mellitus. The prognosis is markedly worse in patients with cirrhosis than in those without cirrhosis at diagnosis (Figure 3); the same is true for diabetes mellitus. It is generally accepted that phlebotomy therapy improves the prognosis. Patients diagnosed and treated in the early non-cirrhotic stage have a normal life expectancy (Figure 3) (Niederau 1985, Niederau 1996). Thus, early diagnosis markedly improves the prognosis (Figure 4). Iron removal by phlebotomy also improves the outcome in patients with liver cirrhosis. The prognosis of liver cirrhosis due to haemochromatosis is markedly better than those with other types of cirrhosis (Powell 1971). Hepatomegaly and elevation of aminotransferases often regress after iron removal (Niederau 1985, Niederau 1996) (Figure 5). Insulin-dependent diabetes mellitus and hypogonadism are irreversible complications despite complete iron removal (Niederau 1996) (Figure 5). Earlier changes in glucose and insulin metabolism, however, may be ameliorated after iron removal. For unknown reasons arthropathy does not respond well to phlebotomy treatment although it may be an early sign of iron overload (Figure 5). The AASLD consensus guidelines recommend to start phlebotomy treatment at ferritin values >300 ng/mL in men and >200 ng/mL in women. The risk for liver fibrosis and cirrhosis is increased only at ferritin levels >1000 ng/mL. Further studies need to determine whether asymptomatic C282Y homozygotes with ferritin values between 300 and 1000 ng/mL need to be treated or whether one might wait and monitor ferritin at that stage.

Juvenile hereditary haemochromatosis

Two genes have been associated with juvenile haemochromatosis: 90% of cases are associated with mutations in hemojuvelin (HJV) (locus name HFE2A, which encodes HJV), while 10% of cases are associated with HAMP (locus name HFE2B, which encodes hepcidin). Despite the nomenclature of HFE2A and HFE2B, juvenile haemochromatosis is not associated with HFE mutations. In order to avoid confusion most physicians use the terms type 2A (hemojuvelin mutations) and type 2B (HAMP mutations). Mutations in hemojuvelin are associated with low levels of hepcidin in urine suggesting that hemojuvelin regulates hepcidin. Hepcidin is the key regulator of intestinal iron absorption and iron release from macrophages. Hepcidin facilitates ferroportin internalisation and degradation. Hepcidin mutations may thereby lead to an increase in ferroportin and thus iron uptake from the intestine. Juvenile haemochromatosis is very rare. A clustering of HJV

mutations can be seen in Italy and Greece although few families account for this phenomenon. Mutations in HJV represent the majority of worldwide cases of juvenile haemochromatosis.

Only a small number of patients have been identified with HAMP-related juvenile haemochromatosis. Juvenile haemochromatosis is characterised by an onset of severe iron overload in the first to third decades of life. Clinical features include hypogonadism, cardiomyopathy, and liver cirrhosis (Diamond 1989, Vaiopoulos 2003). The main cause of death is cardiomyopathy (De Gobbi 2002, Filali 2004). In contrast to HFE type 1 haemochromatosis, both sexes are equally affected. Mortality can be reduced in juvenile haemochromatosis when it is diagnosed early and treated properly. Phlebotomy is the standard therapy in juvenile haemochromatosis as well and is treated similarly to HFE haemochromatosis (Tavill 2001). In patients with juvenile haemochromatosis and anaemia or severe cardiac failure, administration of chelators such as deferoxamine have been tried to reduce mortality; some case reports suggest that this might improve left ventricular ejection fraction (Kelly 1998).

Transferrin receptor 2 (TFR2)-related type 3 haemochromatosis

TFR2-related haemochromatosis is defined as type 3 and is also known as HFE3; however, the term HFE3 should not be used because the HFE gene is not affected in type 3 haemochromatosis. TFR2-related haemochromatosis is inherited in an autosomal recessive manner. TFR2 is a type II 801-amino acid transmembrane glycoprotein expressed in hepatocytes and at lower levels in Kupffer cells (Zhang 2004). A finely regulated interaction between TFR2, TFR1 and HFE is now thought to affect the hepcidin pathway, and, consequently, iron homeostasis (Fleming 2005). Patients with homozygous TFR2 mutations have increased intestinal iron absorption that leads to iron overload. Hepcidin concentrations in urine are low in TFR2 haemochromatosis (Nemeth 2005). TFR2-related haemochromatosis is very rare with only about 20 patients reported worldwide (Mattman 2002). Age of onset in TFR2-related type 3 haemochromatosis is earlier than in HFE-associated type 1 (Piperno 2004, Girelli 2002, Hattori 2003). Progression is, however, slower than in juvenile type 2 (De Gobbi 2002, Roetto 2001, Girelli 2002). The phenotype is similar to type 1. Many patients present with fatigue, arthralgia, abdominal pain, decreased libido, or with biochemical signs of iron overload (Roetto 2001, Girelli 2002, Hattori 2003). Complications of type 3 haemochromatosis include cirrhosis, hypogonadism, and arthropathy. Cardiomyopathy and diabetes mellitus appear to be rather rare. Hepatocellular carcinoma has not been

observed in the small number of cases diagnosed. Most individuals with type 3 haemochromatosis have an Italian or Japanese genetic background. Some of the Japanese males have had liver cirrhosis at diagnosis (Hattori 2003). Similar to type 1 haemochromatosis, the penetration of type 3 haemochromatosis is also considerably less than 100% (Roetto 2001). Standard therapy is iron removal by weekly phlebotomy similar to the management of type 1 disease. Individuals with increased ferritin should be treated similar to those with HFE haemochromatosis.

Type 4 haemochromatosis – Ferroportin Disease

Ferroportin-associated iron overload (also called Ferroportin Disease) was first recognised by Pietrangelo (1999) who described an Italian family with an autosomal dominant non-HFE haemochromatosis. Many family members had iron overload resulting in liver fibrosis, diabetes, impotence, and cardiac arrhythmias. In addition to autosomal dominant inheritance, features distinguishing this from HFE haemochromatosis included early iron accumulation in reticuloendothelial cells and a marked increase in ferritin earlier than what is seen in transferrin saturation (Pietrangelo 1999, Rivard 2003, Montosi 2001, Wallace 2004, Fleming 2001). Several patients showed a reduced tolerance to phlebotomy and became anemic despite elevated ferritin (Pietrangelo 1999, Jouanolle 2003).

In 2001, this form of non-HFE haemochromatosis was linked to mutations of ferroportin (Montosi 2001) that had just been identified as the basolateral iron transporter (Abboud 2000, Donovan 2000). Since that time, numerous mutations in the gene have been implicated in patients from diverse ethnic origins with previously unexplained haemochromatosis. Iron overload disease due to ferroportin mutations has been defined as type 4 haemochromatosis or Ferroportin Disease (for review see Pietrangelo 2004). The iron export is tightly regulated because both iron deficiency and iron excess are harmful. The main regulator of this mechanism is the peptide hepcidin which binds to ferroportin, induces its internalisation and degradation, thereby reducing iron efflux (Nemeth 2004). Increase in iron absorption may be caused either by hepcidin deficiency or its ineffective interaction with ferroportin. All recent studies have shown that hepcidin deficiency appears to be the common characteristic of most types of genetic haemochromatosis (mutations in HFE, transferrin receptor 2, hemojuvelin, or hepcidin itself). The remaining cases of genetic iron overload are due to heterozygous mutations in the hepcidin target, ferroportin. Because of the mild clinical penetrance of the genetic defect there were doubts about the rationale for iron removal therapy. However, a more recent study shows that there may be clinically relevant iron overload with organ damage and liver

cancer in patients carrying the A77D mutation of ferroportin (Corradini 2007). Treatment schemes are similar to those described for other types of genetic haemochromatosis.

Secondary haemochromatosis

Pathophysiology

Most forms of secondary haemochromatosis are due to hemolytic anaemia associated with polytransfusions such as thalassaemia, sickle cell disease, and myelodysplastic syndromes (MDS). Most of these patients need blood transfusions on a regular basis for survival. However, in the long run, multiple blood transfusions often lead to iron overload if patients are not treated with iron chelators. In general, iron overload due to blood transfusions is similar to genetic haemochromatosis; however, secondary iron overload develops much faster than the genetic forms (McLaren 1983), sometimes as soon as after 10–12 blood transfusions (Porter 2001). Subsequently secondary iron overload can result in more rapid organ damage when compared with genetic haemochromatosis. Secondary iron overload can obviously not be treated by phlebotomy because a marked anaemia is the clinical marker of the disease. Secondary iron overload often limits the prognosis of patients with thalassaemia; life expectancy deteriorates with increasing iron concentrations in the liver (Telfer 2000). Therapy with iron chelator may reduce the transfusional iron burden if the frequency of transfusion is not too high. The development of HFE versus secondary haemochromatosis not only differs in terms of the speed of iron accumulation but also in the type of organ damage; in secondary haemochromatosis cardiomyopathy is often the complication that limits the prognosis (Liu 1994). It is interesting that heart disease is also very frequent in juvenile genetic haemochromatosis where there is also rapid iron accumulation. In general, serum ferritin values closely reflect liver iron concentration and may be used as an indication for timing of therapy as well as to check the effects of iron chelation.

For many years, deferoxamine was the only iron chelator available in most countries but in some countries deferiprone is also approved for patients who do not tolerate deferoxamine (Hoffbrandt 2003). The clinical use of deferiprone is limited due to side effects such as agranulocytosis and neutropenia (Refaie 1995). Long-term data prove that deferoxamine can reduce iron overload and its organ complications (Olivieri 1994, Cohen 1981). Deferoxamine, however, needs to be given daily subcutaneously or by IV infusion for several hours. Thus, patients with thalassaemia often report that deferoxamine treatment is worse than thalassaemia itself (Goldbeck

2000). Therefore, adherence problems often limit the beneficial effects of this iron chelator (Cohen 1989).

Without iron chelation, children with thalassaemia often develop a severe cardiomyopathy prior to age 15 (Cohen 1987). After that age, liver cirrhosis is also a significant complication in secondary iron overload due to thalassaemia (Zurlo 1992). Iron chelation should start early to prevent complications of iron overload. By the ages of 3–5, liver iron concentration may reach values associated with a significant risk for liver fibrosis in severe thalassaemia (Angelucci 1995). Children younger than 5 should therefore be cautiously treated with chelators if they have received transfusions for more than a year (Olivieri 1997). Deferoxamine can reduce the incidence and ameliorate the course of iron-associated cardiomyopathy (Olivieri 1994, Brittenham 1994, Miskin 2003).

Deferasirox is an oral iron chelator with high selectivity for iron III (Nick 2003). Deferasirox binds iron in a 2:1 proportion with a high affinity and increases the biliary iron excretion (Nick 2003). This chelator is able to reduce iron overload in hepatocytes and cardiomyocytes (Nick 2003, Hershko 2001). Due to its half-life of 11–18 hours it needs to be taken only once daily (Nisbet-Brown 2003). Deferasirox exerted a similar iron chelation when compared with deferoxamine in patients with thalassaemia; the effect of 40 mg/kg deferoxamine was similar to that of 20 mg/kg deferasirox (Piga 2006). Both in adults and children 20–30 mg/kg/day deferasirox significantly reduced liver iron concentration and serum ferritin (Cappellini 2006). Magnetic resonance imaging showed that 10–30 mg/kg/day deferasirox may also reduce iron concentration in the heart within one year of maintenance therapy. Deferasirox may cause minor increases in serum creatinine as well as gastrointestinal discomfort and skin exanthema which are usually self-limiting. Considering the compliance problems with deferoxamine, deferasirox has a better cost-effectiveness ratio (Vichinsky 2005). Deferasirox is defined as standard therapy both in the guidelines of the National Comprehensive Cancer Network (NCCN) (USA) and in the international guidelines on MDS (Greenberg 2006, Gattermann 2005).

Use of blood from patients with HFE haemochromatosis (type 1) for blood donation

For some decades it has been debated whether blood phlebotomised from patients with HFE haemochromatosis may be used for blood transfusions (Nouel 1991, Barton 1999, Conry-Cantilena 2001, De Buck 2012, Leitmann 2013). In many countries blood from haemochromatosis patients is still not used for blood transfusion because of several arguments and precautions:

For a long time such blood has not been accepted by many blood banks because there was a hypothesis that such blood may be associated with increased risk for the recipient. Indeed, excess iron may increase the risk for bacterial and viral infections (Walker 2000, Khan 2007, Drakesmith 2008). In particular there were some hints that siderophilic bacteria including *Vibrio* sp., *Salmonella* sp. and *Yersinia* sp. grow particularly well in iron-overloaded blood (Nouel 1991, Cauchie 1987, Boelaert 1987, Piroth 1997). There have also been reports that *Yersinia enterocolitica* is responsible for posttransfusion sepsis and death (Leclercq 2005). *In vitro* there is a significantly decreased antibacterial activity against *S. typhimurium* LT2 and a better survival of *Vibrio vulnificus* in blood from iron-overloaded HFE patients when compared with healthy subjects (Jolivet-Gougeon 2007, Jolivet-Gougeon 2008, Bullen 1991).

In contrast, such risks were not present in blood from iron-depleted patients with HFE haemochromatosis (Jolivet-Gougeon 2008, Bullen 1991). A further study showed that the presence of anti-*Yersinia* antibodies was similar in the blood of uncomplicated HFE haemochromatosis patients when compared to blood from control donors (Jolivet-Gougeon 2007). Based on screening tests for antibodies to hepatitis B core antigen, syphilis, human immunodeficiency virus, hepatitis C virus, hepatitis B surface antigen, and human T-lymphotropic virus, no statistically significant difference could be found for HFE donors versus regular donors (Leitman 2003, Sanchez 2001).

It has in addition been argued that the blood donation by haemochromatosis patients is not voluntary because they benefit from the donation (Conry-Cantilena 2001, De Gonzalez 2007, Pennings 2005). Also phlebotomies from haemochromatosis patients does not require a financial compensation and may thus provide a financial advantage for the physician (Leitman 2013). The latter argument needs to be discussed considering that management of haemochromatosis patients as well as the use of their blood vary between industrialised countries (Butzeck 2011, Leitman 2013). In any case, it has been proposed that all phlebotomies should be free to haemochromatosis patients in order to eliminate any financial incentives and the non-voluntary character of the donation (Leitman 2013).

In general, blood banks need to observe rigorously that their criteria for haemochromatosis patients are also applicable to other donors. In a cohort of 130 subjects with HFE polymorphisms referred to a blood centre for management, 76% met all eligibility criteria for allogeneic blood donation and 55% had previously been blood donors before being made aware of their HFE diagnosis (Leitmann 2003). In the latter study, HFE donors were documented to more regularly observing their donation appointments than non-HFE donors, and they were less likely to have low screening hemoglobin of < 12.5 g/dL (Leitman 2003).

Since 2001, many European and U.S. transfusion services have changed

their policy for the management of blood drawn from haemochromatosis patients (Courtois 2001, Radojska 2011, Buring 2002, Guidelines for the Blood Transfusion Services in the United Kingdom 2005, Ministerial Order of the Government of France 2009, FDA guidance for variances for blood collection from individuals with hereditary haemochromatosis 2001). For the USA, the FDA (Food and Drug Administration) issued a guidance in 2001 to allow blood banks to submit variances to federal code to accept blood from HFE patient for blood transfusion (Center for Biologics Evaluation and Research 2013). This guidance contains several criteria (Leitman 2013):

- The donor meets all other general allogeneic donor criteria.
- Phlebotomy is provided free of charge to all HFE patients in that blood centre.
- Incentives for HFE donors are considered untruthful in responding to standardised health history screening questions.
- A medical prescription for phlebotomy therapy including frequency and hemoglobin threshold is provided by the donor's physician.
- A short physical examination is performed at each visit if the patient donates more often than every 8 weeks.

In the 12 years following the publication of this guidance, 163 blood banks in 43 US states have submitted variances and implemented policies for collection of blood from HFE donors (Leitman 2013). HFE donors have been shown to have a considerable satisfaction from knowing that their blood is being used to save lives rather than being discarded (Center for Biologics Evaluation and Research 2013).

It is estimated that routine referral of HFE subjects to blood centres for phlebotomy care could supplement the U.S. blood supply by an additional 1.3 million RBC units per year, possibly help to avoid periodic blood shortages, avoid wastage of safe units, and decrease the costs of care (Leitman 2013).

People with C282Y/H63D and H63D/H63D genotypes and slightly elevated ferritin levels are often referred to the blood centre for phlebotomy treatment (Leitman 2013). These subjects in general do not have organ damage due to iron overload and do need an aggressive phlebotomy therapy like the C282Y homozygotes. In blood centres with active recruitment of HFE patients, blood donations from HFE patients may contribute to 10 – 40% of available blood (Leitman 2013).

Nevertheless there is still no general consensus about the acceptance of haemochromatosis patients as blood donors (Leitman 2013, de Buck 2012). Most recent studies however share the following policy when dealing with a potential acceptance of haemochromatosis patients as blood donors (De Buck 2012, Sackett 1996):

In general all criteria applicable to any other donor need to be rigorously observed also for HFE patients.

Blood from HFE patients should only be used for transfusion when patients have already been iron-depleted and do not have major organ complications.

There are no incentives or financial advantages for the HFE patients and their physicians for the use of phlebotomised blood for donation.

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22. NAFLD and NASH

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Introduction

Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are the most common chronic liver diseases in the West (Tayama 2012, Cusi 2012). They are closely associated with obesity, type 2 diabetes mellitus and metabolic syndrome. The epidemics of diabetes and obesity have also fueled an increasing prevalence of fatty liver disease (Tayama 2012, Cusi 2012). Both NAFLD and NASH are associated with an often asymptomatic elevation of serum ALT and gamma GT. Ultrasound monitoring can suggest the presence of a fatty infiltration of the liver; differentiation between NAFLD and NASH, however, often requires a liver biopsy. Such differentiation is important because NASH is associated with a much higher risk of liver fibrosis and cirrhosis than NAFLD. However, publications also show that most patients with fatty liver disease die from cardiovascular disease and not from liver disease (Pisto 2014, Treeprasertsuk 2013, Haflidadottir 2014, Kim 2013). NAFLD-related hepatocellular carcinoma (HCC) is a relevant complication and may occur also in the absence of cirrhosis (Piscaglia 2015), but is still less prevalent than HCV-related HCC (Beste 2015, Piscaglia 2015, Mittal 2015a, Mittal 2015b, White 2012). Globally, only the HCV-related HCC has increased in the last 15 years, but not NAFL-related HCC (Naghavi 2015). Although NAFLD is a growing cause for liver transplantation (LTX), HCV is still the leading cause for LTX (Wong 2014).

Prevalence

NAFLD is present in 20 to 40% of the general population in industrialised countries and is the most prevalent chronic liver disease (Browning 2004, Chitturi 2004, McCullough 2005). It is more prevalent in obese and diabetic subjects (Bellentani 1994, Wanless 1990, Clark 2002, Chitturi 2004). Among all subjects with NAFLD, features of non-alcoholic steatohepatitis (NASH) can be seen in 10–20%. The prevalence of NASH in Western countries is approximately 2–6%. In the US, NASH is estimated to affect 5–6% of the general population (McCullough 2005). It has been suggested that NASH accounts for more than 50% of cryptogenic cirrhosis (Ratziu 2002). NAFLD may progress to NASH with fibrosis, cirrhosis, and hepatocellular carcinoma (Marchesini 2003, Caldwell 2004). The term

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NASH was introduced in a description of 20 Mayo Clinic patients with a hitherto unnamed disease associated with hepatomegaly, abnormal ALT, a fatty liver histology, lobular hepatitis, and fibrosis mimicking alcoholic hepatitis in the absence of alcohol intake (Ludwig 1980); most patients had obesity and diabetes mellitus.

Demographics and risk factors

In the US, NAFLD is 3-5 times more prevalent in men than in women; such differences in gender might partly be explained by the fact that men have a higher BMI and that some male patients with NAFLD drink more alcohol than they report (Schwimmer 2005, Bahcecioglu 2006, Loguercio 2001). The NAFLD prevalence in the US is particularly high in people of Hispanic (28%) or Asian (20-30%) origin (Schwimmer 2005, Weston 2005). Due to the dramatic increase in obesity in the US and other industrialised countries, there is also a dramatic increase in the prevalence of NAFLD and NASH (Tayama 2012, Cusi 2012). In the US almost 50% of obese boys have NAFLD (Schwimmer 2005). In many countries more than 80% of NAFLD patients have an increased BMI and 30-40% are obese; approximately 50% show signs of insulin resistance, 20-30% have type 2 diabetes, 80% show hyperlipidaemia, and 30-60% have arterial hypertension. Correspondingly there is a strong association between NAFLD and NASH and the metabolic syndrome throughout the world (Marchesini 1999, Bedogni 2005). In comparison with NAFLD patients, NASH patients are older, more obese and more often have high serum liver enzymes, diabetes mellitus and metabolic syndrome (Ratziu 2002, Adams 2005, Hamaguchi 2005, Fassio 2004, Tayama 2012, Cusi 2012).

Pathogenesis

The degree of fatty infiltration in NAFLD is graded according to the percentage of hepatocytes with fat deposits: mild NAFLD involves less than 30% hepatocytes, moderate NAFLD up to 60%, and severe NAFLD above 60% (Ploeg 1993). NAFLD may regress if the cause is eliminated. NASH is associated with insulin resistance, increased circulating levels of leptin, adiponectin, tumour necrosis factor and some interleukins (Friedman 1998, Marra 2004). A meta-analysis confirmed that circulating leptin levels were higher in patients with NAFLD than in controls. Higher levels of circulating leptin were associated with increased severity of NAFLD, and the association remained significant after the exclusion of studies involving paediatric or adolescent populations and morbidly obese individuals being

referred for bariatric surgery (Polyzos 2016). It is thought that there is an increased flow of free fatty acids from visceral fat to the liver contributing to abnormalities in intracellular lipid metabolism (Hashimoto 1999, Vendemia 2001). Insulin resistance and increased free fatty acids may both affect mitochondrial oxidation of fatty acids causing free radical generation in hepatocytes (Grattagliano 2003). Thus, NASH is caused by two mechanisms or toxic "hits": the first mechanism is the hepatic accumulation of triglycerides (NAFLD) due to insulin resistance and the second is thought to be the generation of free radicals with subsequent release of mediators and cytokines (McCullough 2006).

Insulin resistance has been closely linked to non-alcoholic fatty liver disease in both clinical trials and laboratory-based studies (McCullough 2006, Marchesini 2001, Sanyal 2001). The actual process by which NAFLD turns into NASH however remains ill-defined despite this double-hit theory. Likely, genetic factors (similar to those responsible for the metabolic syndrome) as well as exogenic factors (like drugs, moderate amounts of alcohol, and other toxins) may contribute to the evolution of NAFLD into NASH. The role of hepatic iron in the progression of NASH remains controversial, but in some patients, iron may have a role in the pathogenesis of NASH by promoting oxidative stress (Lee 1995, George 1998, Bonkowsky 1999, Younoussi 1999).

Human genetic factors

The genetic determinants of the pathogenesis and disease progression of NAFLD remained obscure until 2008. In 2008, two genome-wide association (GWAS) studies linked the rs738409 polymorphism (IL48M) of patatin-like phospholipase domain containing 3 (PNPLA3) with hepatic fat content and ALT levels (Romeo 2008, Yuan 2008). Later several further studies and a meta-analysis have corroborated such association between the IL48M polymorphism and NAFLD in almost all ethnic groups, and in adults, children and adolescents (Kontronen 2009, Sookoian 2009, Valenti 2010, Romeo 2010a, Romeo 2010b, Rotman 2010, Hotta 2010, Speliote 2010, Davis 2010, Santoro 2010a, Santoro 2010b, Sookoian 2011, Guidice 2011, Lin 2011, Valenti 2012, Peng 2012, Nobili 2013, Kitamoto 2013, Hernaez 2013, Shang 2014, DiStefano 2014, Baclig 2014). Further studies suggest that the IL48M variant is an important risk factor for accumulation of hepatic steatosis in particular when additional factors are present such as free fatty acid release, insulin resistance, visceral obesity, increase in lipogenesis, and changes in lipid metabolism (Guidice 2011, He 2010, Davis 2010, Santoro 2010a, Santoro 2010b, Sevastianova 2012, Valenti 2012, Dongiovanni 2013).

IL48M polymorphism also predisposes to cirrhosis (Shen 2014) and

hepatocellular carcinoma (Falletti 2011, Burza 2012, Trepo 2012, Valenti 2013, Nault 2014, Trepo 2014). Potential mechanisms involved have been reviewed and are not discussed in detail here (Dongiovanni 2013, Nault 2014, Shen 2014). All published data suggest that the 148M PNPLA3 polymorphism favours hepatic carcinogenesis in steatohepatitis as well as in other liver diseases, and the mechanism is partly independent of the predisposition towards fibrogenesis and cirrhosis (Nault 2014, Shen 2014).

The association of the rs738409 SNP in the adiponutrin/PNPLA3 gene with alcoholic and non-alcoholic fatty liver disease and with intracellular triglyceride accumulation is seen also in European populations (Trepo 2014). In parallel, GWAS identified other loci, including neurocan (NCAN), associated with liver fat content and progression of non-alcoholic fatty liver disease (Speliotes 2011). Some of these variants were associated with distinct changes in serum lipid levels, which suggest different and specific impacts on lipid metabolism and NAFLD progression. In particular, the T allele of NCAN rs2228603 was suggested to induce hepatic triglyceride accumulation (Speliotes 2011). In another study (Nischalke 2014) the NCAN rs2228603 and the PNPLA3 IL48M variants were independently associated with increased prevalence of HCC in two genotyped cohorts and were used to stratify patients for risk of liver cancer. This data underlines the importance of steatosis in liver carcinogenesis (Trepo 2014). Other studies show similar findings (Sookoian 2014, Liu 2014, Di Stefano 2014).

The IL48M polymorphism is also a major risk factor for steatosis in chronic hepatitis C virus infection, in particular in non-genotype 3 infections (Cai 2011, Valenti 2011, Müller 2011, Trepo 2011, De Nicola 2014). These studies have further proven the association between the IL48M PNPLA3 variant and fibrosis progression in patients with chronic hepatitis C virus infection.

Data also show evidence that the 148M variant predisposes to steatosis and thereby to progressive fibrosis in chronic hepatitis B, haemochromatosis, primary sclerosing cholangitis and alcoholic liver disease (Valenti 2012, Trepo 2012, Friedrich 2013, Viganò 2013).

Despite the finding that polymorphisms in the adiponutrin/PNPLA3 gene modify steatosis and fibrosis, large GWAS studies were much less successful in identifying human genetic factor leading to obesity, fatty liver, insulin resistance, and diabetes mellitus (Holzapfel 2010, Delahanty 2012). Lifestyle factors were correlated with BMI more closely than genetic factors (Holzapfel 2010).

Microbiome

The gut microbiota, now also called the gut microbiome, is involved in the pathophysiology of non-alcoholic fatty liver disease as well as in obesity

and the metabolic syndrome. All the metabolic products generated by the intestinal microbiome first enter the liver. Studies with germ-free mice have shown that inoculation of microbiota from conventionally raised fat mice results in obesity and fatty liver (Bäckhed 2009). Genetically obese (ob/ob) mice have a decreased ratio of bacteroides versus firmicutes compared with lean (ob/+ and +/+ wild-type) mice (Ley 2009). Inoculation of gut microbiota from these obese mice (ob/ob) to germ-free mice led to an obese phenotype (Turnbaugh 2006). Similar effects occur when such mice are fed a Western diet or are inoculated with microbiota from an obese human (Turnbaugh 2009). It has also been shown by many investigators that the microbiome differs between obese and lean animals and between obese and lean humans (Ley 2005). As yet it is not completely known if intestinal products are the cause or only aggravate NAFLD and NASH. A study proposed that the altered microbiome in obesity might produce more ethanol and might thereby contribute to the development of NASH (Zhu 2012). Another paper shows that inflammasome or interleukin-18 deficiency enhances the progression of NASH and obesity by inducing microbiome dysbiosis (Heno-Mejia 2012). This dysbiosis-induced inflammation enters into the portal circulation through the influx of toll-like receptor (TLR) 4 and TLR9 agonists and thereby leads to an increase in tumour necrosis factor (TNF) (Moschen 2013). It has also been shown for the first time that the composition of the microbiome and the obese/NASH phenotype can be transmitted to wild-type mice co-housed with genetically deficient mice. This report corroborates that the gut microbiome plays an important role in the development of NASH and obesity, probably via changes in the inflammasome (Heno-Mejia 2012).

Metagenome-wide association (MGWAS) studies of gut microbiota showed patients with type 2 diabetes were characterised by a moderate degree of gut microbial dysbiosis, a decrease in the abundance of some universal butyrate-producing bacteria and an increase in various opportunistic pathogens, as well as an enrichment of other microbial functions conferring sulphate reduction and oxidative stress resistance in type 2 diabetes (Qin 2012).

Studies conducted on obese human subjects have confirmed specific changes in the intestinal microbiome, such as a reduction of bacteroidetes and a proportional increase of firmicutes (Ley 2006, Armougom 2009, Santacruz 2010). Moreover, a reduction of bifidobacterium and bacteroides and an increase of staphylococcus, enterobacteriaceae and escherichia coli were detected in overweight compared to normal-weight pregnant women (Santacruz 2010).

A MGWAS study analysed the human gut microbial composition in 123 non-obese and 169 obese Danish individuals (Le Chatelier 2013). The results showed two groups of individuals that differed by the number of gut

microbial genes and thus bacterial richness. Individuals with a low bacterial richness (23% of the population) had more overall visceral adiposity, insulin resistance and dyslipidaemia, and a more pronounced inflammatory phenotype when compared with high bacterial richness individuals. The obese individuals among the lower bacterial richness group also gained more weight over time. Only a few bacterial species were sufficient to distinguish between individuals with high and low bacterial richness, and even between lean and obese subjects. The classifications based on variation in the gut microbiome identified subgroups of individuals in the general white adult population who may be at increased risk of progressing to adiposity-associated co-morbidities (Le Chatelier 2013).

A study evaluated the association between gut dysbiosis and severe NAFLD lesions, i.e. non-alcoholic steatohepatitis (NASH) and fibrosis in 57 patients with biopsy-proven NAFLD (Boursier 2015). The taxonomic composition of gut microbiota was determined using 16S ribosomal RNA gene sequencing of stool samples. Thirty patients had Fo/I fibrosis stage at liver biopsy (10 with NASH), and 27 patients had significant F \geq 2 fibrosis (25 with NASH). *Bacteroides* abundance was significantly increased in NASH and F \geq 2 patients, whereas *Prevotella* abundance was decreased. *Ruminococcus* abundance was significantly higher in F \geq 2 patients. By multivariate analysis, *Bacteroides* abundance was independently associated with NASH and *Ruminococcus* with F \geq 2 fibrosis (Boursier 2015). These results suggest that NAFLD severity associates with gut dysbiosis and a shift in metabolic function of the gut microbiota (Boursier 2015). In particular *Bacteroides* may be associated with NASH and *Ruminococcus* with significant fibrosis.

Natural history

The natural history of NAFLD in the general population is not well-defined since most data come from selected patients and tertiary centres (Dam-Larsen 1996, Lee 1989, Teli 1995). Correspondingly, published mortality and morbidity in hospitalised people with NAFLD are approximately 5 times higher than what is seen in the general population (Matteoni 1999). In the general population the risk for liver-related death in NAFLD appears to be associated mainly with age, insulin resistance, and histological evidence of hepatic inflammation and fibrosis (Adams 2005). Probably around 10% of NAFLD patients will progress to NASH over a period of 10 years (Figure 1). Cirrhosis later develops in 5-25% of patients with NASH and 30-50% of these patients die from liver-related causes over a 10-year period (McCollough 2005, Matteoni 1999). Cirrhosis in patients with NASH can also decompensate into subacute liver failure, progress to hepatocellular cancer

(HCC), and recur after liver transplantation (McCollough 2005). Steatosis alone is reported to have a more benign clinical course, with cirrhosis developing in only 1-3% of patients (Day 2004, Day 2005, McCollough 2005, Matteoni 1999). Patients with NASH and fibrosis also have a significant risk for hepatocellular carcinoma (El-Serag 2004) (Figure 1).

There is no doubt that the incidence and prevalence of NAFLD and NASH are increasing in almost all industrialised countries, sometimes in an epidemic manner. Some papers suggest that NASH may soon be the leading cause of cirrhosis, HCC and LTX. There is also growing evidence that HCC in NAFLD and NASH may even develop without cirrhosis. These suggestions would imply that NAFLD related HCC and NAFLD related liver mortality should rapidly increase in the near future. A critical view on recent publications however sheds some doubts on these suggestions.

A retrospective cohort study evaluated trends in the aetiology of HCC among adult recipients of liver transplantation (LTX) in the U.S. from 2002 to 2012 (Wong 2014). During that period 61,868 adults underwent LTX including 10,061 patients with HCC. The proportion of HCV-related HCC increased steadily from 2002 to 2012, and HCV remained the leading aetiology of HCC (43.4% in 2002, 46.3% in 2007, 49.9% in 2012). NASH-related HCC also increased significantly (8.3% in 2002, 10.3% in 2007, 13.5% in 2012) (Wong 2014). Thus, NASH is still only the second leading aetiology of HCC leading to LTX in the U.S. and HCV-related HCC is still more than three-times more prevalent than NAFLD-related HCC.

A PubMed survey analysed original reports published from January 1992 to December 2011 evaluating the association between NAFLD, NASH, cryptogenic cirrhosis presumed to be NASH-related, and the risk of HCC (White 2012). There were 17 cohort studies (3 population based, 9 clinic based, and 5 natural history), 18 case-control and cross-sectional studies, and 26 case series. NAFLD or NASH cohorts with few or no cases of cirrhosis cases had a minimal risk for HCC: the cumulative HCC mortality was only 0 – 3% for study periods for up to 20 years. Cohorts with NASH and cirrhosis had a consistently higher risk with a cumulative incidence ranging from 2.4% over 7 years to 12.8% over 3 years (White 2012). However, the risk for HCC was substantially lower in these cohorts than for cohorts with hepatitis C-related cirrhosis. This study concluded that there is epidemiologic evidence to support an association between NAFLD or NASH and an increased risk of HCC; such risk seemed to be limited to individuals with cirrhosis (White 2012).

This data is in contrast to another multicentre prospective study which assessed the clinical features of patients with NAFLD-related HCC (NAFLD-HCC) and compared them to those of HCV-related HCC (Piscaglia 2015). A total of 756 patients with either NAFLD (n=145) or HCV-related chronic liver disease (n=611) were enrolled in several Italian centres. Cirrhosis was

present here in only about 50% of NAFLD-HCC, but in almost all cases of HCV-HCC (Piscaglia 2015).

A retrospective study looked at a cohort of Veterans Affairs (VA) patients with the diagnosis of cirrhosis ($n = 129,998$) or HCC ($n = 21,326$) from 2001 to 2013 (Beste 2015). Cirrhosis prevalence and mortality, and HCC incidence and mortality increased from 2001 to 2013, driven by HCV, with a much smaller contribution from NAFLD (Beste 2015).

A further study analysed a cohort of 1500 patients who developed HCC between 2005 and 2010 from Veterans Administration (VA) hospitals in the U.S.A. (Mittal 2015a). NAFLD was the underlying risk factor for HCC in 120 patients (8.0%); the annual proportion of NAFLD-related HCC remained relatively stable (7.5%-12.0%). In contrast, the proportion of HCC cases associated with HCV increased from 61.0% in 2005 to 74.9% in 2010 (Mittal 2015a). The proportion of HCC cases associated with only alcohol abuse decreased from 21.9% in 2005 to 15.7% in 2010, and the annual proportion of HCC cases associated with hepatitis B remained relatively low and stable (1.4%-3.5%). A significantly lower proportion of patients with NAFLD-related HCC had cirrhosis (58.3%) compared to patients with alcohol- or HCV-related HCC (72.4% and 85.6%, respectively; $P < 0.05$). NAFLD was only the third most common risk factor for HCC in the VA population. The proportion of NAFLD-related HCC was relatively stable from 2005 through 2010 (Mittal 2015a).

Further details of this analysis were published separately (Mittal 2015b). This study part looked for evidence of cirrhosis and risk factors for HCC in the VA cohort. Patients without cirrhosis were assigned to categories of level 1 evidence for no cirrhosis (very high probability) or level 2 evidence for no cirrhosis (high probability), which were based on findings from histological analyses, laboratory tests, non-invasive markers of fibrosis, and imaging features. A total of 43 of the 1500 patients with HCC (3%) had level 1 evidence for no cirrhosis, and 151 (10%) had level 2 evidence for no cirrhosis; the remaining 1203 patients (80%) had confirmed cirrhosis. Compared with patients with HCC in presence of cirrhosis, greater proportions of HCC patients without evidence of cirrhosis had metabolic syndrome, NAFLD, or no identifiable risk factors. HCC patients with NAFLD had 5.4-fold risk of having HCC in the absence of cirrhosis, compared to patients with HCV-related HCC. However, only 13% of patients with HCC in the VA system did not appear to have cirrhosis. NAFLD and metabolic syndrome were the main risk factors for HCC in the absence of cirrhosis.

There have been several large studies which analysed the long-term outcome of patients with fatty liver disease. They uniformly demonstrated that most patients with NAFLD and also with NASH do not die from liver-related problems but from cardiovascular events (Pisto 2014, Treeprasertsuk 2013, Haflidadottir 2014, Kim 2013).

A large Finnish study analysed a population-based, randomly recruited cohort (Oulu Project Elucidating Risk of Atherosclerosis, OPERA) (Pisto 2014). The study was initiated in 1991 and included 988 middle-aged Finnish participants. Total mortality and hospital events were followed up to 2009 based on the registry of the National Institute for Health and Welfare and the National death registry. The severity of hepatic steatosis was measured by ultrasound and divided into three severity groups. During follow-up between 1991-2009, 13.5% of the participants with non-fatty liver, 24.2% of participants with moderate liver fat content and 29.2% of the participants with severe fatty liver experienced a cardiovascular event ($p < 0.001$). Liver fat content predicted the risk for cardiovascular events even when adjusted for age, gender, smoking, alcohol consumption, LDL cholesterol, BMI, and systolic blood pressure (Pisto 2014).

The Rochester Epidemiology Project also analysed whether the severity of liver fibrosis in NAFLD predicts all-cause mortality, cardiac complications, and/or liver complications (Treeprasertsuk 2013) in a cohort of NAFLD patients during 1980-2000. The NAFLD fibrosis score (NFS) was used to separate NAFLD patients with and without advanced fibrosis. A total of 302 NAFLD patients (mean age: 47 ± 13 year) were included with a follow-up period of 12.0 ± 3.9 years. NFS was < -1.5 at baseline in 181 patients (60%), while NFS was > -1.5 in 121 patients (40%). A total of 39 patients (13%) died during follow-up. The leading causes of death were non-hepatic malignancy ($n = 13/39$; 33.3%) and coronary heart disease (CHD) ($n = 8/39$; 20.5%); only 5/39 patients died from liver disease (12.8%). Thirty patients had new-onset CHD, whereas 8 of 30 patients (27%) died from CHD-related causes. In a multivariate analysis, a higher NFS at baseline and the presence of new-onset CHD significantly predicted death. There was a significant, graded relationship between NFS and mortality (Treeprasertsuk 2013). The use of metformin or simvastatin during follow-up was associated with fewer deaths in patients with NAFLD. A German study also showed that clinically relevant coronary artery disease is frequently associated with the presence of NAFLD (Friedrich-Rust 2017).

A further retrospective study analysed patients who underwent a liver biopsy between 1984-2009 at the National University Hospital of Iceland with NAFLD or AFLD (Haflidadottir 2014). A total of 151 had NAFLD and 94 had AFLD with median survival of 24 years and 20 years, respectively ($p > 0.05$). A total of 10/151 (7%) patients developed cirrhosis in the NAFLD group and 19/94 (20%) in AFLD group ($p = 0.03$). The most common cause of death in the NAFLD group was cardiovascular disease (48%). In contrast liver disease was the most common cause of death in the AFLD group (36%), whereas liver-related deaths occurred only in 7% of the NAFLD group. Survival of AFLD patients was significantly shorter compared to the NAFLD patients after adjusting for gender and age at diagnosis (HR 2.16, $p = 0.009$).

(Haflidadottir 2014).

Another large study analysed the long-term impact of NAFLD on mortality using the National Health and Nutrition Examination Survey conducted in 1988–1994 including subsequent follow-up data for mortality through December 31, 2006 (Kim 2013). NAFLD was defined by ultrasound in the absence of other liver diseases. The presence and severity of liver fibrosis in subjects with NAFLD was determined by the NAFLD fibrosis score (NFS), the AST-platelet ratio index (APRI), and the FIB-4 score. Out of 11,154 participants, 34% had NAFLD – the majority (72%) had NFS consistent without significant fibrosis ($NFS < -1.455$), whereas 3% had a score indicative of advanced fibrosis ($NFS > 0.676$). After a median follow-up of 14.5 years, NAFLD was not associated with higher mortality [age- and sex-adjusted hazard ratio (HR) 1.05, $p > 0.1$] (Kim 2013). In contrast, there was a progressive increase in mortality with advancing fibrosis scores. Compared to subjects without fibrosis, those with advanced fibrosis had a 69% increase in mortality (HR 1.69 for NFS, HR 1.85 for APRI, HR 1.66 for FIB-4) after adjustment for other known predictors of mortality (Kim 2013). These increases in mortality were almost entirely from cardiovascular causes (HR 3.46 for NFS, HR 2.53 for APRI, HR 2.68 for FIB-4). Thus, ultrasound-diagnosed NAFLD is in general not associated with increased mortality. However, advanced fibrosis as determined by non-invasive fibrosis markers was a significant predictor of mortality, mainly – or even only – from cardiovascular causes (Kim 2013).

Indeed up to 20–50% of all HCC cases may develop in patients with fatty liver disease in the absence of cirrhosis. This percentage is higher when compared with HCV-related HCC. Almost all publications, however also show that HCV-related HCC is still much more frequent than NAFLD-related HCC; the same is also true for the causes of liver transplantation. The majority of publications also show that most patients with fatty liver disease die from cardiovascular and not from liver disease (Pisto 2014, Treeprasertsuk 2013, Haflidadottir 2014, Kim 2013). The risk of a cardiovascular death increases with the degree of NAFLD-related liver fibrosis. The worldwide incidence of HCV-related HCC is still increasing while the incidence of NAFLD-related HCC has not increased, but has decreased in the past two decades (Naghavi 2015). The latter unexpected data came from the Global Burden of Disease Study 2013 which estimated yearly deaths for 188 countries between 1990 and 2013 (Naghavi 2015). Significant declines were noted for liver cancer due to alcohol use and liver cancer due to other causes (obviously mainly due to fatty liver disease) while significant increases were noted for liver cancer due to hepatitis C. Thus, globally liver cancer due to NAFLD and NASH did not increase but decreased between 1990 and 2013 (Naghavi 2015).

Diagnosis

NAFLD and NASH require valid reporting about alcohol consumption. Since only approximately 10% of Western populations are completely abstinent from alcohol, one needs to set a threshold above which one assumes that alcohol at least contributes to the pathogenic process of NAFLD and NASH. Most authors use a daily alcohol ingestion of 20 g as such a threshold (Figure 2); others use lower values such as 10 g/day or as high as 40 g/day for men.

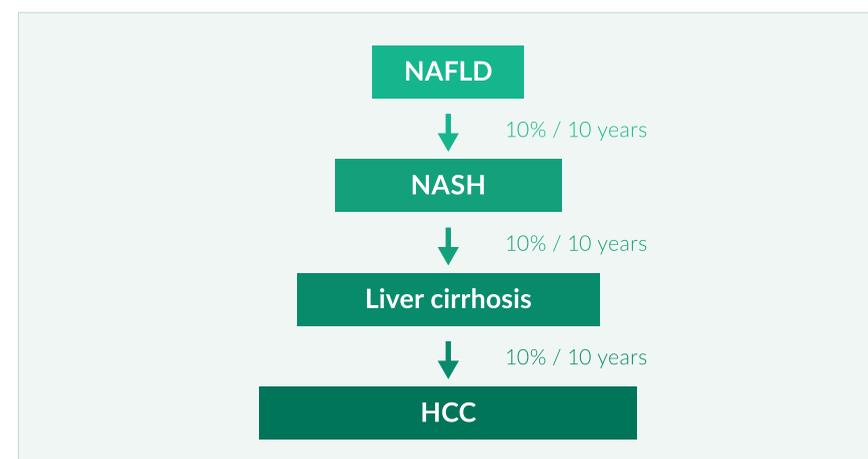


Figure 1. Natural history of NASH

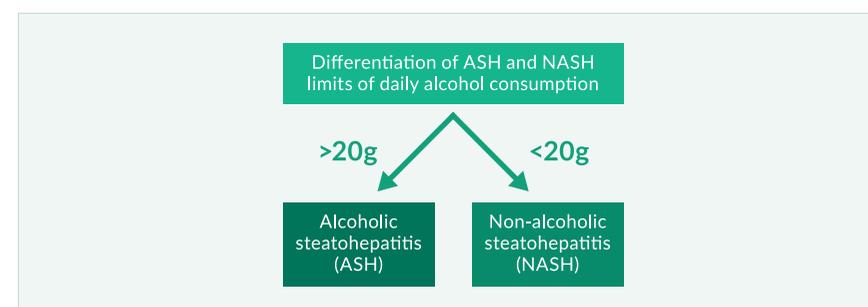


Figure 2. Differentiation of alcoholic liver disease (ASH) and NASH

The workup of NAFLD and NASH also includes assessment of drug use, exclusion of HBV and HCV infections, haemochromatosis, autoimmune liver disease and, in younger patients, Wilson's disease. In special groups of patients NASH may be accompanied by drug- and alcohol-induced liver disease and by HCV and HBV infections. The combination of NAFLD/NASH and HCV infection plays a particularly important clinical role because

in this situation the rate of liver fibrosis is increased and the success of antiviral therapy is diminished (Ramesh 2004). NASH can be induced by various drugs and toxins including corticosteroids, amiodarone, methotrexate, tetracycline, tamoxifen, and valproate (Pessayre 2002). Thus, one needs to carefully assess the full clinical history of patients. In practice NAFLD is often diagnosed by combining elevated levels of ALT and gamma GT with the sonographic appearance of an increase in echodensity of the liver. However, a considerable number of patients with NAFLD and even with NASH and fibrosis have normal serum liver enzymes (Abrams 2004). Usually ALT is higher than AST unless there is already severe fibrosis or cirrhosis. Fasting serum glucose should be checked in all patients with NAFLD and NASH; one will also often find elevated serum insulin, insulin resistance, and/or diabetes (Table 1).

Table 1. Non-invasive predictors of NASH

<p>HAIR index (hypertension; ALT >40 U/l; insulin resistance) HAIR score for each patient (0–3) calculated by adding hypertension = 1, ALT 40 IU > 1, and IR index 5.0 > 1. Score ≥ 2 are 80% sensitive, 89% specific for NASH (Dixon 2001)</p>
<p>BAAT index (BMI >28; Age >50 years; ALT >2x UNL; increased triglycerides) BMI $\geq 28 = 1$ point; Age $\geq 50 = 1$ point; ALT $\geq 2 \times$ ULN = 1 point; TG ≥ 150 mg/dL = 1 point Score ≤ 1 has 100% negative predictive value for NASH (Ratziu 2000)</p>
<p>FIB4 score = age (years) x AST [U/L]/(platelets [10^9/L] x (ALT[U/L])) Using a lower cutoff value of 1.45, a FIB-4 score <1.45 had a negative predictive value of 90% for advanced fibrosis (Ishak fibrosis score 4–6). A FIB4 score >3.25 has a 97% specificity and a positive predictive value of 65% for advanced fibrosis (Sterling 2006; Vallet-Pichard 2007)</p>
<p>BARD score AST/ALT ratio ≥ 0.8–2 points; a BMI ≥ 28 – 1 point; and the presence of diabetes – 1 point. Score ranges from 0 to 4 points. Scores equaling 0 or 1 have a high (96%) negative predictive value (NPV) for advanced fibrosis (Harrison 2008)</p>
<p>NFS calculated = $-1.675 + 0.037 \times$ age [years] $+ 0.094 \times$ BMI [kg/m²] $+ 1.13 \times$ impaired fasting glucose or diabetes [yes = 1, no = 0] $+ 0.99 \times$ AST/ALT ratio $- 0.013 \times$ platelet [10^9/L] $- 0.66 \times$ albumin g/dL) > 0.676 high probability advanced fibrosis, $-1.455 \leq$ NFS ≤ 0.676 indeterminate probability of advanced fibrosis, and NFS < -1.455 as low probability of advanced fibrosis (Angulo 2007)</p>
<p>APRI = [AST/upper limit of normal]/ platelet count [10^9/L] x 100 0.5 for low and 1.5 for high probability of advanced fibrosis (Wai 2003)</p>
<p>The Enhanced Liver Fibrosis (ELF) score includes the three laboratory markers N-terminal peptide of procollagen III (P3NP/PIIINP), hyaluronic acid (HA), and tissue inhibitor of matrix metalloproteinase 1 (TIMP-1). These markers are not in the routine panel in clinical practice. The ELF Score is calculated = $2.278 + 0.851 \ln$ (CHA) $+ 0.751 \ln$ (CPIIINP) $+ 0.394 \ln$ (CTIMP-1) A low ELF score (< 7.7) has a high negative predictive value for moderate to severe fibrosis and a high score (> 11.2) has a high positive predictive value for advanced fibrosis or cirrhosis (Lichtinghagen 2013).</p>

Many authors also recommend to routinely look for metabolic syndrome which is diagnosed when three of the following features are seen (Greenland 2003):

- waist circumference ≥ 102 cm for men and ≥ 88 cm for women,
- fasting glucose level ≥ 6.1 mmol/L,
- triglyceridaemia ≥ 1.7 mmol/L,
- decreased high-density lipoprotein cholesterol (< 1.0 mmol/L in women; < 0.9 mmol/L in men)
- hypertension $\geq 135/80$ mmHg.

Ultrasound of the liver has a high sensitivity and specificity (both approaching 90%) for detection of fatty infiltration but does not allow assessment for the presence or degree of inflammation and fibrosis (Davies 1991). Therefore, diagnosis of fat in the liver is easily made by ultrasound but diagnosis of NAFLD or NASH cannot be made without a liver histology. In addition, liver biopsy is still the best way to reliably differentiate NASH from NAFLD (Harrison 2003). Today most pathologists use the Brunt description to score the histological degree of NASH (Brunt 1999) (Table 2).

Table 2. Histological Brunt score (Brunt 1999)

Grade	Steatosis	Ballooning of hepatocytes	Degree of inflammation
1	<33%	Minimal	Mild
2	34–66%	Present	Moderate
3	>66%	Marked	Portal moderate, lobular moderate
Stage	Fibrosis		
1	Perisinusoidal		
2	Perisinusoidal and portal/periportal		
3	Bridging septa		
4	Extensive bridging fibrosis, cirrhosis		

Since NAFLD is a very frequent but also relatively benign disease, one aims to identify risk factors for NASH in order to avoid doing liver biopsies in all NAFLD patients. Risk factors for NASH include older age, excessive obesity, diabetes mellitus, other hepatotoxins, and clinical, laboratory or sonographic signs suggesting severe liver disease. Liver biopsy remains the gold standard for characterising liver histology in patients with NAFLD (Chalasanani 2012). However, it is expensive and carries some morbidity and a small mortality risk. Thus, it should be performed in those patients who benefit most from diagnostic, therapeutic and prognostic perspectives.

Liver biopsy should be considered in patients with NAFLD who are at increased risk to have steatohepatitis and advanced fibrosis. The presence of metabolic syndrome and the NAFLD Fibrosis Score may be used for identifying patients who are at risk for steatohepatitis and advanced fibrosis (Chalasani 2012). Liver biopsy should also be considered in patients with suspected NAFLD in whom competing etiologies for hepatic steatosis and co-existing chronic liver diseases cannot be excluded without a biopsy (Chalasani 2012).

There has been increasing interest in non-invasive methods to identify liver fibrosis in patients with NAFLD (Gambino 2011; EASL 2016) – these include APRI score, BARD score, HAIR score, BAAT score, NAFLD Fibrosis Score (NFS), Enhanced Liver Fibrosis (ELF) panel, and transient elastography (“Fibroscan”). The NAFLD Fibrosis Score (NFS) is based on six routinely available variables (age, BMI, diabetes, platelet count, albumin, AST/ALT ratio) and is calculated using a published formula (<http://naflscore.com>). In a meta-analysis of 13 studies consisting of > 3000 patients, the NFS was useful to predict advanced fibrosis or cirrhosis (Gambino 2011). NFS and FIB-4 score have been externally validated in ethnically different NAFLD populations. According to European guidelines NFS, FIB-4, Enhanced Liver Fibrosis (ELF) and FibroTest predict overall mortality, cardiovascular mortality, and liver-related mortality. NFS predicts incident diabetes, and changes in NFS are associated with mortality. The tests are most useful to distinguish advanced (F3-4) vs. non-advanced fibrosis (F0-2). They not reliably differentiate fibrosis stages F1 from F2 or F0 from F1 (Guha 2008). The negative predictive values (NPVs) for excluding advanced fibrosis are higher than the positive predictive values (PPVs) (Guha 2008; McPherson 2013); thus the EASL recommends to use the non-invasive tests for first-line risk stratification to exclude severe fibrosis (EASL 2016).

Similarly, transient elastography is also an acceptable non-invasive technique to exclude advanced fibrosis and cirrhosis (EASL 2016). A meta-analysis showed that transient elastography has a high sensitivity and specificity for identifying fibrosis in NAFLD patients (Gambino 2011) although it has some problems in individuals with a very high BMI (Chalasani 2012). These problems have partly been solved with a new probe developed for such very obese patients. The technique has been approved by the FDA, but is still not reimbursed in many countries.

The combination of non-invasive scores and transient elastography adds to the diagnostic accuracy of the use of the single procedures and might save diagnostic liver biopsies (EASL 2016). The German NAFLD guidelines recommend to first determine the NFS (Roeb 2015). If its value is lower than -1.455 no other test has to be done since advanced fibrosis is very unlikely. If the NFS is higher than -1.455 transient elastography should be done. If the transient elastography value is higher than 9.6 kPa advanced fibrosis

is reliably confirmed and liver biopsy is not necessary any longer. Liver biopsy is only recommended if transient elastography values are between 7.9 and 9.6 kPa.

Diet, physical exercise and lifestyle recommendations

Table 3. Treatment options for NASH (modified from Chalasani 2012 and Gao 2014)

Moderate weight loss induced by dietary changes and/or physical exercise
Abstinence from major alcohol consumption
Good control of diabetes mellitus
Pioglitazone or vitamin E may be given with caution
Liraglutide may be used when diabetes mellitus type 2 and/or obesity are also present
Surgery for massive obesity (e.g., gastric bypass surgery)
Liver transplantation

Weight loss generally reduces hepatic steatosis, achieved either by hypocaloric diet alone or in conjunction with increased physical activity (Chalasani 2012) (Table 3). Loss of at least 3-5% of body weight appears necessary to improve steatosis, but a greater weight loss (up to 10%) may be needed to improve necroinflammation. Exercise alone in adults with NAFLD may reduce hepatic steatosis but its ability to improve other aspects of liver histology remains unknown (Chalasani 2012). Several studies have shown that rapid weight loss (very low calorie diet or starving) increases the risk of progression of liver disease and even liver failure (Grattagliano 2000, James 1998, Neuschwander-Tetri 2003). Patients should therefore be educated not to induce rapid weight loss, but to aim at a weight loss of less than 10% of their body weight over 6-12 months (Okita 2001). It is unclear whether special diets are helpful; probably it is more important that the patients simply eat healthy foods like vegetables and fruits, rich in fibre and complex carbohydrates with a low glycemic index; they should avoid meat, saturated fat and products with less complex carbohydrates. Lifestyle modifications should include an increase in physical activity and sports.

There have been several studies, which further evaluated different modes of weight loss due to dietary changes and exercise on fatty liver disease:

A randomised crossover 6-week dietary intervention study examined the effect of a Mediterranean diet (high in monounsaturated fatty acids) on steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease (Ryan 2013). The Mediterranean diet reduced hepatic steatosis and

improved insulin sensitivity (Ryan 2013). Another randomised factorial 2 × 2, parallel-group study evaluated the effects of aerobic exercise training and dietary changes on liver fat content in patients with type 2 diabetes (Bozetto 2012): 1) high-carbohydrate/high-fibre/low-glycaemic index diet (CHO/fibre group), 2) high-MUFA diet (MUFA group), 3) high-carbohydrate/high-fibre/low-glycaemic index diet plus physical activity programme (CHO/fibre + Ex group), and 4) high-MUFA diet plus physical activity programme (MUFA + Ex group). Liver fat content decreased more in MUFA (-29%) and MUFA + Ex (-25%) groups than in CHO/fibre (-4%) and CHO/fibre + Ex groups (-6%). Statistics showed a significant effect on liver fat for diet, with no effects for exercise training or diet-exercise interaction. Thus, an isocaloric diet enriched in MUFA compared with a diet higher in carbohydrate and fibre was associated with a reduction of hepatic fat content in type 2 diabetic patients independent of an aerobic training programme and should be considered for the nutritional management of hepatic steatosis in people with type 2 diabetes (Bozetto 2012).

The effects 16 weeks of exercise training were compared with an observation group in a randomised trial (Sullivan 2012). Exercise training resulted in a decrease in hepatic triglyceride content by about 10%, but did not change total body weight or percent body fat. Another randomised controlled trial from Japan evaluated the effect of calorie restriction-induced weight loss with or without aerobic exercise on liver fat content in subjects with visceral adiposity (Yoshimura 2014). Both calorie restriction-induced weight loss without aerobic exercise as well as calorie restriction-induced weight loss with aerobic exercise reduced liver fat content; however, there was no additive effect of exercise training. Another randomised controlled intervention trial compared the effects of 4-months aerobic or resistance training on hepatic fat content in type 2 diabetic subjects with NAFLD (Bacchi 2013). After the training, hepatic fat content was markedly reduced, to a similar extent, in both the aerobic and resistance training groups (mean relative reduction from baseline 32.8% vs. 25.9%). In addition, hepatic steatosis (fat content >5%) disappeared in about one-quarter of the patients in each intervention group. Insulin sensitivity during euglycemic clamp was increased, while total body fat mass and haemoglobin A1c levels were reduced comparably in both intervention groups (Bacchi 2013). Thus, resistance training and aerobic training are equally effective in reducing hepatic fat content among type 2 diabetic patients with NAFLD. One other study examined the effects of aerobic versus resistance exercise without caloric restriction on abdominal adiposity, ectopic fat, and insulin sensitivity in 45 adolescent boys (Lee 2012). Both aerobic and resistance training prevented the significant weight gain observed in control subjects. Compared with controls, total and visceral fat and intrahepatic lipid were reduced in both exercise groups. Both exercise programmes also improved

insulin sensitivity (Lee 2012). A further study showed that energy-matched moderate and high intensity exercise training improves nonalcoholic fatty liver disease risk independent of changes in body mass or abdominal adiposity (Winn 2018).

Alcohol and coffee

Several studies suggest a beneficial effect of light alcohol consumption (on average less than one drink per day) on the presence and severity of NAFLD (Suzuki 2007, Dunn 2008, Gunji 2009, Cotrim 2009, Dunn 2009, Moriya 2011). There are no studies reporting the effect of ongoing alcohol consumption on disease severity or natural history of NAFLD or NASH (Chalasanani 2012). The effects of light drinking on the cardiovascular system and cancer risks, if any, have not been investigated in individuals with NAFLD. Heavier alcohol consumption is certainly harmful also in obese patients (further literature in Chalasanani 2012).

With the results of studies, coffee consumption does not need to be limited and may even have a positive impact on the development of liver fibrosis (Molloy 2012, Birerdinc 2012, Catalano 2010).

Pharmacological treatment

As yet, no drug has been approved by FDA or EMA to treat NASH. However, the 2012 US guidelines (Chalasanani 2012) recommend that vitamin E and/or pioglitazone may be given in some patients for treatment of NASH. These recommendations are based in particular on two NIH-sponsored, randomised controlled clinical trials (RCTs) with vitamin E and pioglitazone, the PIVENS and the TONIC trial (Sanyal 2010, Lavine 2011).

Glitazones. The PIVENS study was a large multicentre RCT that randomised 247 non-diabetic patients with NASH to pioglitazone (30 mg/day), vitamin E (800 IU/day) or placebo for 24 months (Sanyal 2010). The primary endpoint was an improvement in >2 NAS points with at least 1 point improvement in hepatocellular ballooning and a 1 point improvement in either the lobular inflammation or steatosis score, and no increase in the fibrosis score. This goal was achieved in 19% of the placebo patients compared to 34% of the pioglitazone-treated patients ($p=0.04$ vs. placebo) and in 43% of the vitamin E-treated patients ($p=0.001$ vs. placebo). Because the study consisted of two primary comparisons (pioglitazone vs. placebo and vitamin E vs. placebo), a p -value of 0.025 was considered to be significant a priori. Therefore vitamin E but not pioglitazone met the primary endpoint although there were some histological benefits associated with pioglitazone

(Sanyal 2010). It is noteworthy that pioglitazone was associated with a 4.7 kg weight gain compared to placebo ($p < 0.001$). A meta-analysis including 5 RCTs showed that pioglitazone significantly improved steatosis and inflammation, but not fibrosis (Boettcher 2012). Other studies also suggest that pioglitazone improves histological inflammation and fibrosis, and ameliorates cardio-metabolic endpoints in patients not responding to lifestyle intervention (Musso 2012, Chalasani 2012). The other large multicentre RCT, the TONIC study, used the sustained reduction of ALT as the primary endpoint and a change in histology as secondary endpoint (Lavine 2011). The TONIC study compared the efficacy of vitamin E or metformin to placebo for treatment of nonalcoholic fatty liver disease in children and adolescents (8-17 years of age). Although the primary outcome of a reduction of ALT was not different among the three groups, there was a significant improvement in histology ($p < 0.006$) with vitamin E treatment compared to placebo over 96 weeks. In this study, metformin administered at 500 mg twice daily had no effect on aminotransferases and histology (Lavine 2011).

Vitamin E. The US guidelines (Chalasani 2012) state that vitamin E at a daily dose of 800 IU/day improves histology in non-diabetic adults with biopsy-proven NASH and should be considered as first-line treatment. It is also mentioned that vitamin E is not recommended to treat NASH in diabetic patients, NAFLD without liver biopsy, NASH cirrhosis or cryptogenic cirrhosis until further data supporting its effectiveness become available. In addition, the guidelines discuss the controversy as to whether vitamin E increases cancer risks (Chalasani 2012). According to the same guideline (Chalasani 2012) pioglitazone can be used in patients with biopsy-proven NASH. However, it needs be noted that the majority of patients who participated in pioglitazone trials were non-diabetic and that long-term safety and efficacy of pioglitazone in patients with NASH is not established.

Metformin and ursodeoxycholic acid (UDC). Metformin and UDC should not be used for treatment of NASH according to current US guidelines (further literature in Chalasani 2012). Obeticholic acid (OCA) is a semisynthetic derivative of the primary human bile acid chenodeoxycholic acid, the natural agonist of the farnesoid X receptor, which is a nuclear hormone receptor that regulates glucose and lipid metabolism. A double-blind placebo-controlled proof-of-concept study evaluated the effects of OCA on insulin sensitivity in patients with nonalcoholic fatty liver disease and type 2 diabetes mellitus (Mudaliar 2013). OCA treatment for 6 weeks increased insulin sensitivity and reduced markers of liver inflammation and fibrosis in patients with type 2 diabetes mellitus and nonalcoholic fatty liver disease (Mudaliar 2013). However pruritus is a frequent adverse event challenging long term adherence.

Drugs to induce weight loss. In general, all drugs that induce weight loss might be beneficial in NAFLD and NASH, in particular when diet and

lifestyle modification do not work (Table 2). Both sibutramine and orlistat have shown to improve some characteristics of NAFLD and NASH such as the sonographic degree of liver steatosis as well as the histological degree of steatosis and fibrosis (Sabuncu 2003, Derosa 2004, Hussein 2007, Harrison 2007). All the latter agents are not approved for use in NASH and NAFLD. Liraglutide is also approved for obese patients for weight loss, but is not reimbursed for that indication in many countries. The GLP1 analogues however may have a beneficial effect on NAFLD and NASH as well (see following paragraph).

Antioxidants. Antioxidants and cytoprotective agents have also been proposed to treat NAFLD and NASH including vitamin E, vitamin C, vitamin D, pentoxifylline, glutathione, betaine, N-acetylcysteine, S-adenosyl-L-methionine and ursodeoxycholic acid. In a Cochrane analysis, none of these agents showed significant benefit in validated randomised studies (Lirussi 2007). Vitamin D deficiency has been proposed to be involved in the pathogenesis of NASH, and studies proposed that vitamin D supplementation may be useful for treatment of NASH (Barchetta 2012, Roth 2012). There is also one randomised controlled trial suggesting that pentoxifylline might be useful for therapy of non-alcoholic fatty liver disease (Zein 2011). However, larger randomised controlled trials are needed to prove a beneficial role for pentoxifylline in NASH.

Ezetimibe. An open-label randomised controlled clinical trial investigated the efficacy of ezetimibe on NAFLD pathology and insulin sensitivity (Takeshita 2014). The fibrosis stage and ballooning score were significantly improved by ezetimibe. However, ezetimibe treatment significantly increased HbA1c and was associated with a significant increase in hepatic long-chain fatty acids. These findings shed light on previously unrecognised actions of ezetimibe that should be examined further in future studies (Takeshita 2014).

Silybinin. A multicentre, phase 3, double-blind clinical trial assessed the effects of reasil (a silybin phytosome complex consisting of silybin plus phosphatidylcholine, coformulated with vitamin E) versus placebo in patients with histologically documented NAFLD (Loguercio 2012). Patients receiving reasil for 12 months showed significant improvements in liver enzymes, HOMA and liver histology when compared to placebo.

Drug recommendations in guidelines. The Chinese NAFLD guidelines (Gao 2013), the Japanese guidelines (Watanabe 2015), the German guidelines (Roeb 2015) and the informal EASL background information (www.easl.eu) do not support the routine use of any pharmacological agent for NAFLD/NASH patients in view of the available studies. The Chinese guidelines state that there is still insufficient evidence supporting the use of antioxidants and hepatoprotective medications as routine treatment for NAFLD and NASH. Agents included are polyene phosphatidylcholine,

vitamin E, silymarin, adenosylmethionine and reduced glutathione. Of all the medications, vitamin E administered at a daily dose of 800 IU/day may be considered as the best liver protectant. According to the Chinese guidelines (Gao 2013) pioglitazone can also be used for the treatment of steatohepatitis in NASH patients. However, there is also a higher rate of congestive heart failure, weight gain and oedema in patients treated with pioglitazone when compared with controls, and the long-term safety and efficacy of pioglitazone in patients with NASH remain uncertain.

Novel pharmacological approaches

Interestingly, NAFLD and NASH are now also the focus of large pharmaceutical companies like Roche, BMS, NGM, MSD and Gilead which are sponsoring studies with interesting new compounds (Ratziu 2012, Stefan 2014). As of April 2018, four drugs are being studied in phase 3 trial and more than 30 in phase 2 trials (www.clinicaltrials.gov).

Inhibition of 11β -hydroxysteroid dehydrogenase type 1. A randomised controlled trial showed that inhibition of 11β -hydroxysteroid dehydrogenase type 1 (11β -HSD1, also known as HSD11B1) by RO5093151 (a Roche agent) reduced liver-fat content in patients with this disorder (Stefan 2014). This study suggests that targeting of 11β -HSD1 might be a promising approach for the treatment of non-alcoholic fatty liver disease.

Caspase inhibitors. In nonalcoholic steatohepatitis (NASH), the extent of hepatocyte apoptosis correlates with disease severity. Reducing hepatocyte apoptosis with the selective caspase inhibitor GS-9450 (a Gilead agent) has a potential for altering the course of the liver disease. In a phase 3, double-blind study, 124 subjects with biopsy-proven NASH were randomised to placebo or various doses of GS-9450 for 4 weeks (Ratziu 2012). GS-9450 significantly reduced ALT levels in NASH patients (Ratziu 2012). Thus, selective caspase inhibitors may be a promising treatment option for NASH.

The pan-caspase protease inhibitor emricasan has been shown to inhibit apoptosis, inflammation and fibrosis in NASH models (Anstee 2019, Witek 2009). A preliminary report of a phase 2 clinical trial showed significantly decreased serum ALT and cCK18 levels in NAFLD patient (Shiffman 2015). The therapeutic effects of this drug have also been examined in various other fibrotic liver diseases where it has been shown to reduce hepatic venous pressure gradient (HVPG). A phase 2 trial in NASH patients with fibrosis (NCT02686762) is ongoing to evaluate the efficacy and safety of emricasan (10 mg and 100 mg/d for 72 wk) with a special focus on fibrosis without worsening of NASH.

Glucagon-like peptide-1 (GLP-1) analogues. Glucagon-like peptide-1

(GLP-1) analogues reduce hepatic steatosis, concentrations of liver enzymes and insulin resistance in murine models of fatty liver disease. These analogues are licensed for type 2 diabetes and obesity, but their efficacy in patients with NASH was unknown. A multicentre, double-blinded, randomised, placebo-controlled phase 2 trial from four UK centres assessed the effects of s.c. injections of liraglutide (1.8 mg daily) compared with placebo for patients who were overweight and had clinical evidence of NASH (Armstrong 2015). Between 2010-2013, 26 patients were randomly assigned to receive liraglutide and 26 to placebo (Armstrong 2015). Nine (39%) of 23 patients who received liraglutide and underwent end-of-treatment liver biopsy had resolution of NASH compared with two (9%) of 22 such patients in the placebo group (relative risk [RR] 4.3; $p=0.019$). Two (9%) of 23 patients in the liraglutide group versus eight (36%) of 22 patients in the placebo group had progression of fibrosis (RR 0.2; $p=0.04$) (Armstrong 2015). Most adverse events were grade 1 (mild) to grade 2 (moderate) in severity, transient, and similar in the two treatment groups for all organ classes and symptoms, with the exception of gastrointestinal disorders in 21 (81%) of 23 patients in the liraglutide group and 17 (65%) of 22 patients in the placebo group, which included diarrhoea (ten [38%] patients in the liraglutide group vs five [19%] in the placebo group), constipation (seven [27%] vs none), and loss of appetite (eight [31%] vs two [8%]). This study was now fully published in the *Lancet* in 2016 (Armstrong 2016). Liraglutide was safe, well tolerated, and often led to histological resolution of non-alcoholic steatohepatitis, warranting extensive, longer-term studies. Liraglutide has been approved in the US and the EU for treatment of both diabetes mellitus type 2 and for treatment of obesity. Many NASH patients also have diabetes mellitus type 2 and/or obesity. Thus, already today it is possible to treat such patients with liraglutide. In the author's opinion, liraglutide and similar GLP-1 analogues are the most promising pharmacological agents for treatment of NASH and may today already be used when NASH is associated with diabetes or obesity. This personal view is also supported by a meta-analysis (Carbone 2016) which analysed all original studies investigating treatment of adults with NAFLD using GLP-1 analogues. Key outcomes were a change in serum alanine transaminase (ALT), as a marker of liver inflammation, and improvement in disease status measured by imaging or histology. Four studies met all inclusion and exclusion criteria. There were a total of 136 participants with NAFLD and concomitant type 2 diabetes mellitus (T2DM). Meta-analysis (random-effects model) revealed a significant decrease in serum ALT following treatment (mean reduction 14.1 IU/L, $P<0.0001$). In two studies with imaging and tissue data, treatment was found to significantly reduce steatosis, inflammation, and fibrosis. The significant decrease in a key biochemical marker of hepatic inflammation following treatment with incretin-based therapies, as well as improvements in imaging and histology,

suggests these agents may be effective options for managing NAFLD with comorbid type 2 diabetes and/or obesity.

A randomised, placebo-controlled phase 2 trial (NCT0243711) analysed the efficacy and safety of another GLP1-analogue semaglutide in subjects with obesity without type 2 diabetes. Semaglutide induced mean weight losses of -6.0% (0.05 mg) to -13.8% (0.4 mg) (-2.3% with placebo) at week 52. The effect of semaglutide on liver enzymes in subjects with elevated baseline ALT was now evaluated in a post hoc sub-analysis from that trial (Newsome 2018). In subjects with obesity and high ALT, semaglutide at 0.2–0.4 mg once daily s.c. reduced ALT to an extent that was broadly comparable across weight loss categories, and resulted in dose-related ALT normalisation in up to 46% of subjects after 52 weeks. These data suggest a potential role for semaglutide in the treatment of NAFLD with elevated liver enzymes.

Farnesoid X nuclear receptor agonists. The farnesoid X receptor is a nuclear receptor which regulates bile, cholesterol, glucose and lipid metabolism (Pellicciari 2004, Ballestri 2016). These receptors act via multiple pathways inhibiting hepatic lipogenesis, gluconeogenesis, glycogenolysis, maintaining cholesterol balance, and improving insulin sensitivity (Fuchs 2012, Makri 2016). The bile acid derivative 6-ethylchenodeoxycholic acid (obeticholic acid) is a potent activator of the farnesoid X nuclear receptor that reduces liver fat and fibrosis in animal models of fatty liver disease. Obeticholic acid (OCA) is a farnesoid X receptor agonist. It is a synthetic derivative of natural bile acid chenodeoxycholic acid (CDCA); its potency is 100-times greater than that of CDCA. In various animal models, OCA has shown anti-inflammatory and anti-fibrotic properties and also improved insulin resistance and hepatic steatosis (Ali 2015). In an animal model, OCA was shown to reduce hepatic inflammation and fibrosis and also decreased intrahepatic vascular resistance and improved portal hypertension (Verbeke 2016). In an animal model with advanced cirrhosis, treatment with OCA was shown to reduce gut bacterial translocation from 78.3% to 33.3% ($p < 0.01$) indicating its effect in maintaining intestinal barrier integrity in a further animal model (Ubeda 2016).

A phase 2 multicentre, double-blind, placebo-controlled, parallel group, randomised clinical trial compared treatment with obeticholic acid given orally (25 mg daily) or placebo for 72 weeks at medical U.S. centres in patients with non-cirrhotic NASH (Neuschwander-Tetri 2015). The primary outcome was improvement in liver histology defined as a decrease in NASH activity score by at least 2 points without worsening of fibrosis from baseline to the end of treatment. A planned interim analysis of change in AST at 24 weeks undertaken before end-of-treatment (72 weeks) biopsies supported the decision to continue the trial (relative change in AST -24%, 95% CI -45 to -3). A planned interim analysis of the primary outcome showed improved efficacy of obeticholic acid ($p=0.0024$) and supported a decision

not to do end-of-treatment biopsies and end treatment early in 64 patients, but to continue the trial to obtain the 24-week post-treatment measures (Neuschwander-Tetri 2015). A total of 141 patients were randomly assigned to receive obeticholic acid and 142 to placebo. Fifty (45%) of 110 patients in the obeticholic acid group who were meant to have biopsies at baseline and 72 weeks had improved liver histology compared with 23 (21%) of 109 such patients in the placebo group (RR 1.9; $p=0.0002$); 33 (23%) of 141 patients in the obeticholic acid developed pruritus compared with nine (6%) of 142 in the placebo group (Neuschwander-Tetri 2015). Also, total cholesterol and LDL cholesterol were significantly increased in the obeticholic acid group when compared with baseline and placebo, while HDL cholesterol was decreased (Neuschwander-Tetri 2015). This lipid profile may be a significant problem using obeticholic acid in NASH since most NASH patients die from cardiovascular and not from liver complications (Pisto 2014, Treeprasertsuk 2013, Hafliadottir 2014, Kim 2013). Nevertheless, a large phase 3 trial is ongoing.

Various further experimental and clinical trials evaluate other FXR agonists such as GW4064, PX20606, GS-9674 and INT-767. In animal models, these agonists have been shown to improve histological features of NASH such as steatosis and fibrosis (Gege 2014, Carino 2017, Haga 2017, Schwable 2017). Further clinical trials will probably follow.

Polyunsaturated fatty acids. A study by Sanyal et al. (2014) analysed the effect of n-3 polyunsaturated fatty acids (ethyl-eicosapentanoic acid = EPA-E) which are known to reduce insulin resistance, lipogenesis and inflammation. This phase 2b multicentre, prospective, double-blind, randomised, placebo-controlled trial at 37 sites in North America included subjects with NASH and NAFLD activity scores ≥ 4 . A total of 243 subjects were randomly assigned to groups given placebo ($n = 75$), low-dosage EPA-E (1800 mg/d; $n = 82$), or high-dosage EPA-E (2700 mg/d; $n = 86$) for 12 months. EPA-E had no significant effects on steatosis, inflammation, ballooning, fibrosis scores, and liver enzymes.

Phosphodiesterase-4 inhibitors. Another trial looked at the effects of the phosphodiesterase-4 inhibitor ASP9831 in patients with NASH after it had shown potent anti-inflammatory and antifibrotic effects in preclinical studies. The phase 2 study evaluated the efficacy and safety of the phosphodiesterase-4 inhibitor ASP9831 in patients with NASH who were assigned randomly to groups given either 50 mg ($n = 33$) or 100 mg ($n = 33$) ASP9831 twice daily, or placebo ($n = 30$) for 12 weeks. After 12 weeks there was no significant change in mean ALT or AST or other biomarkers in any group.

p38 MAPK inhibitors. Chronic inflammation contributes to progression of NAFLD and NASH. The p38 mitogen activated kinases (p38 MAPK) promotes inflammation in the liver (Sabio 2010a, Sabio 2010b,

Sabio 2014). Some p38 MAPK isoforms have been shown to contribute to the development of NAFLD and NASH in animal models of fatty liver disease (Gonzalez-Teran 2013, Risco 2012). Human studies have also shown that the hepatic expression of p38 protein is increased in obese individuals with steatosis. Deletion of p38-c and -d in myeloid cells prevented neutrophil migration to the liver and protected these animals against diet-induced steatosis and inflammation (Gonzalez-Teran 2016). Therefore, p38 MAPK may be a promising target for therapy of NAFLD and NASH.

PPAR- α and - δ agonists (elafibranor). The peroxisome proliferator-activated receptors (PPARs) are a family of nuclear receptors that function as transcription factors regulating the expression of various genes thereby regulating e.g. cellular differentiation, development, and metabolism of carbohydrates, lipids, and proteins (Michalik 2006, Dunning 2014). PPAR- α which is mainly expressed in liver regulates many aspects of lipid metabolism. PPAR- δ which is present in various tissues is involved in fatty acid oxidation and insulin sensitivity. In animal models, PPAR has been shown to be hepato-protective by reducing lipid accumulation, inflammation, and fibrosis (Staele 2013, Montagner 2016, Piccinin 2016). In a randomised clinical trial (NCT01694849), a daily dose of 80 or 120 mg elafibranor or placebo was given to non-cirrhotic NASH patients for 52 weeks (Ratziu 2016). The study failed to meet the primary endpoint of resolution of NASH without worsening of fibrosis. However, it was observed that patients with an initial NAS of ≥ 4 treated with 120 mg/d elafibranor showed a significant improvement in liver inflammation. Currently the efficacy of elafibranor is analysed in a phase 3 clinical trial (NCT02704403) in patients with NASH and fibrosis.

NOX-1/4 inhibitors. The NADPH oxidase (NOX) catalyses the production of ROS (Aoyama 2012). In animal models induction of these enzymes by activated hepatic stellate cells promote fibrosis and inflammation (Paik 2011). In a murine model, the NOX-1/4 inhibitor GKT137831 decreased ROS production and fibrotic gene expression and probably thereby reduced hepatic inflammation and fibrosis (Aoyama 2012). Thus, NOX-1/4 inhibitors may have a beneficial effect on liver fibrosis in NAFLD and NASH.

Galectin-3 antagonists. Galectins bind to terminal galactose residues on glycoproteins and are usually expressed in immune cells. Their expression is increased in the presence of inflammation and fibrosis (DiLella 2011, Henderson 2009). Galectin-3 knockout mice show reduced hepatic fibrosis after liver injury. GR-MD-02, a galectin-3 inhibitor, has shown a decrease fibrosis, hepatic steatosis and collagen deposition in various animal models with NASH (Traber 2013). A randomised clinical study analysed the efficacy and safety of the galectin-3 inhibitor GR-MD-02 vs. placebo in patients with NASH and advanced fibrosis (Harrison 2016). GR-MD-02 was safe and well tolerated with evidence of a pharmacodynamic effect. These results provide

support for a Phase 2 development programme in advanced fibrosis due to NASH (Harrison 2016).

Acetyl CoA carboxylase inhibitor. Malonyl coenzyme A plays an important role in fatty acid metabolism in maintaining a balance between lipogenesis and lipid oxidation (Foster 2012). It promotes fatty acid synthesis and inhibits β -oxidation of lipids. Malonyl CoA is generated from acetyl CoA and the key enzyme regulating this process is acetyl CoA carboxylase (ACC). Therefore, inhibiting ACC prevents fatty acid synthesis and promotes its oxidation. In a murine model of NAFLD, inhibition of ACC has been shown to decrease hepatic steatosis, lipogenesis and increase insulin sensitivity and fatty acid oxidation (Foster 2012). Administration of the ACC isozyme 1 and 2 inhibitor ND-630 to diet-induced obese rats and Zucker diabetic rats reduced hepatic steatosis, lowered hemoglobin A1C (0.9% reduction), and improved insulin sensitivity (Harriman 2016). Also in a crossover, randomised, double-blind trial, administration of a single dose of NDI-010976 (a highly potent and selective inhibitor of both ACC1 and ACC2) to overweight/obese subjects inhibited *de novo* lipogenesis in a dose dependent manner (Stiede 2017). Together, all these results suggest its usefulness in treating metabolic syndrome, type 2 diabetes mellitus, and fatty liver disease. Thus, large long term clinical trials in humans are needed.

FGF-21 and FGF-19 analogues. The fibroblast growth factors (FGF) are a family of cell signaling proteins that are involved in a wide variety of processes, most notably as crucial elements for normal development (Burgess 1989). The fibroblast growth factor 21 (FGF-21) is secreted mainly by the liver. It is a starvation-induced peptide hormone with pleiotropic effects whose levels are mainly increased during fasting (Inagaki 2015, Nies 2016). FGF-21 concentrations have been reported to be increased in patients with NAFLD. A reduction of FGF-21 was associated with a metabolic worsening in an animal model of NASH (Liu 2016). FGF21 expression in the liver was reported to be increased in NASH patients (Gallego-Duran 2018). The hepatokine FGF21 is by the liver and regulates sugar intake and preferences for sweet foods via signaling through FGF21 receptors in the suprachiasmatic nucleus and paraventricular nucleus of the hypothalamus as well as the nucleus accumbens and ventral tegmental area (Talukdar 2016, von Holstein-Rathlou 2016, Soberg 2017). Treatment with the FGF-21 analogue BMS-986036 was reported to improve insulin sensitivity, hepatic steatosis and to decrease lipogenesis (Mu 2012). In another animal model of NASH the FGF-21 variant LY240531 was shown to increase fatty acid oxidation by enhancing hepatic mitochondrial oxygen consumption (Liu 2016). Also, various inflammatory markers and AST and ALT levels were reduced, suggesting an attenuation of liver injury (Lee 2016). In a randomised, double-blind, placebo-controlled phase II trial (NCT02413372) (Sanyal 2017)

patients with NASH and stage 1-3 fibrosis received the pegylated FGF21 BMS-986036 at 10 mg/day or 20 mg/week for 16 weeks; BMS-986036 dose-dependently reduced the hepatic fat fraction when compared with placebo and was also associated with improvements of adiponectin serum Pro-C3, liver stiffness, lipids, ALT, and AST. The drug was well tolerated without SAEs, discontinuations or deaths (Sanyal 2017). In another phase 2 study of obese patients with type 2 diabetes mellitus, BMS-986036 improved insulin sensitivity and lipids (Charles 2017). An integrated safety analysis of phase 2 studies showed that BMS-986036 was generally safe and well tolerated, supporting further clinical trials to evaluate the safety and efficacy of BMS-986036 in NASH (Halegoua-Demarzi 2018).

The activation FXR in terminal ileum by bile acid induces FGF19 secretion which then decreases bile acid synthesis and gluconeogenesis (Nies 2016). This process results in the activation of the FGFR4 receptor which has a proliferative effect on hepatocytes with the potential for carcinogenesis (Wu 2010). FGF19 also has important metabolic effects affecting glucose and lipid metabolism when used in experimental mouse models (Tomlinson 2002, Fu 2004, Kir 2011). A randomised, double-blind, placebo-controlled phase 2 study (NCT02443116) assessed the safety and efficacy of the engineered FGF19 analogue NGM282 for the treatment of NASH (Harrison 2018). Patients were randomly assigned (1:1) to receive either 3 mg or 6 mg s.c. NGM282 or placebo. Primary endpoint was the absolute change from baseline to week 12 in liver fat content measured by MRI. NGM282 resulted in a rapid and significant reduction of liver fat content with an “acceptable” safety profile in patients with NASH. Further studies of NGM282 are warranted in this patient population (Harrison 2018).

CCR2 and CCR5 inhibitor (cenicriviroc). CCR2 and CCR5 are chemokine receptors which are mainly expressed in immune cells such as monocytes, macrophages, Kupffer cells, natural killer cells, and T cells. They also stimulate hepatic stellate and may thereby initiate fibrosis. Cenicriviroc (CVC) acts as an inhibitor of both the CCR2 and the CCR5 receptor. CVC has been shown to decrease fibrosis and inflammation in various animal models of diet-induced NASH or substance-induced NASH (Lefebvre 2013, Lefebvre 2016, Seki 2009). The ongoing CENTAUR trial (NCT02217475) analyses the efficacy and safety of CVC in NASH patients with fibrosis [255]. Preliminary results after 1 year on study drug have been published (Friedman 2018) and data after 2 years have been shown at the EASL meeting in 2018 (Ratzi 2018). After 1 year of CVC treatment, twice as many subjects achieved improvement in fibrosis without worsening of NASH when compared with placebo (Friedman 2018). A majority of subjects achieving ≥ 1 stage fibrosis improvement at year 1 maintained that benefit at year 2 with CVC, with greater effect in those with advanced fibrosis (Ratziu 2018).

Inhibition of acetyl-coenzyme A carboxylase. *De novo* lipogenesis is an important factor that drives pathogenesis of NASH (Lambert 2014). The rate-limiting step of lipogenesis is catalysed by enzyme acetyl-coenzyme A carboxylase (ACC) (Fullerton 2013). Preclinical and early-phase human studies suggested that an inhibition of ACC may improve steatosis, liver biochemistry, inflammation, fibrosis, and insulin sensitivity (Harriman 2016, Bates 2017, Stiede 2017). More studies analysed the efficacy and safety of the ACC inhibitor GS-0976 in patients with NASH (Loomba 2017, Lawitz 2017). GS-0976 significantly improved liver steatosis and stiffness as well as fibrosis markers in patients with NASH (Loomba 2017, Lawitz 2017).

SCD-1 inhibitors. Stearoyl-CoA desaturase (Δ -9-desaturase) (SCD) catalyses the rate-limiting step in the formation of monounsaturated fatty acids (Paton 2017). SCD-1 is an important metabolic control point. Inhibition of its expression may improve the treatment of a variety of metabolic diseases (Flowers 2017). The SCD1 inhibitor aramchol improved hepatic fat accumulation by decreasing lipogenesis and increasing fatty acid oxidation (Safadi 2014). This drug was found to significantly decrease liver fat content in NAFLD patients when given for 3 months; the effect of the drug on fibrosis was not determined (Safadi 2014). A phase 2 clinical trial of this drug is ongoing to assess the efficacy and safety of aramchol in NASH patients with fibrosis (NCT02279524).

Lysyl oxidase-like 2 inhibitor. Lysyl oxidase-like 2 inhibitors cause cross linkage of collagen and thereby reduce its degradation (Moon 2014). The lysyl oxidase-like 2 enzyme had been shown to promote fibrosis in various liver diseases. Simtuzumab, a monoclonal antibody of lysyl oxidase-like 2, decreased liver fibrosis in animal NASH models (Barry-Hamilton 2017). Two trials examine the efficacy of this drug for decreasing fibrosis and preventing progression to cirrhosis in such patients (NCT01672866, NCT01672879). One of the trials failed to show an effect of simtuzumab on liver fibrosis in NASH patients (Loomba 2017).

Apoptosis signal regulating kinase 1 (ASK1). Apoptosis signal regulating kinase 1 (ASK1) promotes apoptosis and fibrosis induced by hyperglycaemia, TGF- β or ROS. ASK1 is activated in patients with NASH. In animal models of NASH the ASK1 inhibitor GS4997 reduced hepatic steatosis, fibrosis, body weight, fasting blood glucose, insulin resistance, lipogenesis, cholesterol biosynthesis, and plasma AST/ALT levels (Karnik 2014, Karnik 2015, Xiang 2016). A phase 2 study (NCT02466516) evaluated the safety and efficacy of selonsertib, a selective inhibitor of apoptosis signal-regulating kinase 1, alone or in combination with simtuzumab, in patients with NASH and stage 2-3 fibrosis (Loomba 2017). Due to the lack of effect of simtuzumab on histology, selonsertib groups with and without simtuzumab were pooled. After 24 weeks of treatment, the proportion of patients with a ≥ 1 stage reduction of fibrosis in the 18-mg selonsertib group

was 13 of 30 (43%; 95% confidence interval, 26-63); in the 6-mg selonsertib group, 8 of 27 (30%; 95% confidence interval, 14-50); and in the simtuzumab-alone group, 2 of 10 (20%; 95% confidence interval, 3-56). Improvement in fibrosis was associated with reductions in MRI liver stiffness, collagen content and lobular inflammation on biopsy, as well as in improvement in serum biomarkers of apoptosis and necrosis. These findings suggest that selonsertib may reduce liver fibrosis in patients with nonalcoholic steatohepatitis and stage 2-3 fibrosis (Loomba 2017).

Pre-clinical data suggest that combinations of an ASK1 inhibitor with an ACC inhibitor or a FXR agonist are more effective than monotherapies. A study evaluated the safety and efficacy of these combinations in subjects with NASH. Patients with NASH received a 12-week treatment with the apoptosis-signal regulating kinase (ASK1) inhibitor (selonsertib) in combination with the acetyl-CoA carboxylase inhibitor (GS-0976) or the farnesoid X receptor agonist (GS-9674) (Lawitz 2018). These combinations were safe and led to improvements in hepatic steatosis, liver biochemistry, and fibrosis markers. Since the responses were similar with monotherapies, as yet only the safety data are promising for such combinations of drugs.

Sirtuins. Sirtuins (SIRT) are information regulator proteins. SIRT-1, one member of this protein family, has anti-inflammatory effects and increases insulin sensitivity (Morris 2013). A decreased liver expression of SIRT-1 was observed in an animal model of NAFLD (Colak 2011). Since the SIRT-1 activator resveratrol improved hepatic steatosis and insulin sensitivity (Li 2014), SIRT-1 may be a potential target for treatment of NASH and NAFLD.

Alterations of the intestinal microbiome

Several studies have demonstrated that probiotic strains, in particular those of the lactobacillus and bifidobacterium, exert beneficial effects in subjects with the metabolic syndrome (detailed literature in Ma 2013). Indeed, they seem to promote weight loss, reduce visceral adiposity, improve glucose tolerance and modulate low-grade intestinal inflammation (Ma 2013). In a randomised pilot study patients with histologically proven NASH were randomised to receive probiotics or usual care for 6 months (Wong 2013). Probiotics reduced liver fat and AST level in NASH patients. In another randomised, double-blind, placebo-controlled clinical studies in 52 patients with NAFLD synbiotic supplementation in addition to lifestyle modification was superior to lifestyle modification alone for the treatment of NAFLD, at least partially through attenuation of inflammatory markers (Eslamparast 2014).

Despite encouraging results from these studies and of a meta-analysis evaluating the role of probiotics for the treatment of NAFLD (Ma 2013) other meta-analyses of available randomised clinical trials are more cautious and do not recommend the use of probiotics for the treatment of obesity and fatty liver disease as yet (Millin 2012, Floch 2011). A systematic review analysed randomised clinical trials (RCTs) testing probiotics, prebiotics or both (synbiotics) in adult NAFLD patients (Buss 2014). After the screening process, 9 full-text articles were included in the review, but 6 studies were excluded for methodological problems. Three randomised controlled trials were finally included in the analysis. Patients in these three studies were randomised to receive different formulations of probiotics, synbiotics or placebo. Reductions in aminotransferases were observed in the treated group in 2 of the 3 studies. However, in one study reductions were also detected in the control group. This latest meta-analysis concludes that the current evidence precludes recommendations on the use of pre- and probiotics in clinical practice (Buss 2014). The guidelines also do not recommend such use of pre- and probiotics in clinical practice yet (Chalasanani 2012, Gao 2014).

A pilot human study evaluated the effects of infusing intestinal microbiota from lean donors to male recipients with metabolic syndrome on the recipients' microbiota composition and glucose metabolism. Subjects were assigned randomly to groups that were given small intestinal infusions of allogenic or autologous microbiota. Six weeks after infusion of microbiota from lean donors, insulin sensitivity of recipients increased along with levels of butyrate-producing intestinal microbiota. Intestinal microbiota might be developed as therapeutic agents to increase insulin sensitivity (Vrieze 2012).

Another pilot study explored the role of microbiota in pregnancy characterising faecal bacteria of 91 pregnant women of varying pre-pregnancy BMIs and gestational diabetes status and their infants (Koren 2012). Similarities between infant-mother microbiota increased with children's age, and the infant microbiota was unaffected by mother's health status. Gut microbiota changed dramatically from first (T1) to third (T3) trimesters, with an overall increase in proteobacteria and actinobacteria, and reduced richness. T3 stool showed strongest signs of inflammation and energy loss. When transferred to germ-free mice, T3 microbiota induced greater adiposity and insulin resistance compared to T1. These findings indicate that host-microbial interactions that impact host metabolism can occur and may have an impact in pregnancy (Koren 2012).

The composition of the intestinal microbiome determines the efficacy of energy harvest from food. Changes in dietary composition have been associated with changes in the composition of gut microbiota. The capacity to explore the microbiome was markedly improved by the development of metagenomic approach which has led to the first human gut microbial gene catalogue. This approach helps to stratifying individuals by their gut

genomic profile into different enterotypes. However, most previous analyses were carried out in non-intervention settings. A pilot study (Cotillard 2013) investigates the temporal relationships between food intake, gut microbiota and metabolic and inflammatory phenotypes, during diet-induced weight-loss and weight-stabilisation interventions in 38 obese and 11 overweight individuals. Individuals with reduced microbial gene richness (40%) showed a low-grade inflammation. Furthermore, dietary intervention improved the low gene richness and clinical phenotypes. Low gene richness may therefore have a potential as an efficacy marker of an intervention (Cotillard 2013).

It has been corroborated that an animal-based diet can rapidly increase the abundance of bile-tolerant micro-organisms (alisticipes, bilophilus and bacteroides) and may decrease the levels of firmicutes that metabolise dietary plant polysaccharides (roseburia, eubacterium rectale and ruminococcus bromii) (David 2014). Microbial activity mirrored differences between herbivorous and carnivorous mammals. This data demonstrates that the gut microbiome can rapidly respond to altered diet, potentially facilitating the diversity of human dietary lifestyles (David 2014).

In another study a murine model of high-fat diet-induced NAFLD was used to look at the effects of alterations in the intestinal microbiome on NAFLD characteristics (Jian 2014). Mice treated with antibiotics exhibited altered bile acid composition, with an increase in conjugated bile acid metabolites that inhibited intestinal farnesoid X receptor (FXR) signalling. Compared with control mice, animals with intestine-specific FXR disruption had reduced hepatic triglyceride accumulation in response to a high-fat diet. Other parts of this study demonstrated that inhibition of the intestinal FXR/ceramide axis mediates gut microbiota-associated NAFLD development, linking the microbiome, nuclear receptor signalling and NAFLD. This work suggests that inhibition of intestinal FXR is a potential therapeutic target for NAFLD treatment (Jiang 2014).

Surgery for obesity

Bariatric surgery has shown to improve NASH (Liu 2007, de Almeida 2006, Furuya 2007). The US guidelines (Chalasani 2012) state that bariatric surgery is not contraindicated in otherwise eligible obese individuals with NAFLD or NASH.

Until recently there were no randomised studies evaluating bariatric surgery in NAFLD and NASH patients. The first randomised controlled study compared the effects of a step 1 American Heart Association diet plus exercise and intragastric balloon placement to a step 1 American Heart Association diet plus exercise and sham intragastric balloon placement over a period of 6 months (Lee 2012). At 6 months, BMI and the nonalcoholic

fatty liver disease activity score were significantly lower in the intragastric balloon placement group when compared with the sham-treated group with an additional trend toward improvement in the steatosis score. There was no change in the median lobular inflammation, hepatocellular ballooning or fibrosis score in either group (Lee 2012). This being the only randomised study, it appears premature to recommend bariatric surgery as a standard option to treat NASH.

A number of long-term studies have been published all of which support the beneficial metabolic value of bariatric surgery some of them with a randomised controlled trial (RCT) design (Adams 2017, Kalinowski 2017, Klebanoff 2017, Pereli 2018, Schauer 2017).

Liver transplantation (LTX) for NASH

LTX is the final option for patients with end-stage liver disease due to cirrhosis and complications of portal hypertension with NASH. Due to the increase in the prevalence of NASH, there is also an increase in LTX due to end-stage liver disease caused by NASH (Burke 2004). Additional reports corroborate that NASH-related LTX has further increased (Wong 2014). However, NASH is still only the second or third leading cause of LTX in the U.S. (for further discussion and references see chapter on Natural History). NASH can recur after LTX, particularly if patients have previously undergone jejunioileal bypass surgery (Kim 1996, Requart 1995, Weston 1998, Contos 2001, Burke 2004). LTX does not cure the metabolic defect that causes NASH.

Follow-up of NAFLD and NASH patients

Patients with NASH cirrhosis should be screened for gastroesophageal varices and for hepatocellular cancer (HCC) (Chalasani 2012) according to current practice guidelines (Garcia-Tsao 2007, Bruix 2011). Although a HCC may occur even in NASH patients without cirrhosis, the relative paucity of such an event does probably not justify to also screen non-cirrhotic NASH patients for HCC. Current evidence does not support to routinely repeat liver biopsy in patients with NAFLD or NASH (Chalasani 2012).

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23. Wilson's disease

Claus Niederau

Introduction

In 1912, Kinnear Wilson was the first to describe an inherited lethal disease associated with progressive lenticular degeneration, chronic liver disease and cirrhosis (Wilson 1912). In the same year, Kayser and Fleischer detected that patients with Wilson's Disease (WD) often have brownish corneal copper deposits now called Kayser-Fleischer rings (Fleischer 1912).

WD is an autosomal recessive error of the metabolism. Its gene ATP7B encodes a copper-transporting ATPase (Bull 1993, Tanzi 1993, Petrukhin 1993, Yamaguchi 1993). The genetic defect of the ATP7B protein reduces biliary copper excretion leading to copper accumulation in the cornea and various organs including the liver, brain and kidney. The alteration of the ATP7B protein also reduces the incorporation of copper into ceruloplasmin. The corresponding presence of apoceruloplasmin (ceruloplasmin with no copper incorporation) leads to a decrease in circulating levels of ceruloplasmin due to the reduced half-life of the apoprotein. Thus, despite copper accumulation in many organs, circulating levels of copper and ceruloplasmin are decreased in most WD patients.

The prevalence of WD is rare, estimated at 3 per 100,000 in the general population (Fryzman 1990). The clinical presentation may vary. Some WD patients are diagnosed with liver problems while others present with neurologic or psychiatric symptoms; many patients show both hepatic and neurological disease (Figure 1). Episodes of hemolysis and renal abnormalities may also occur. WD typically affects children and younger adults, and is rarely seen in adults older than 40. WD is fatal unless appropriately treated. Drugs for treatment of WD are copper chelators such as penicillamine, and trientine (Walshe 1956). More recently, zinc has been used to reduce intestinal copper absorption and to detoxify free circulating copper. Patients with fulminant liver failure or decompensated cirrhosis may have to undergo liver transplantation (LTX), which cures WD.

Clinical presentation

Screening for WD is only useful in families with an affected member. In all other circumstances diagnostic procedures are only done when symptoms and findings suggest WD. These include liver disease,

neurological symptoms, renal abnormalities and episodes of hemolysis. WD is diagnosed in the vast majority of patients between the ages of 5 and 35. There are rare reports of patients diagnosed at ages 3–5 (Kalach 1993, Wilson 2000) and at ages of up to about 60 years (Gow 2000). Late-onset WD is a frequently overlooked condition (Ferenci 2007). Diagnostic workup does not rely on a single test but includes identification of corneal Kayser-Fleischer rings, reduced serum ceruloplasmin and copper as well as a quantitative determination of liver copper concentration (Scheinberg 1952, Walshe 1956, Saito 1987, Stremmel 1991, Roberts 2003) (Figure 2).

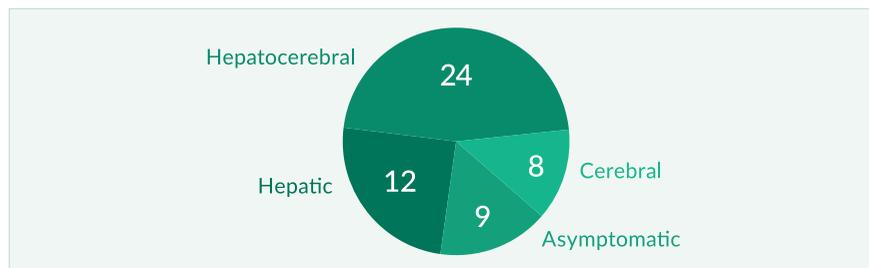


Figure 1. Clinical course of WD in 53 patients (modified from Stremmel 1991)

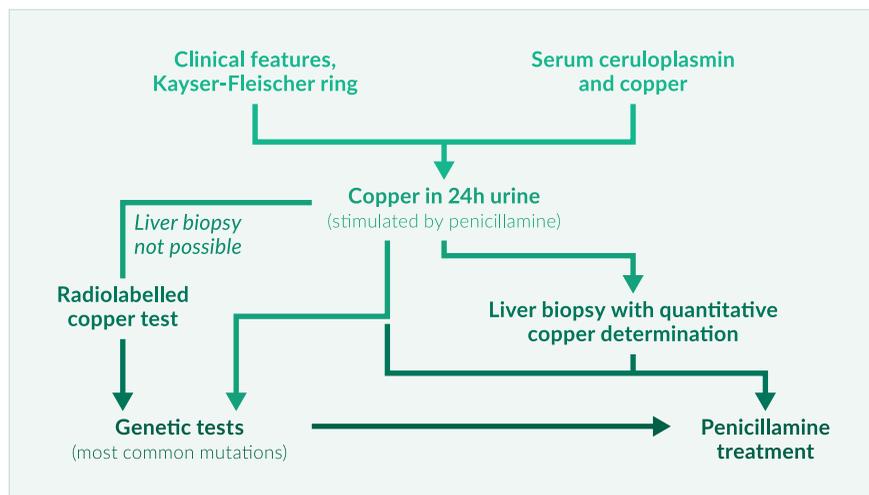


Figure 2. Diagnostic workup for WD

Genetic tests are usually only done in relatives of a confirmed WD patient. It is easy to diagnose WD in subjects who present with liver cirrhosis, typical neurologic manifestations and Kayser-Fleischer rings; many of these patients present at ages 5 to 35 and have decreased serum copper and ceruloplasmin (Sternlieb 1990). However, a considerable number of WD patients present only with liver disease and may not have

Kayser-Fleischer rings or decreased serum levels of ceruloplasmin (Steindl 1997). Under these circumstances diagnosis may be difficult; measurement of 24-hour urinary copper excretion often helps to support the suspicion of WD. Liver biopsy with measurement of quantitative copper concentration should be done to corroborate the diagnosis (Stremmel 1991, Roberts 2003).

In general, WD patients diagnosed primarily with liver disease are children and adolescents and are younger than those diagnosed due to neurological symptoms (Merle 2007). Many patients who present only with CNS symptoms are 20–40 years old. Patients with WD may present with a wide spectrum of liver disease ranging from asymptomatic elevation of serum aminotransferases to fulminant liver failure. Serum aminotransferases are elevated in most WD patients irrespective of age (Schilsky 1991). Other WD patients may present with findings and symptoms of autoimmune hepatitis including autoimmune antibodies and elevated IgG (Scott 1978, Milkiewicz 2000). The clinical picture might also resemble acute or chronic viral hepatitis, without the viral serum markers. Even liver histology is not predictive or typical for WD unless copper concentration is measured. Histological findings may range from fatty liver changes to severe necro-inflammatory and fibrotic disease and complete cirrhosis. In particular, children and adolescents with chronic active hepatitis of unknown aetiology or autoimmune hepatitis and adult patients with a suspicion of autoimmune hepatitis or non-response to immunosuppressants should be evaluated for WD (Roberts 2003).

WD has to be excluded in patients with fulminant liver failure of unknown aetiology, especially at ages under 35 years; WD patients with such presentation usually have some sort of liver disease (Rector 1984, Ferlan-Maroult 1999, Roberts 2003) associated with Coombs negative hemolytic anaemia and severely increased prothrombine time non-responsive to vitamin K and progressive renal failure (Sallie 1992). Some patients have bilirubin levels of more than 40 mg/dL while serum alkaline phosphatase is normal or just slightly elevated (Berman 1991). In contrast to many types of toxic liver failure, liver failure in WD usually does not start with high increases in aminotransferases. In many WD patients AST levels exceed ALT levels (Emre 2001, Berman 1991). In most cohorts, for unexplained reasons, the ratio of females to males is approximately 2:1 (Roberts 2003). Serum ceruloplasmin may be decreased while serum copper and 24-hour urinary excretion of copper is usually elevated. It is extremely helpful when one can identify Kayser-Fleischer rings in this situation; these patients need to be studied with a slit lamp by an experienced ophthalmologist. Patients with acute liver failure need a diagnostic workup as rapidly as possible; if there is a strong suspicion or diagnosis of WD, the patient should be transferred to a transplant centre the same day.

Neurological symptoms in WD often resemble those seen in Parkinson's

disease including tremor and rigor. Many patients report that symptoms start with problems in handwriting and dysarthria. Neurological symptoms may be associated with slight behavioural alterations, which may later proceed to manifest psychiatric disease including depression, anxiety and psychosis. With the progression of CNS involvement WD patients may develop seizures and pseudobulbar palsy associated with severe dysphagia, aspiration and pneumonia. Although many older WD patients present with neurological disease, the diagnostic workup often shows significant liver involvement or even complete liver cirrhosis.

Renal involvement of WD may present with aminoaciduria and nephrolithiasis (Azizi 1989, Nakada 1994, Cu 1996). There may be various other non-neurological and non-hepatic complications of WD such as osteoporosis and arthritis, cardiomyopathy, pancreatitis, hypoparathyroidism, and miscarriages (for literature see Roberts 2003).

Kayser-Fleischer rings are caused by corneal copper deposition (Figure 3). Sometimes, one can see the rings directly as a band of brown pigment close to the limbus. In other patients the ring can only be identified using a slit lamp. Very rarely similar rings may be seen in non-WD patients, e.g., in some patients with neonatal or chronic cholestasis (Tauber 1993). Kayser-Fleischer rings are detectable in 50–60% of WD patients in most large cohorts (Tauber 1993, Roberts 2003). Many young WD patients with liver disease do not have such rings (Giacchino 1997) whereas almost all patients with primarily neurologic symptoms do have them (Steindl 1997). WD patients may also have other less specific eye changes including sunflower cataracts (Cairns 1963). Kayser-Fleischer rings usually regress with chelation therapy or after LTX (Stremmel 1991, Schilsky 1994).

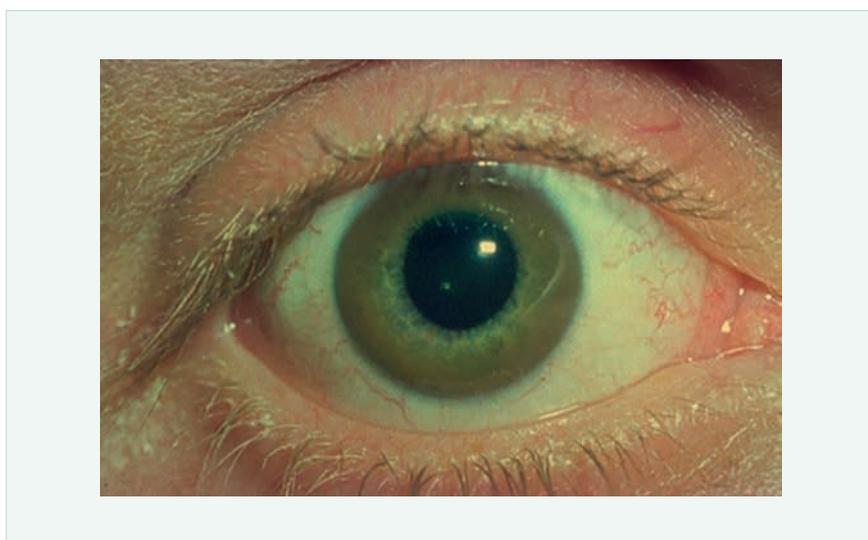


Figure 3. Kayser-Fleischer ring in a patient with WD

Diagnosis

Scoring system

Diagnosis of Wilson's disease may be difficult. Therefore, a scoring system has been established (Ferenci 2003) (Table 1) which is now recommended by recent EASL guidelines (EASL 2012) (Table 2).

Table 1. Scoring system – 8th International Meeting on Wilson's disease (Ferenci 2003)

KF rings	Absent 0		Present 2
Neurologic symptoms	Absent =0	Mild =1	Severe =2
Serum ceruloplasmin	>0.2 g/L =0	0.1–0.2 g/L =1	<0.1 g/L =2
Coombs negative hemolytic anaemia	Absent =0	Present=1	
Liver copper (in the absence of cholestasis)	0.8 μ mol/g =1	0.8–4 μ mol/g =1	>4 μ mol/g =2
Rhodanine positive granules	Absent =0	Present =1	
Urinary copper (in absence of acute hepatitis)	Normal =0 Normal =0	1–2x ULN =1 Normal, but >5xULN	>2x ULN =2 after D-penicillamine =2
Mutation analysis	None =0	1 chromosome =1	both chromosomes =4
Total score			
≥4:	Diagnosis established		
3:	Diagnosis possible, more tests needed		
2:	Diagnosis very unlikely		

Serum ceruloplasmin

Ceruloplasmin, the major circulating copper transporter, is synthesised and secreted mainly by hepatocytes. The 132 kd protein consists of six copper atoms per molecule of ceruloplasmin (holoceruloplasmin) while the remaining part of the protein does not carry copper (apoceruloplasmin). Ceruloplasmin acts as an acute phase reactant and may thus be increased by any inflammatory process; it may also rise in pregnancy and with the use of oestrogens and oral contraceptives. One also needs to remember that the normal range of serum ceruloplasmin is age-dependent: it is usually low in infants until the age of 6 months; in older children it may be somewhat higher than in adults. As explained previously, serum levels of ceruloplasmin are generally decreased in WD; however, this finding alone

is unreliable because low serum ceruloplasmin may be seen without WD and serum ceruloplasmin may even be increased in severe WD and liver failure. Non-specific reductions of ceruloplasmin are usually associated with protein deficiency or any end-stage liver disease. Long-term parenteral nutrition may also lead to decreased levels of ceruloplasmin. Low serum ceruloplasmin is also a hallmark of Menkes' disease, a very rare X-linked inborn error of metabolism that leads to a defect in copper transport due to mutations in ATP7A (Menkes 1999). Very rarely, one cannot measure serum ceruloplasmin at all. This aceruloplasminaemia is a very rare genetic disease caused by mutations in the ceruloplasmin gene; however, patients with aceruloplasminaemia develop iron and not copper overload (Harris 1998).

Most patients with WD have a serum ceruloplasmin lower than 20 µg/dL; this finding is diagnostic for WD however only when there are other findings such as a Kayser-Fleischer corneal ring. In one prospective screening study, ceruloplasmin was measured in 2867 patients presenting with liver disease: only 17 of them had reduced ceruloplasmin levels and only 1 of these subjects had WD (Cauza 1997). Thus decreased ceruloplasmin had a positive predictive value of only 6% in the 2867 patients tested. In two cohorts, about 20% of WD had normal ceruloplasmin and no Kayser-Fleischer rings (Steindl 1997, Gow 2000). Most reports, however, show that more than 90% of WD patients have a reduced serum ceruloplasmin (Walshe 1989, Lau 1990, Stremmel 1991). Measurement of ceruloplasmin as a single marker cannot reliably differentiate homozygotes from heterozygotes.

Serum copper

Corresponding to the decrease in serum ceruloplasmin, total serum copper is also usually decreased in WD. Similar to the diagnostic problems in interpreting ceruloplasmin data in WD patients with fulminant liver failure, serum copper may also be normal in this situation – even if serum ceruloplasmin is decreased. In acute liver failure, circulating copper may in fact be elevated because it is massively released from injured hepatocytes. If ceruloplasmin is reduced, a normal or elevated serum copper usually suggests that there is an increase in free serum copper (not bound to ceruloplasmin). The free copper concentration calculated from total copper and ceruloplasmin values has also been proposed as a diagnostic test and for monitoring of WD. It is elevated above 25 µg/dL in most untreated patients (normal values are below 10–15 µg/dL). The amount of copper associated with ceruloplasmin is 3.15 µg of copper per mg of ceruloplasmin. Thus free copper is the difference between the total serum copper in µg/dL and three times the ceruloplasmin concentration in mg/dL (Roberts 1998).

Increases in serum free copper, however, are not specific for WD and

can be seen in all kinds of acute liver failure as well as in marked cholestasis (Gross 1985, Martins 1992). Thus, serum copper is not recommended as a primary tool for diagnosis of Wilson's disease (Ferenci 2003, EASL 2012) (Table 2). Serum copper, however, is still recommended as a tool to monitor treatment (EASL 2012) (Table 3). The calculation of the free copper concentration critically depends on the adequacy of the methods used for measuring total serum copper and ceruloplasmin; often labs simply state that one of the tests is below a certain value, which makes it impossible to calculate the amount of free copper.

Urinary copper excretion

Most WD patients have an increase in urinary copper excretion above 100 µg/24 hours, which is thought to represent the increase in circulating free copper (not bound to ceruloplasmin). Some studies suggest that about 20% of WD patients may have 24-hr urinary copper excretion between 40–100 µg/24 h (Steindl 1997, Giacchino 1997, Gow 2000, Roberts 2003). However, some increase in urinary copper excretion can be found in severe cholestasis, chronic active hepatitis and autoimmune hepatitis (Frommer 1981). It has been suggested that urinary copper excretion stimulated by penicillamine may be more useful than the non-stimulating test. In children, 500 mg of oral penicillamine is usually given at the beginning and then at 12 hours during the 24-hour urine collection. All WD children looked at had levels above 1600 µg copper/24 h and all patients with other liver diseases, including autoimmune hepatitis and cholestatic liver disease, had lower values. It is not clear whether this test has a similar discriminative power in adults where it has been used in various modifications (Tu 1967, Frommer 1981).

Hepatic copper concentration

Hepatic copper content above 250 µg/g dry weight liver is still the gold standard for diagnosis of WD and is not seen in heterozygotes or other liver diseases with the exception of Indian childhood cirrhosis (Martins 1992). Biopsies (larger than 1 cm in length) for measurements of hepatic copper determination should be taken with a disposable Tru-Cut needle, placed dry in a copper-free container and shipped frozen (Song 2000, Roberts 2003).

Radiolabelled copper

In WD, incorporation of radiolabelled copper into ceruloplasmin is

significantly reduced. This test is rarely used because of the difficulty in obtaining the isotope and because of legal restrictions.

Liver biopsy findings

Histological findings in WD range from some steatosis and hepatocellular necrosis to the picture as seen in severe autoimmune hepatitis with fibrosis and cirrhosis. Patients diagnosed at a young age usually have extensive liver disease; older patients who first present with neurological symptoms often have abnormalities in liver biopsy as well (Stremmel 1991, Steindl 1997, Merle 2007). Detection of copper in hepatocytes, e.g. by staining with rhodamine using routine histochemistry, does not allow a diagnosis of WD (Geller 2000) (Figure 4).

Neurology and MRI of the CNS

Neurologic symptoms in WD include Parkinson's-like abnormalities with rigidity, tremor and dysarthria. In more severely affected patients there may be muscle spasms, contractures, dysphonia, and dysphagia. In patients with pronounced neurological symptoms, magnetic resonance imaging (MRI) often identifies abnormalities in basal ganglia such as hyperintensity on T2-weighted imaging (Aisen 1995, van Wassanaer 1996). MRI of the CNS is superior to computed tomography to diagnose WD.

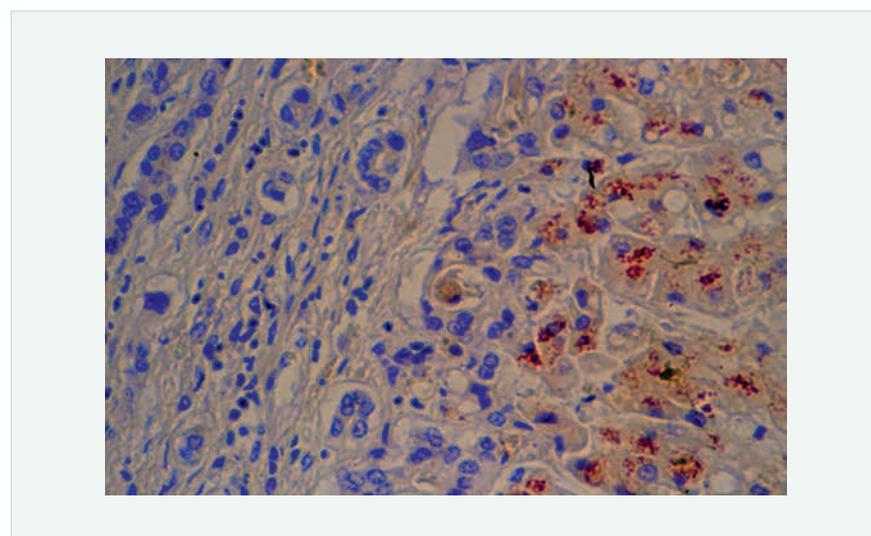


Figure 4. Liver histology (rhodamine staining for copper) in a WD patient

Genetic Studies

The use of mutation analysis in WD is limited by the fact that more than 200 ATP7B mutations have been described (see www.medgen.med.ualberta.ca/database.html). When the mutation is known in a specific patient, gene analysis may be useful for family screening or prenatal analysis (Thomas 1995, Shab 1997, Loudianos 1994). Some populations in Eastern Europe show predominance of the H1069Q mutation (for literature see Roberts 2003). Recently genetic analysis is recommended as a basic tool for diagnosis of Wilson's disease (Ferenci 2003, EASL 2012) (Table 2).

Table 2. EASL recommendations for diagnosis of Wilson's disease (EASL 2012)

Wilson's disease should be considered in any individual with liver abnormalities or neurological movement disorders of uncertain cause. Age alone should not be the basis for eliminating a diagnosis of Wilson's disease.
Kayser-Fleischer rings should be sought by slit-lamp examination by a skilled examiner. The absence of Kayser-Fleischer rings does not exclude the diagnosis of Wilson's disease, even in patients with predominantly neurological disease.
Neurologic evaluation and imaging of the brain, preferably by MR imaging, should be considered prior to treatment in all patients with neurologic Wilson's disease and should be part of the evaluation of all patients presenting with neurological symptoms consistent with Wilson's disease.
A low serum ceruloplasmin level should be taken as evidence for diagnosis of Wilson's disease. Borderline levels require further evaluation. Serum ceruloplasmin within the normal range does not exclude the diagnosis.
Basal 24-hour urinary excretion of copper >1.6 μmol is typical in symptomatic patients. In children with mild hepatic disease basal 24-hour urinary excretion of copper can only be mildly elevated or may even be normal.
Hepatic parenchymal copper content >4 $\mu\text{mol/g}$ dry weight provides critical diagnostic information and should be obtained in cases where the diagnosis is not straightforward and in younger patients. In untreated patients, normal hepatic copper content almost excludes Wilson's disease.
Whole-gene sequencing is currently possible and haplotype analysis should be the primary mode for Wilson's disease.

Treatment

Before 1948, all patients with Wilson's Disease died shortly after diagnosis. In 1948, intramuscular administration of the copper chelator BAL (dimercaprol) was introduced as the first treatment of WD (Cumming 1951, Denny-Brown 1951) followed by the oral chelators penicillamine (1955), trientine (1969) and tetrathiomolybdate (1984). Other treatment modalities include oral zinc salts (1961) and liver transplantation (1982). Today, most patients with WD remain on a lifelong pharmacologic therapy usually including a copper chelator and/or a zinc salt (Figure 5). LTX is reserved for

fulminant liver failure and irreversible decompensation of liver cirrhosis. Patients with a successful LTX do not need WD treatment because LTX heals the biochemical defect. Today, most physicians use oral chelators for initial treatment of symptomatic patients; many physicians start therapy with penicillamine while some prefer trientine. Both drugs are probably equally effective, with trientine having fewer side effects. In patients with advanced neurological disease some authors recommend tetrathiomolybdate for primary therapy. Combination therapy of chelators and zinc salts might have additive effects, acting on both urinary copper excretion and its intestinal absorption. After removal of most accumulated copper and regression of the most severe clinical problems the chelator dose may be reduced and later replaced by zinc. Patients presenting without symptoms may be treated with a low dose of a chelator or with a zinc salt from the beginning. Compliance problems have been shown to regularly cause recurrence of symptomatic WD and may lead to fulminant liver failure, need for LTX or death.

Recent EASL guidelines summarise the treatment recommendations for Wilson's disease (EASL 2012) (Table 3).

Table 3. Excerpts from the EASL recommendations for therapy of Wilson's disease (EASL 2012)

Initial treatment for symptomatic patients with Wilson's disease should include a chelating agent (D-penicillamine or trientine). Trientine may be better tolerated. Zinc may have a role in neurologically symptomatic patients. If zinc is used, careful monitoring of transaminases is needed, with changing to chelators if transaminases are increasing.
Treatment of presymptomatic patients or those with neurological disease on maintenance therapy can be accomplished with a chelating agent or with zinc.
Treatment is lifelong and should not be discontinued, unless liver transplantation is performed.
Patients should avoid foods and water with high concentrations of copper.
Patients with acute liver failure should be treated with liver transplantation when the revised King's score is 11 or higher.
Patients with decompensated cirrhosis, unresponsive to chelation treatment, should be evaluated promptly for liver transplantation.
Treatment for Wilson's disease should be continued during pregnancy, but dose reduction is advisable for D-penicillamine and trientine.
For routine monitoring, serum copper and ceruloplasmin, liver enzymes and function test, blood count and urine analysis as well as physical and neurological examinations should be performed at least twice annually.
The 24-hour urinary copper excretion on medication and after 2 days of cessation of therapy should be measured at least yearly. The estimated serum non-ceruloplasminbound copper may be a useful monitoring parameter.

Penicillamine. Penicillamine was the first oral copper chelator shown to be effective in WD (Walshe 1955). Total bioavailability of oral penicillamine

ranges between 40 and 70% (Bergstrom 1981). Many studies have shown that penicillamine reduces copper accumulation and provides clinical benefit in WD (Walshe 1973, Grand 1975, Sternlieb 1980). Signs of liver disease often regress during the initial 6 months of treatment. Non-compliance has been shown to cause progression of liver disease, liver failure, death and LTX (Scheinberg 1987). However, neurological symptoms may deteriorate at the start of penicillamine treatment; it remains controversial how often this neurological deterioration occurs and whether it is reversible; the rate of neurological worsening ranges from 10–50% in different cohorts (Brewer 1987, Walshe 1993). Some authors even recommend not using penicillamine in WD patients with neurological disease (Brewer 2006). Penicillamine is associated with many side effects that lead to its discontinuation in up to 30% of patients (for literature see Roberts 2003). An early sensitivity reaction may occur during the first three weeks including fever, cutaneous exanthema, lymphadenopathy, neutropenia, thrombocytopenia and proteinuria. In such early sensitivity, penicillamine should be replaced by trientine immediately. Nephrotoxicity is another frequent side effect of penicillamine, which occurs later and includes proteinuria and signs of tubular damage. In this case penicillamine should be immediately discontinued. Penicillamine may also cause a lupus-like syndrome with hematuria, proteinuria, positive antinuclear antibody and Goodpasture's Syndrome. More rarely the drug can damage the bone marrow leading to thrombocytopenia or total aplasia. Dermatologic side effects include elastosis perforans serpiginosa, pemphigoid lesions, lichen planus and aphthous stomatitis. There have also been reports of myasthenia gravis, polymyositis, loss of taste, reduction of IgA and serous retinitis due to administration of penicillamine.

In order to minimise its side effects penicillamine should be started at 250 mg daily; the dose may be increased in 250 mg steps every week to a maximal daily amount of 1000 to 1500 mg given in 2 to 4 divided doses daily (Roberts 2003). Maintenance doses range from 750 to 1000 mg/d given as 2 divided doses. In children the dose is 20 mg/kg/d given in 2 or 3 divided doses. Penicillamine should be given 1 hour before or 2 hours after meals because food may inhibit its absorption. After starting penicillamine therapy serum ceruloplasmin at first may decrease. Treatment success is checked by measuring 24-hr urinary copper that should range between 200–500 µg/day. In the long run, ceruloplasmin should increase and free copper should regress towards normal with penicillamine therapy (Roberts 2003).

Trientine (triene). The chemical structure of the copper chelator trientine (triethylene tetramine dihydrochloride, AKA triene) differs from penicillamine. Trientine has usually been used as an alternative or substitute for penicillamine, in particular when penicillamine's major side effects are not tolerable (Walshe 1982). Triene only rarely has side effects. Similar to

penicillamine, long-term treatment with trientine may cause hepatic iron accumulation in persons with WD. Trientine is poorly absorbed from the gastrointestinal tract, and only 1% appears in the urine (Walshe 1982). Doses range from 750 to 1500 mg/d given in 2 or 3 divided doses; 750 or 1000 mg are given for maintenance therapy (Roberts 2003). In children, a dose of 20 mg/kg/d is recommended. Similar to penicillamine, trientine should be given 1 hour before or 2 hours after meals. The effectiveness of copper chelation by triene is measured as described for penicillamine. Triene chelates several metals such as copper, zinc and iron by urinary excretion and it effectively removes accumulated copper from various organs in persons with WD as well as in severe liver disease (Walshe 1979, Scheinberg 1987, Santos 1996, Saito 1991). It is still unclear whether penicillamine is a more effective copper chelator when compared to triene; probably the difference in effectiveness is small (Walshe 1973, Sarkar 1977). Potential deterioration of neurological disease may also be seen after starting triene therapy; the worsening however is less frequent and less pronounced than that seen after starting with penicillamine.

Zinc. Most physicians substitute penicillamine or triene with zinc for maintenance therapy when most copper accumulation has been removed. Zinc can also be given as initial therapy in asymptomatic patients diagnosed by family screening. A recent report however shows that WD symptoms may occur despite zinc prophylaxis in asymptomatic patients (Mishra 2008). In a recent study from India, 45 WD patients were on both penicillamine and zinc sulfate. The majority of patients (84%) had neuropsychiatric manifestations. The mean duration of treatment with penicillamine and zinc, before stopping penicillamine, was 107 months. All patients had to stop penicillamine due to the financial burden. The patients then only received zinc sulfate for 27 months and 44 of the 45 patients (98%) remained stable. Only one patient reported worsening in dysarthria (Sinha 2008). Zinc does not act as an iron chelator but inhibits intestinal copper absorption and has also been suggested to bind free toxic copper (Brewer 1983, Schilksky 1989, Hill 1987). Zinc rarely has any side effects. It is still unclear whether zinc as monotherapy is an effective “decoppering” agent in symptomatic patients. There are some hints that hepatic copper may accumulate despite zinc therapy including reports about hepatic deterioration with a fatal outcome (Lang 1993, Walshe 1995). Therefore some authors use zinc in combination with a chelator. Neurological deterioration is rather rare under zinc therapy (Brewer 1987, Czlonkowska 1996). The recommended doses of zinc vary in the literature: according to AASLD practice guidelines dosing is in milligrams of elemental zinc (Roberts 2003). For larger children and adults, 150 mg/d is administered in divided doses. Compliance with doses given thrice daily may be problematic; zinc has to be taken at least twice daily to be effective (Brewer 1998). Other authors recommend using zinc sulfate at 150 mg thrice

daily as a loading dose and 100 mg thrice daily for maintenance. Further recommendations suggest giving 50 mg as zinc acetate thrice daily in adults. The type of zinc salt used has been thought to make no difference with respect to efficacy (Roberts 2003). However, zinc acetate has been suggested to cause the least gastrointestinal discomfort. When zinc is combined with a chelator the substances should be given at widely spaced intervals, potentially causing compliance problems. Effectiveness of the zinc treatment should be checked as described for penicillamine and zinc (Roberts 2003).

Tetrathiomolybdate. Tetrathiomolybdate is an experimental copper chelator not approved by FDA or EMA. It has been suggested as the initial treatment of WD patients with neurological involvement. Early reports say that tetrathiomolybdate stabilises the neurological disease and reduces circulating free copper in a matter of weeks (Brewer 1994, Brewer 1996). A more recent randomised study supports this view and also suggests that zinc monotherapy is insufficient for treatment of neurological WD (Brewer 2006).

Vitamin E, other antioxidants and diet. Since serum and hepatic concentrations of vitamin E levels may be reduced in WD (von Herbay 1994, Sokol 1994) it has been suggested to complement vitamin E intake. Some authors have also recommended taking other antioxidants; studies have not proven their effectiveness as yet.

WD patients should avoid food with high copper content (nuts, chocolate, shellfish, mushrooms, organ meats, etc). Patients living in older buildings should also check whether the water runs through copper pipes. Such dietary and lifestyle restrictions do not replace chelator or zinc therapy (Roberts 2003).

Fulminant hepatic failure and LTX. Most WD patients with fulminant liver failure need LTX urgently in order to survive (Sokol 1985, Roberts 2003). However, in a long-term cohort study only two patients died prior to LTX being available (Stremmel 1991). It is a difficult clinical question whether WD patients with liver failure can survive without LTX. The prognostic score used to help with this difficult decision includes bilirubin, AST, and INR (Nazer 1986). In any case, WD patients with signs of fulminant liver failure need to be transferred immediately (same day!) to a transplant centre.

WD patients with a chronic course of decompensated cirrhosis follow the usual rules for LTX. LTX cures the metabolic defects and thus copper metabolism returns to normal afterwards (Groth 1973). Prognosis for WD after LTX is excellent, in particular when patients survive the first year (Eghtesad 1999). It is still unclear under which circumstances LTX may be helpful for WD patients with neurological complications, which do not respond to drug therapy. In some patients CNS symptoms regress after LTX while other patients do not improve (for literature see Brewer 2000).

Asymptomatic Patients. All asymptomatic WD subjects – usually identified by family screening – need to be treated by chelators or zinc in order to prevent life-threatening complications (Walshe 1988, Brewer 1989, Roberts 2003). It is unclear whether therapy should begin in children under the age of 3 years.

Maintenance Therapy. After initial removal of excessive copper by chelators, some centres replace the chelators with zinc for maintenance therapy. It is unclear when such change is advisable and whether it might be better to reduce the dose of chelators instead of replacing them with zinc. It is generally accepted that replacement of chelators with zinc should only be done in patients who are clinically stable for some years, have normal aminotransferase and liver function, a normal free copper concentration and a 24-hr urinary copper repeatedly in the range of 200–500 µg while on chelators (Roberts 2003). Long-term treatment with zinc may be associated with fewer side effects than chelator treatment. Many patients on trientine, however, do have significant side effects, and this author believes one does need to replace trientine with zinc in such patients. In any case, therapy either with a chelator or with zinc needs to be maintained indefinitely; any interruption may lead to lethal liver failure (Walshe 1986, Scheinberg 1987).

Pregnancy. Treatment must be maintained during pregnancy because an interruption has been shown to carry a high risk of fulminant liver failure (Shimono 1991). Maintenance therapy with chelators (penicillamine, trientine) or with zinc usually results in a good outcome for mother and child, although birth defects have (rarely) been documented (for literature see Sternlieb 2000). It is recommended that the doses of both chelators be reduced, if possible by about 50%, in particular during the last trimester to avoid potential problems in wound healing (Roberts 2003). Zinc does not need to be reduced.

Monitoring of treatment

Monitoring should be done closely during initial treatment in all WD patients to look for efficacy (Table 4) and side effects. During the maintenance phase patients should be checked at least twice a year.

Table 4. Monitoring the treatment efficacy in WD

Clinical improvement (neurologic features, liver disease, hematology)
Regression of Kayser-Fleischer Ring
Circulating free copper <10 µl/dL
24-hr urinary copper excretion (200–500 µg/day on chelators)
Decrease in liver copper content

Clinical examinations include neurological, ophthalmologic and psychiatric consultations (Figure 5). Patients with liver involvement need to be checked carefully for signs of liver failure.

Laboratory tests include measurements of serum copper and ceruloplasmin, calculation of free (non-ceruloplasmin-bound) copper (see above), and 24-hr urinary copper excretion (Roberts 2003). While on chelating therapy 24-hr urinary copper excretion should initially range between 200 and 500 µg; such a value can also suggest that the patient is adherent to the drug. After removal of copper accumulation, urinary copper excretion may be lower. Prognosis of WD is dependent on the initial severity of the disease and then on adherence to the life-long treatment. Patients treated prior to severe and potentially irreversible neurological and hepatic complications have a good prognosis approaching a normal life expectancy (Figure 6). Irreversible liver disease often can be treated successfully by LTX while some patients with severe neurological disease do not get better despite optimal therapy.

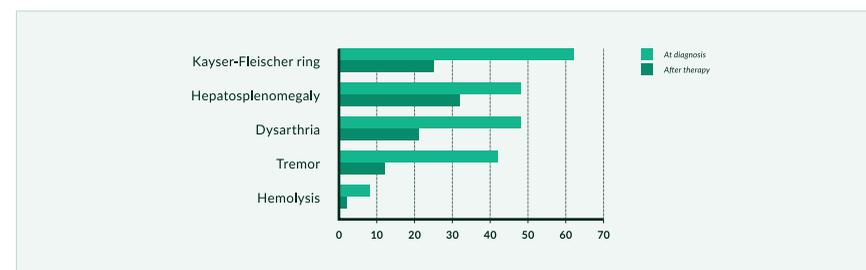


Figure 5. Findings prior to and after beginning chelating therapy in 53 WD patients (modified from Stremmel 1991)

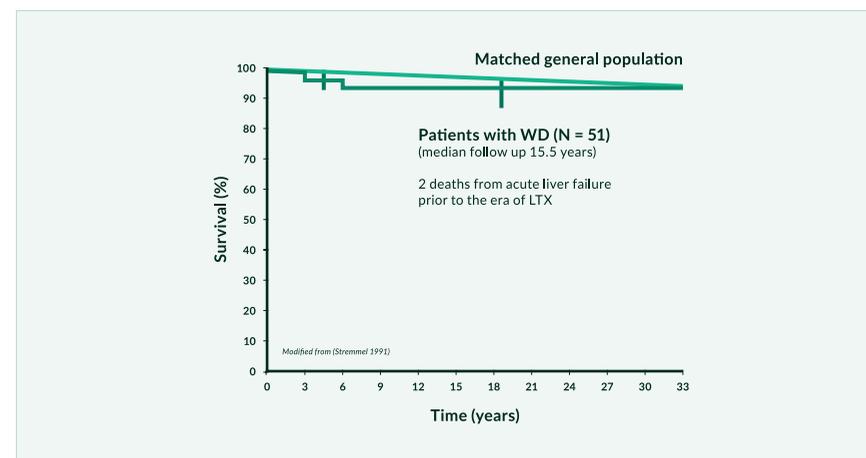


Figure 6. Cumulative survival in 51 WD patients versus a matched general population (modified from Stremmel 1991)

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24. Autoimmune liver diseases: AIH, PBC and PSC

Christian P. Strassburg

Autoimmune hepatitis (AIH)

Autoimmune hepatitis (AIH) is a chronic inflammatory disease, in which a loss of tolerance against hepatic tissue is presumed. Autoimmune hepatitis (AIH) was first described as a form of chronic hepatitis in young women showing jaundice, elevated gamma globulins and amenorrhoea, which eventually led to liver cirrhosis (Waldenström 1950). A beneficial effect of steroids was described in the reported patient cohort and thus the groundwork was laid for the first chronic liver disease found to be curable by drug therapy. AIH was later recognised in combination with other extrahepatic autoimmune syndromes, and the presence of antinuclear antibodies (ANA) led to the term lupoid hepatitis (Mackay 1956). Systematic evaluations of the cellular and molecular immunopathology, of the clinical symptoms and of laboratory features has subsequently led to the establishment of autoimmune hepatitis as a clinical entity on its own, which is serologically heterogeneous, treated by an immunosuppressive therapeutic strategy (Strassburg 2000). An established (Alvarez 1999a) and recently simplified (Hennes 2008b) revised scoring system allows for a reproducible and standardised approach to diagnosing AIH in a scientific context but has limitations in everyday diagnostic applications. The use and interpretation of seroimmunological and molecular biological tests permits a precise discrimination of autoimmune hepatitis from other etiologies of chronic hepatitis, in particular from chronic viral infection as the most common cause of chronic hepatitis worldwide (Strassburg 2002). Today, AIH is a treatable chronic liver disease in the majority of cases. Much of the same initial treatment strategies of immunosuppression still represent the standard of care. The largest challenge regarding treatment is the timely establishment of the correct diagnosis.

Definition and diagnosis of autoimmune hepatitis

In 1992, an international panel met in Brighton, UK, to establish diagnostic criteria for AIH because it was recognised that several features including histological changes and clinical presentation are also prevalent

in other chronic liver disorders (Johnson 1993). In this and in a revised report the group noted that there is no single test for the diagnosis of AIH. In contrast, a set of diagnostic criteria was suggested in the form of a scoring system designed to classify patients as having probable or definite AIH (Table 1). According to this approach the diagnosis relies on a combination of indicative features of AIH and the exclusion of other causes of chronic liver diseases. AIH predominantly affects women of any age, and is characterised by a marked elevation of serum globulins, in particular gamma globulins, and circulating autoantibodies. It should be noted that AIH regularly affects individuals older than 40 but should be considered in all age groups (Strassburg 2006). The clinical appearance ranges from an absence of symptoms to a severe or fulminant presentation (Stravitz 2011) and responds to immunosuppressive treatment in most cases. An association with extrahepatic autoimmune diseases such as rheumatoid arthritis, autoimmune thyroiditis, ulcerative colitis and diabetes mellitus and a family history of autoimmune or allergic disorders has been reported (Strassburg 1995).

Autoantibodies are one of the distinguishing features of AIH. The discovery of autoantibodies directed against different cellular targets including endoplasmic reticulum membrane proteins, nuclear antigens and cytosolic antigens has led to a suggested subclassification of AIH based upon the presence of three specific autoantibody profiles. According to this approach, AIH type 1 is characterised by the presence of antinuclear antibodies (ANA) and/or anti-smooth muscle antibodies (SMA) directed predominantly against smooth muscle actin. AIH type 2 is characterised by anti-liver/kidney microsomal autoantibodies (LKM-1) directed against cytochrome P450 CYP2D6 (Manns 1989, Manns 1991) (Figure 1) and with lower frequency against UDP-glucuronosyltransferases (UGT) (Strassburg 1996). AIH type 3 (Manns 1987, Stechemesser 1993) is characterised by autoantibodies against a soluble liver antigen (SLA/LP) identified as UGA suppressor serine tRNA-protein complex (Gelpi 1992, Wies 2000, Volkmann 2001, Volkmann 2010). However, this serological heterogeneity does not influence the decision of whom to treat or of what strategy to employ.

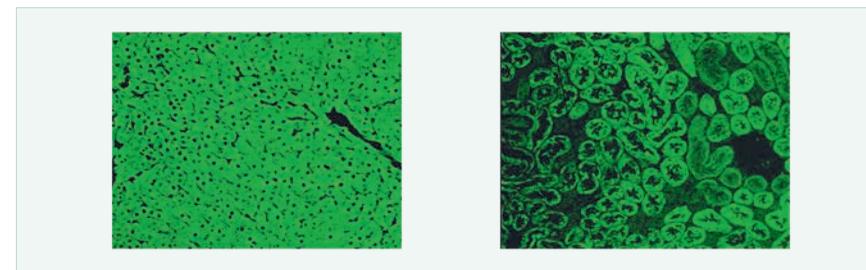


Figure 1. Indirect immunofluorescence showing LKM-1 autoantibodies on rat kidney and liver cryostat sections. Serum of a patient with autoimmune hepatitis type 2. A) Using rat hepatic cryostat sections a homogeneous cellular immunofluorescence staining is visualised excluding the hepatocellular nuclei (LKM-1). B) Typical indirect immunofluorescence pattern of LKM-1 autoantibodies detecting the proximal (cortical) renal tubules but excluding the distal tubules located in the renal medulla, which corresponds to the tissue expression pattern of the autoantigen CYP2D6

Although the histological appearance of AIH is characteristic, there is no specific histological feature that can be used to prove the diagnosis (Dienes 1989). Percutaneous liver biopsy is recommended initially for grading and staging (EASL 2015), as well as for therapeutic monitoring when this is considered necessary for therapeutic planning. Histological features usually include periportal hepatitis with lymphocytic infiltrates, plasma cells, and piecemeal necrosis. With advancing disease, bridging necrosis, panlobular and multilobular necrosis may occur and ultimately lead to cirrhosis. A lobular hepatitis can be present, but is only indicative of AIH in the absence of copper deposits or biliary inflammation. However, biliary involvement does not rule out AIH. The presence of granulomas and iron deposits argue against AIH.

Viral hepatitis should be excluded by the use of reliable, commercially available tests. Hepatitis E is frequently found in AIH patients and should be considered (van Gerven 2016). The exclusion of other hepatotropic viruses such as cytomegalovirus, Epstein-Barr and herpes may only be required in cases suspicious of such infections or if the diagnosis of AIH based on the above-mentioned criteria remains inconclusive.

The probability of AIH usually decreases whenever signs of bile duct involvement are present, such as elevation of alkaline phosphatase, histological signs of cholangiopathy and detection of AMA. If one or more components of the scoring system are not evaluated, only a probable diagnosis can be made (Table 1).

Epidemiology and clinical presentation

Based on limited epidemiological data, the prevalence is estimated to range between 20 to 50 cases per million among the Caucasian population in

Western Europe and North America (Jepsen 2015). The prevalence of AIH is similar to that of systemic lupus erythematosus, primary biliary cholangitis and myasthenia gravis, which also have an autoimmune aetiology (Nishioka 1997, Nishioka 1998). Among the Caucasian population in North American and Western European, AIH accounts for up to 20% of cases with chronic hepatitis (Cancado 2000). However, chronic viral hepatitis remains the major cause of chronic hepatitis in most Western societies.

Autoimmune hepatitis is part of the syndrome of chronic hepatitis, which is characterised by sustained hepatocellular inflammation for at least six months and an elevation of ALT and AST of 1.5 times the upper limit of normal. In about 49% of AIH patients an acute onset of AIH is observed and rare cases of fulminant AIH have been reported. In most cases, however, the clinical presentation is not spectacular and is characterised by fatigue, right upper quadrant pain, jaundice and occasionally also by palmar erythema and spider naevi. In later stages, the consequences of portal hypertension dominate, including ascites, bleeding oesophageal varices and encephalopathy. A specific feature of AIH is the association of extrahepatic immune-mediated syndromes including autoimmune thyroiditis, vitiligo, alopecia, nail dystrophy, ulcerative colitis, rheumatoid arthritis, and also diabetes mellitus and glomerulonephritis.

Table 1. International criteria for the diagnosis of AIH (Alvarez 1999)

Parameter	Score
Gender	
Female	+ 2
Male	0
Serum biochemistry	
Ratio of elevation of serum alkaline phosphatase to aminotransferase	
>3.0	- 2
1.5-3	0
<1.5	+ 2
Total serum globulin, γ-globulin or IgG (x upper limit of normal)	
>2.0	+ 3
1.5-2.0	+ 2
1.0-1.5	+ 1
<1.0	0
Autoantibodies (titres by immunofluorescence on rodent tissues)	
Adults	
ANA, SMA or LKM-1	
>1:80	+ 3
1:80	+ 2
1:40	+ 1
<1:40	0
Antimitochondrial antibody	
Positive	- 4
Negative	0

Parameter	Score
Hepatitis viral markers	+ 3
Negative	- 3
Positive	
History of drug use	
Yes	- 4
No	+ 1
Alcohol (average consumption)	
<25 gm/day	+ 2
>60 gm/day	- 2
Genetic factors: HLA-DR3 or -DR4	+ 1
Other autoimmune diseases	+ 2
Response to therapy	
Complete	+ 2
Relapse	+ 3
Liver histology	
Interface hepatitis	+ 3
Predominant lymphoplasmacytic infiltrate	+ 1
Rosetting of liver cells	+ 1
None of the above	- 5
Biliary changes	- 3
Other changes	- 3
Seropositivity for other defined autoantibodies	+ 2

Interpretation of aggregate scores: definite AIH – greater than 15 before treatment and greater than 17 after treatment; probable AIH – 10 to 15 before treatment and 12 to 17 after treatment

Natural history and prognosis

Data describing the natural history of AIH are scarce. The last placebo-controlled immunosuppressive treatment trial containing an untreated arm was published in 1980 (Kirk 1980). The value of these studies is limited considering that these patients were only screened for then available epidemiological risk factors for viral hepatitis and were not characterised by standardised diagnostic criteria and available virological tests. Nevertheless, these studies reveal that untreated AIH had a very poor prognosis and 5- and 10-year survival rates of 50% and 10% were reported. They furthermore demonstrated that immunosuppressive treatment significantly improved survival.

Up to 30% of adult patients had histological features of cirrhosis at diagnosis. In 17% of patients with periportal hepatitis, cirrhosis developed within five years, but cirrhosis develops in 82% when bridging necrosis or necrosis of multiple lobules is present. The frequency of remission (86%) and treatment failure (14%) are comparable in patients with and without cirrhosis at presentation. Importantly, the presence of cirrhosis does not

influence 10-year survival and those patients require a similarly aggressive treatment strategy (Geall 1968, Soloway 1972).

Almost half of the children with AIH already have cirrhosis at the time of diagnosis. Long-term follow-up revealed that few children can completely stop all treatment and about 70% of children receive long-term treatment (Homborg 1987, Gregorio 1997). Most of these patients relapse when treatment is discontinued, or if the dose of the immunosuppressive drug is reduced. About 15% of patients develop chronic liver failure and are transplanted before the age of 18 years.

In elderly patients, a more severe initial histological grade has been reported (Strassburg 2006). The risk of hepatocellular carcinoma varies considerably between the different diseases PBC, PSC and AIH. Particular PSC is regularly complicated by cholangiocarcinoma, gall bladder carcinoma and rarely hepatocellular carcinoma (Zenoussi 2014). In contrast, occurrence of HCC in patients with AIH is a rare event and develops only in long-standing cirrhosis.

Who requires treatment?

Autoimmune hepatitis (AIH) is a remarkably treatable chronic liver disease (Manns 2001, Czaja 2010). Untreated, the prognosis of active AIH is dismal, with 5- and 10-year survival rates between 50 and 10% and a well-recognised therapeutic effect exemplified by the last placebo-controlled treatment trials (Soloway 1972, Kirk 1980). For these reasons the indication for treatment is given in any patient who has an established AIH diagnosis, elevations of aminotransferase activities (ALT, AST), an elevation of serum IgG and histological evidence of interface hepatitis or necroinflammatory activity. This has been discussed in the newest version of the AASLD (Manns 2010a) and the EASL (EASL 2015) AIH guidelines. An initial liver biopsy is recommended for confirmation of the diagnosis and for grading and staging. Biopsies are also helpful for observation of aminotransferase activities in serum reflecting inflammatory activity in the liver, which is not always closely correlated.

Who does not require treatment?

Very few patients with an established AIH diagnosis should not be treated. Rare cases, in which the initiation of standard therapy should be weighed against potential side effects, are contraindications with steroids or azathioprine, or for certain other immunosuppressants (see below). In decompensated liver cirrhosis of patients on the waiting list for liver

transplantation and in individuals with complete cirrhosis and absent inflammatory activity treatment does not appear beneficial (Manns 2010a, EASL 2015).

Standard treatment strategy

Independent of the clinically- or immunoserologically-defined type of AIH, standard treatment is implemented with predniso(lo)ne alone or in combination with azathioprine. Both strategies are as effective (Manns 2001, Manns 2010a). The basic premise is based upon the findings of studies of almost three decades ago that indicated the effectiveness of steroids in AIH. Since that time, no single multicentre randomised treatment trial in AIH patients has been performed. Advances of alternative treatments are based on small cohorts and on the need to develop strategies for difficult-to-treat patients. The use of prednisone or its metabolite prednisolone, which is used more frequently in Europe, is effective since chronic liver disease does not seem to have an effect on the synthesis of prednisolone from prednisone. The exact differentiation between viral infection and autoimmune hepatitis is important. Treatment of replicative viral hepatitis with corticosteroids must be prevented as well as administration of interferon in AIH, which can lead to dramatic disease exacerbation.

Standard induction treatment and suggested follow-up examinations are summarised in Table 2. Please note the differences in preferred regimen in Europe and the US, which are delineated in the AASLD AIH Guideline (Manns 2010a). Therapy is usually administered over the course of two years. The decision between monotherapy and combination therapy is guided principally by side effects. Long-term steroid therapy leads to cushingoid side effects. Cosmetic side effects decrease patient compliance considerably (Table 3). Serious complications such as steroid diabetes, osteopenia, aseptic bone necrosis, psychiatric symptoms, hypertension and cataract formation also have to be anticipated in long-term treatment. Side effects are found in 44% of patients after 12 months and in 80% of patients after 24 months of treatment. However, predniso(lo)ne monotherapy is possible in pregnant patients. Azathioprine, on the other hand, leads to a decreased dose of prednisone. It bears a theoretical risk of teratogenicity. In addition, abdominal discomfort, nausea, cholestatic hepatitis, rash and leukopenia can be encountered. These side effects are seen in 10% of patients receiving a dose of 50 mg per day. From a general point of view, a postmenopausal woman with osteoporosis, hypertension and elevated blood glucose would be a candidate for combination therapy. In young women, pregnant women or patients with haematological abnormalities, prednisone monotherapy may be the treatment of choice.

Table 2. Treatment regimen and follow-up examinations of autoimmune hepatitis regardless of autoantibody type

	Monotherapy			Combination therapy		
Prednis(ol)-one	60 mg reduction by 10 mg/week to maintenance of 20 mg/wk reduction by 5 mg to 10 mg find lowest dose in 2.5 mg decrements			30–60 mg reduction as in monotherapy		
Azathioprine	n.a. (maintenance with azathioprine: monotherapy: 2 mg/kg body weight)			1 mg/kg of body weight (Europe) 50 mg (US)		
Examination	Before therapy	During therapy before remission q 4 weeks	Remission on therapy q 3–6 months	Cessation of therapy q 3 weeks (x 4)	Remission post-therapy q 3–6 months	Evaluation of relapse
Physical	+		+	+	+	+
Liver biopsy	+		(+/-)			+
Blood count	+	+	+	+	+	
Aminotransferases	+	+	+	+	+	+
Gamma glutamyl-transferase	+	+	+			
Gamma-globulin	+	+	+	+	+	+
Bilirubin	+	+	+	+	+	+
Coagulation studies	+	+	+	+	+	
Autoantibodies	+	+/-				+
Thyroid function tests	+	+/-				+

Table 3. Side effects

Prednis(ol)one	Azathioprine
acne	nausea
moon-shaped face	vomiting
striae rubra	abdominal discomforts
dorsal hump	hepatotoxicity
obesity	rash
weight gain	leukocytopenia
diabetes mellitus	teratogenicity (?)
cataracts	oncogenicity (?)
hypertension	

One of the most important variables for treatment success is adherence. The administration of treatment is essential since most cases of relapse are the result of erratic changes of medication and/or dose. Dose reduction is aimed at finding the individually appropriate maintenance dose. Since histology lags 3 to 6 months behind the normalisation of serum parameters, therapy has to be continued beyond the normalisation of aminotransferase levels. Usually, maintenance doses of predniso(lo)ne range between 10 and 2.5 mg. After 12 to 24 months of therapy predniso(lo)ne can be tapered over the course of 4 to 6 weeks to test whether a sustained remission has been achieved. Tapering regimens aiming at withdrawal should be attempted with great caution and only after obtaining a liver biopsy that demonstrates a complete resolution of inflammatory activity. Relapse of AIH and risk of progression to fibrosis is almost universal when immunosuppression is tapered in the presence of residual histological inflammation. Withdrawal should be attempted with caution to prevent recurrence and subsequent fibrosis progression and should be discussed with the patient and closely monitored.

Outcomes of standard therapy can be classified into four categories: remission, relapse, treatment failure and stabilisation.

Remission is a complete normalisation of all inflammatory parameters including histology. The achievement of aminotransferase activities within two-fold of the upper limit of normal is not recommended as treatment goal, rather, normalisation should be aimed at. Remission is ideally the goal of all treatment regimens and ensures the best prognosis. Remission can be achieved in 65 to 75% of patients after 24 months of treatment. Remission can be sustained with azathioprine monotherapy of 2 mg/kg bodyweight (Johnson 1995). This prevents cushingoid side effects. However, side effects such as arthralgia (53%), myalgia (14%), lymphopenia (57%) and myelosuppression (6%) have been observed. Complete remission is not achieved in about 20% of patients and these patients continue to carry a risk of progressive liver injury.

Relapse is characterised by an increase in aminotransferase levels and the recurrence of clinical symptoms either while on treatment, following tapering of steroid doses to determine the minimally required dose, or, after a complete withdrawal of therapy. Relapse happens in 50% of patients within six months of treatment withdrawal and in 80% after three years. Relapse is associated with progression to cirrhosis in 38% and liver failure in 14%. Relapse requires reinitiation of standard therapy, consideration of dosing as well as diagnosis, and perhaps a long-term maintenance dose with predniso(lo)ne or azathioprine monotherapy.

Treatment failure characterises a progression of clinical, serological and histological parameters during standard therapy. This is seen in about 10% of patients. In these cases the diagnosis of AIH has to be carefully

reconsidered to exclude other etiologies of chronic hepatitis. In these patients experimental regimens can be administered or liver transplantation will become necessary.

Stabilisation is the achievement of a partial remission. Since 90% of patients reach remission within three years, the benefit of standard therapy has to be reevaluated in this subgroup of patients. Ultimately, liver transplantation provides a definitive treatment option.

Treatment of elderly patients

The presentation of acute hepatitis, clinical symptoms of jaundice, abdominal pain and malaise have a high likelihood of attracting medical attention and subsequently leading to the diagnosis of AIH (Nikias 1994). More subtle courses of AIH may not lead to clinically relevant signs and may develop unnoticed other than via routine work-up for other problems or via screening programmes. The question of disease onset in terms of initiation of immune-mediated liver disease versus the clinical consequences that become noticeable after an unknown period of disease progression is not easily resolved. Thus, “late onset” AIH may simply just reflect a less severe course of the disease with slower progression to cirrhosis. While LKM positive patients display a tendency towards an earlier presentation, both acute and subtle (earlier and late presentation) variants appear to exist in ANA positive AIH. In practice, the diagnostic dilemma is that AIH is still perceived by many as a disease of younger individuals and that therefore this differential diagnosis is less frequently considered in elderly patients with cryptogenic hepatitis or cirrhosis. Another relevant question resulting from these considerations is the issue of treatment. Standard therapy in AIH consists of steroids alone or a combination with azathioprine. In maintenance therapy azathioprine monotherapy can also be administered but induction with azathioprine alone is not effective. From a general standpoint most internists will use caution when administering steroids to elderly patients, especially in women in whom osteopenia or diabetes may be present.

Recommendations for the treatment of AIH suggest that side effects be weighed against the potential benefit of therapy, and that not all patients with AIH are good candidates for steroid treatment (Manns 2001). Controversy exists surrounding the benefit of therapy in this group of elderly patients. One cohort reported on 12 patients aged over 65 out of a total of 54 AIH patients. Cirrhosis developed after follow-up in 26% irrespective of age although the histological grade of AIH activity was more severe in the elderly group. Although 42% of the patients over 65 did not receive therapy, deaths were only reported in the younger group (Newton 1997). Another cohort of 20 patients aged over 65, reported a longer time to

diagnosis (8.5 vs. 3.5 months) with patients presenting mainly with jaundice and acute onset AIH but that they showed a comparable response rate to immunosuppression to that of younger patients (Schramm 2001). The authors also noted that the prevalence of the HLA A1-B8 allotype was less frequent in older patients suggesting a role for immunogenetics.

This point was further elaborated by a report analysing 47 patients with ANA positive AIH aged 60 years and older, as well as 31 patients aged 30 years and younger in whom DR4+/DR3- prevalence was 47% (older) versus 13% (younger) patients (Czaja 2006). Steroid responsiveness was better in the older patients, in line with previous findings in the same cohort (Czaja 1993). Cirrhosis and extrahepatic immune-mediated syndromes including thyroid and rheumatologic disease (47% vs. 26%) were more prevalent in older AIH patients. However, although more treatment failures were observed in the younger patients (24% vs 5%), the rates of remission, sustained remission and relapse were similar. Interestingly, an assessment of age-stratified prevalence showed an increase after the age of 40 from 15% to over 20%.

From all this data, AIH in elderly patients appears to be characterised by a distinct clinical feature, a distinct immunogenetic profile, favourable response rates and higher rates of cirrhosis present at diagnosis, all of which contribute to the heterogeneity of AIH. A UK cohort of 164 AIH patients included 43 individuals aged 60 years (Al-Chalabi 2006). The different age groups showed no significant differences regarding serum biochemistry, autoantibody titres, time to establishment of diagnosis, and mode of presentation. The authors provided a substratification of patients below and above 40 years of age and reported that older patients had a higher median histological stage and a comparable median grade but that younger patients had more median relapse episodes and a higher median stage at follow-up biopsy. The most distinguishing clinical sign was a higher prevalence of ascites in the older group. However, rates of complete, partial and failed response were similar, and the median number of relapses was higher in younger patients, which nevertheless did not lead to differences in liver-related deaths in either group (12% vs. 15%). In comparison to the study of ANA positive AIH patients from the US (Czaja 2006), the differing findings regarding HLA association are noteworthy. In the UK study there was no differential distribution of HLA DR3 and DR4 and this questions the suggested hypothesis of a primary influence of immunogenetics on the observed clinical distinctions. The reasons for the clinical differences of AIH in older and younger patients are unclear. They may include differences in hepatic blood flow and alterations involving the regulation of cellular immunity during ageing (Talor 1991, Prelog 2006). In summary, these data suggest that AIH in elderly patients should be considered and treated (Strassburg 2006).

Alternative treatments

When standard treatment fails or drug intolerance occurs, alternative therapies such as cyclosporine, tacrolimus, cyclophosphamide, mycophenolate mofetil, rapamycin, UDCA, and budesonide can be considered (Table 4). The efficacy of most of these options has not yet been definitively decided and is only reported in small case studies.

Budesonide

Budesonide is a synthetic steroid with high first-pass metabolism in the liver, in principle with limited systemic side effects compared to conventional steroids. In comparison to prednisone the absolute bioavailability of budesonide is less than 6-fold lower (Thalen 1979) but it has an almost 90% first-pass metabolism in the liver, a higher affinity to the glucocorticoid receptor, acts as an anti-inflammatory and immunosuppressive drug and leads to inactive metabolites (6-OH-budesonide, 16-OH-prednisolone). In a pilot study treating 13 AIH patients with budesonide over a period of 9 months the drug was well-tolerated and aminotransferase levels were normalised (Danielson 1994). However, in a second study budesonide therapy was associated with a low frequency of remission and high occurrence of side effects (Czaja 2000) in 10 patients who had previously been treated with azathioprine and steroids and had not reached a satisfactory remission. This study concluded that budesonide was not a good treatment option in those patients. A third study reported that remission was induced with budesonide combination therapy in 12 previously untreated patients (Wiegand 2005). The authors performed kinetic analyses and reported that the area under the curve (AUC) of budesonide was increased in those with high inflammatory activity and cirrhosis. This finding plausibly demonstrates that in patients with portosystemic shunts in portal hypertension the effect of high hepatic first-pass metabolism that would limit typical steroid side effects is reduced.

Table 4. Alternative drugs in autoimmune hepatitis

Compound	Advantage	Disadvantage
Budesonide	High first pass effect Immunosuppressive action Inactive metabolites	Cirrhosis (portosystemic shunts) and side effects
Cyclosporine	Satisfactory experience Potent immunosuppressant Transplant immunosuppressant	Renal toxicity
Tacrolimus	Potent immunosuppressant Transplant immunosuppressant	Renal toxicity
Mycophenolic acid	Favourable toxicity profile Transplant immunosuppressant	Disappointing effectiveness
Cyclophosphamide	Effective	Continuous therapy Hematological side effects

The main advantage of budesonide for the future treatment of autoimmune hepatitis would therefore be to replace prednisone in long-term maintenance therapy and induction therapy to reduce steroid side effects. To this end the first multicentre placebo-controlled randomised AIH treatment trial in 3 decades was performed with a total of 207 non-cirrhotic patients from 30 centres in nine European countries and Israel (Manns 2010b). In this trial 40 mg prednisone (reduction regimen) and azathioprine was compared to 3 mg budesonide (TID initially, reduced to BID) in combination with azathioprine. The data shows that budesonide in combination with azathioprine is efficient in inducing stable remission, is superior in comparison to a standard prednisone tapering regimen beginning with 40 mg per day and leads to a substantially superior profile of steroid-specific side effects. From these data, budesonide has emerged as an alternative first line treatment strategy for non-cirrhotic patients with AIH (Manns 2010b, EASL 2015). Budesonide is licensed for the use in AIH in many countries. Effective treatment of children with budesonide has been reported (Wojnarowski 2013).

Deflazacort

This alternative corticosteroid has also been studied for immunosuppression in AIH because of its feature of fewer side effects than conventional glucocorticoids. In a pilot study 15 patients with AIH type I were treated with deflazacort, who had been previously treated with prednisone with or without azathioprine until they reached a biochemical remission. Remission was sustained for two years of follow-up. However, the long-term role of second-generation corticosteroids to sustain remission

in AIH patients with reduced treatment-related side effects requires further controlled studies (Rebollo Bernardez 1999).

Cyclosporine A

Cyclosporine A (CyA) is a lipophilic cyclic peptide of 11 residues produced by *Tolypocladium inflatum* that acts on calcium-dependent signaling and inhibits T cell function via the interleukin 2 gene (Strassburg 2008). Out of the alternative AIH drugs considerable experience has been reported with CyA. CyA was successfully used for AIH treatment and was well tolerated (Alvarez 1999b, Debray 1999). The principal difficulty in advocating widespread use of CyA as first line therapy relates to its toxicity profile, particularly with long-term use (increased risk of hypertension, renal insufficiency, hyperlipidaemia, hirsutism, infection, and malignancy) (Alvarez 1999b, Debray 1999, Fernandez 1999, Heneghan 2002).

Tacrolimus

Tacrolimus is a macrolide lactone compound with immunosuppressive qualities exceeding those of CyA. The mechanism of action is similar to that of CyA but it binds to a different immunophilin (Strassburg 2008). The application of tacrolimus in 21 patients treated for one year led to an improvement of aminotransferase and bilirubin levels with a minor increase in serum BUN and creatinine levels (Van Thiel 1995). In a second study with 11 steroid-refractory patients, improvement of inflammation was also observed (Aqel 2004). A recent study demonstrated the effectiveness of tacrolimus in difficult to treat patients (Than 2016). However, although tacrolimus represents a promising immunosuppressive candidate drug, larger randomised trials are required to assess its role in the therapy of AIH.

Mycophenolic acid

Mycophenolate is a noncompetitive inhibitor of inosine monophosphate dehydrogenase, which blocks the rate-limiting enzymatic step in *de novo* purine synthesis and is widely used in solid organ transplantation. Mycophenolate has a selective action on lymphocyte activation, with marked reduction of both T and B lymphocyte proliferation. In a pilot study, seven patients with AIH type 1 who either did not tolerate azathioprine or did not respond to standard therapy with a complete normalisation of aminotransferase levels, were treated with mycophenolate in addition to

steroids. Normalisation of aminotransferase levels was achieved in five out of seven patients within three months. These preliminary data suggested that mycophenolate may represent a promising treatment strategy for AIH (Richardson 2000). However, in a retrospective study, there was no statistically significant benefit for mycophenolate treatment in 37 patients with AIH and azathioprine failure or intolerance who were treated with mycophenolate (Hennes 2008a). Less than 50% reached remission and in the azathioprine non-responders failure was 75%. Mycophenolate has been demonstrated to be most effective as a second line therapy in patients found to be intolerant to azathioprine. There is some evidence that mycophenolate can be used as first line therapy (Zachou 2016). There is limited data available on the use of mTOR inhibitors such as everolimus in AIH (Ytting 2015).

Cyclophosphamide

The induction of remission with 1–1.5 mg per kg per day of cyclophosphamide in combination with steroids has been reported. However, the dependency of continued application of cyclophosphamide with its potentially severe haematological side effects renders it a highly experimental treatment option (Kanzler 1996).

Anti-TNF α antibodies

There is some emerging evidence that anti-TNF antibodies are capable of inducing remission in AIH patients in whom standard or alternative therapeutic options have been exhausted (Efe 2010, Umekita 2011, Weiler-Norman 2013). However, the development of AIH has also been observed under treatment with anti-TNF antibodies (Ramos-Casals 2008). Future studies will have to define the role of this therapeutic option in difficult-to-treat cases of AIH.

Ursodeoxycholic acid

Ursodeoxycholic acid is a hydrophilic bile acid with putative immunomodulatory capabilities. It is presumed to alter HLA class I antigen expression on cellular surfaces and to suppress immunoglobulin production. Uncontrolled trials have shown a reduction in histological abnormalities, clinical and biochemical improvement but not a reduction of fibrosis in four patients with AIH type 1 (Calmus 1990, Nakamura 1998,

Czaja 1999). However, its role in AIH therapy or in combination with immunosuppressive therapy is still unclear.

Other alternative treatment strategies include methotrexate, anti-TNF α antibodies, and rituximab, but there is currently insufficient data on any of these.

Overlap syndromes and treatment

Overlap syndrome describes a disease condition that is not completely defined (Strassburg 2006). A valid definition is difficult (Boberg 2011). It is characterised by the coexistence of clinical, biochemical or serological features of autoimmune hepatitis (AIH), primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), and depending on the definition, also viral hepatitis C (HCV) (Ben-Ari 1993, Colombato 1994, Duclos-Vallee 1995, Chazouilleres 1998, Angulo 2001, Rust 2008). In adult patients an overlap of PBC and AIH is most frequently encountered although it is unclear whether this is true co-existence of both diseases or an immunoserological overlap characterised by the presence of antinuclear (ANA) as well as antimitochondrial (AMA) antibodies (Poupon 2006, Gossard 2007, Silveira 2007, Al-Chalabi 2008). In many AMA negative patients with a cholestatic liver enzyme profile ANA are present. This has been termed autoimmune cholangiopathy or AMA negative PBC (Micheletti 1994).

Apart from coexisting, autoimmune liver diseases can also develop into each other, i.e., the sequential manifestation of PBC and autoimmune hepatitis. The true coexistence of AIH and PSC has only been conclusively shown in paediatric patients (Gregorio 2001). It can be hypothesised whether a general predisposition toward liver autoimmunity exists which has a cholestatic, a hepatitic and a bile duct facet, which may be variable depending upon unknown host factors. The diagnosis of an overlap syndrome relies on the biochemical profile (either cholestatic with elevated alkaline phosphatase, gamma glutamyltransferase and bilirubin, or hepatitic with elevated aspartate aminotransferase and alanine aminotransferase levels in addition to elevated gamma globulins), the histology showing portal inflammation with or without the involvement of bile ducts, and the autoantibody profile showing AMA or autoantibodies associated primarily with AIH such as liver-kidney microsomal antibodies (LKM), soluble liver antigen antibodies (SLA/LP) or ANA. In cholestatic cases cholangiography detects sclerosing cholangitis. In an overlap syndrome the classical appearance of the individual disease component is mixed with features of another autoimmune liver disease. Immunoglobulins are usually elevated in all autoimmune liver diseases.

Regarding a therapeutic strategy, the leading disease component is

treated. In an overlap syndrome presenting as hepatitis, immunosuppression with prednisone (or combination therapy with azathioprine) is initiated. In cholestatic disease ursodeoxycholic acid is administered. Both treatments can be combined when biochemistry and histology suggest a relevant additional disease component (Chazouilleres 1998). Validated therapeutic guidelines for overlap syndromes are not available. It is important to realise that treatment failure in AIH may be related to an incorrect diagnosis or an overlap syndrome of autoimmune liver diseases (Potthoff 2007). Several studies show that treatment of the AIH component of overlap syndromes is important to avoid progression to cirrhosis (Chazouilleres 2006, Gossard 2007, Silveira 2007, Al-Chalabi 2008).

Liver transplantation

In approximately 10% of AIH patients liver transplantation remains the only life-saving option (Strassburg 2004). The indication for liver transplantation in AIH is similar to that in other chronic liver diseases and includes clinical deterioration, development of cirrhosis, bleeding oesophageal varices and coagulation abnormalities despite adequate immunosuppressive therapy (Neuberger 1984, Sanchez-Urdazpal 1991, Ahmed 1997, Prados 1998, Tillmann 1999, Vogel 2004). There is no single indicator or predictor for the necessity of liver transplantation. Candidates for liver transplant are usually patients who do not reach remission within four years of continuous therapy. Indicators of a high mortality associated with liver failure are histological evidence of multilobular necrosis and progressive hyperbilirubinaemia. In Europe, 4% of liver transplants are for AIH (Strassburg 2009). The long-term results of liver transplantation for AIH are excellent. The five-year survival is up to 92% (Sanchez-Urdazpal 1991, Prados 1998, Ratziu 1999) and well within the range of other indications for liver transplantation. The European liver transplant database indicates 76% survival in five years and 66% survival after 10 years (1647 liver transplantations between 1988 and 2007). When these numbers are considered it is necessary to realise that patients undergoing liver transplantation usually fail standard therapy and may therefore have a reduced life expectancy after liver transplant compared to those who achieve stable complete remission on drug therapy.

Recurrence and *de novo* AIH after liver transplantation

The potential of AIH to recur after liver transplantation is beyond serious debate (Schreuder 2009). The first case of recurrent AIH after liver

transplant was reported in 1984 (Neuberger 1984) and was based upon serum biochemistry, biopsy findings and steroid reduction. Studies published over the years indicate that the rate of recurrence of AIH ranges between 10–35%, and that the risk of AIH recurrence is perhaps as high as 68% after five years of follow-up (Wright 1992, Devlin 1995, Götz 1999, Milkiewicz 1999, Manns 2000, Vogel 2004). It is important to consider the criteria upon which the diagnosis of recurrent AIH is based. When transaminitis is chosen as a practical selection parameter many patients with mild histological evidence of recurrent AIH may be missed. It is therefore suggested that all patients with suspected recurrence of autoimmune hepatitis receive a liver biopsy, biochemical analyses of aminotransferases as well as a determination of immunoglobulins and autoantibody titres (Vogel 2004). Significant risk factors for the recurrence of AIH have not yet been identified although it appears that the presence of fulminant hepatic failure before transplantation protects against the development of recurrent disease. Risk factors under discussion include steroid withdrawal, tacrolimus versus cyclosporine, HLA mismatch, HLA type, and LKM-1 autoantibodies. An attractive risk factor for the development of recurrent AIH is the presence of specific HLA antigens that may predispose toward a more severe immunoreactivity. In two studies recurrence of AIH appeared to occur more frequently in HLA DR3 positive patients receiving HLA DR3 negative grafts. However, this association was not confirmed in all studies. There have not been conclusive data to support the hypothesis that a specific immunosuppressive regimen represents a risk factor for the development of recurrent AIH (Gautam 2006). However, data indicate that patients transplanted for AIH require continued steroids in 64% versus 17% of patients receiving liver transplants for other conditions (Milkiewicz 1999).

Based on these results and other studies it would appear that maintenance of steroid medication in AIH patients is indicated to prevent not only cellular rejection but also graft-threatening recurrence of AIH (Vogel 2004). Steroid withdrawal should therefore be performed only with great caution. The recurrence of AIH is an important factor for the probability of graft loss. Apart from HCV and primary sclerosing cholangitis a recent report found AIH recurrence to represent the third most common reason for graft loss (Rowe 2008). Transplanted patients therefore require a close follow-up and possibly an immunosuppressive regimen including steroids, although this is controversial and not backed by prospective studies (Campsen 2008).

In addition to AIH recurrence the development of *de novo* autoimmune hepatitis after liver transplantation has been reported (Kerker 1998, Jones 1999a, Salcedo 2002). The pathophysiology of this is also elusive. From a treatment point of view *de novo* autoimmune hepatitis, which

appears to occur mostly in patients transplanted with PBC but may just be the serendipitous occurrence of AIH, is responsive to steroid treatment (Salcedo 2002).

Primary biliary cholangitis

Introduction

The former designation “primary biliary cirrhosis” is no longer used because it labels patients as having cirrhosis where this is often not the case. However, the acronym PBC remains unchanged (Beuers 2015). PBC is a chronic inflammatory, cholestatic disease of the liver with an unknown cause. The clinical observation of a broad array of immune-mediated symptoms and phenomena suggests the disease to be of autoimmune aetiology, in the course of which progressive and irreversible destruction of small interlobular and septal bile ducts progressively and irreversibly ensues (Table 5). As in other autoimmune diseases PBC affects women in over 80% of cases and is associated with varying extrahepatic autoimmune syndromes in up to 84%. These extrahepatic manifestations of immune-mediated disease include the dry gland syndrome (sicca syndrome with xerophthalmia and xerostomia) but also collagen diseases, autoimmune thyroid disease, glomerulonephritis and ulcerative colitis (Table 6).

Table 5. Clinical profile of primary biliary cholangitis (PBC)

Sex	90% female
Age	40–59 yrs pruritus jaundice skin pigmentation
Elevation	alkaline phosphatase (AP), aspartate aminotransferase (AST), bilirubin, IgM antimitochondrial antibodies (AMA) associated immune-mediated syndromes
Liver biopsy	cellular bile duct infiltration granulomas possible copper deposits

The striking female predominance (Donaldson 1996, Mackay 1997, Uibo 1999) and familiar clustering of PBC (Kato 1981, Jones 1999b, Tsuji 1999) suggest that inheritable genetic factors play a role in this disease. This has focused attention on the immunogenetics of PBC in order to further define host risk factors (Manns 1994). Studies have suggested an instability of lymphocytic DNA in PBC patients (Notghi 1990). Immunogenetic analyses,

however, have only come up with relatively weak associations with specific human leukocyte antigen haplotypes. An additional hypothesis is an alteration of bile acid composition and bile fluid composition, which would indicate a role for transporter proteins in the development of PBC. Bicarbonate rich bile is believed to be protective for biliary epithelium.

Table 6. Extrahepatic immune-mediated syndromes in PBC and overlap with rheumatic diseases

Dry gland "sicca" syndrome
Sjögren's syndrome
Rheumatoid arthritis
Autoimmune thyroid disease
Renal tubular acidosis
Mixed connective tissue disease (MCTD)
Polymyositis
Polymyalgia rheumatic
Pulmonary fibrosis
CREST syndrome
Systemic lupus erythematosus (SLE)
Pernicious anaemia
Ulcerative colitis
Exogenous pancreatic insufficiency
Myasthenia gravis

Definition and prevalence of PBC

PBC is an inflammatory, primarily T cell-mediated chronic destruction of intrahepatic microscopic bile ducts of unknown aetiology (Strassburg 2000). It affects women in 80% of cases who exhibit elevated immunoglobulin M, antimitochondrial antibodies directed against the E2 subunit of pyruvate dehydrogenase (PDH-E2), a cholestatic liver enzyme profile with elevated alkaline phosphatase, gamma glutamyltransferase as well as serum bilirubin levels, and a variable course of disease leading to cirrhosis over the course of years or decades. A prominent feature is the presence of extrahepatic immune-mediated disease associations. In later stages, pronounced fatigue, pruritus, marked hyperbilirubinaemia and the consequences of portal hypertension such as ascites, bleeding oesophageal varices, and encephalopathy develop (Strassburg 2004).

The prevalence is estimated at 65 per 100,000 in women and 12 per 100,000 in men with an incidence of 5 per 100,000 in women and 1 per 100,000 in men. The prevalence and incidence appear to vary regionally and

appears to be increasing (Boonstra 2012). An increase of PBC incidence in recent years may be the result of more specific testing of antimitochondrial antibody reactivity (Strassburg 2004).

Diagnostic principles of PBC

Suspicion of PBC arises when cholestasis and cirrhosis are present in middle-aged women (Figure 2). Ultrasound is employed to rule out mechanical cholestasis. The presence of antimitochondrial antibodies (AMA) against PDH-E2 is diagnostic of PBC. AMA against E2 subunits of members of the inner mitochondrial membrane-expressed oxoacid dehydrogenase complex (PDH, branched chain ketoacid dehydrogenase [BCKD], and ketoglutarate dehydrogenase [OADC]) are present in 95% of PBC patients. AMA negative PBC can exhibit antinuclear autoantibodies with specificity against nuclear dot antigen (SPT00), a 210 kDa nuclear membrane protein (gp210), or nucleoporin p62. In AMA negative PBC a biopsy is indicated to contribute to the establishment of the diagnosis; in the presence of AMA against PDH-E2, histology is used primarily for the staging of cirrhosis and is not necessary (Strassburg 2004). The diagnosis is established when 2 of the main criteria (cholestatic biochemistry, AMA or PBC-specific autoantibody, typical histology) are met.

Diagnostic role of AMA in PBC

The main aim of AMA determinations is the detection of PBC-specific AMA and the exclusion of AMA of low diagnostic relevance for the disease. As a screening test the determination of AMA using indirect immunofluorescence testing on rat kidney cryostat sections or immobilised Hep-2 cells (Strassburg 1999). The indirect immunofluorescence on rat kidney sections leads to the staining of the distal and proximal tubuli (note: proximal staining only is indicative of liver/kidney microsomal antibodies, LKM). When positive AMA immunofluorescence is detected, further analysis should include subclassification using molecularly defined antigen preparations. The detection of PDH-E2, BCKD-E2 can be achieved by ELISA using recombinant antigen or reference sera. If both are negative, testing should include OGD-E2. The final step is performed using western blot Analyses to confirm the findings. By western blot the indicative 74 kDa (PDH-E2), 52 kDa (BCKD-E2) and 48 kDa (OGD-E2) bands can be visualised. This multi-step regimen secures a rational and reliable diagnosis of PBC-specific AMA excluding those found in drug-induced and infectious diseases.

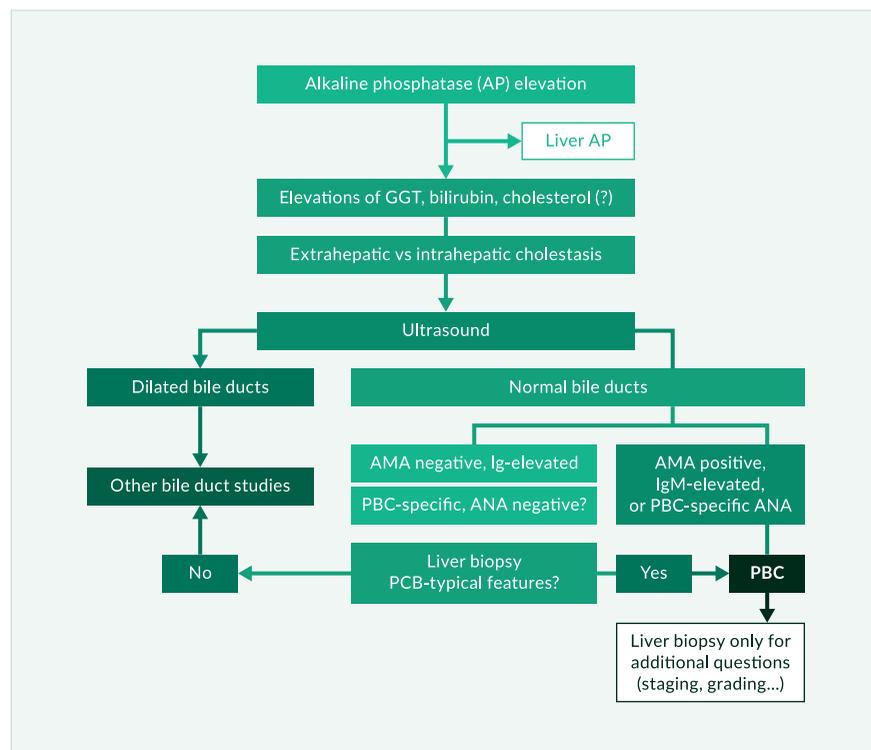


Figure 2. Diagnostic algorithm of PBC including clinical presentation, ultrasound and serology

In the majority of cases the determination of anti-PDH-E2 is sufficient to secure the diagnosis. Studies will have to evaluate whether the future application of a single PDH-E2 ELISA as highly specific screening test in suspected PBC represents an efficient and economic diagnostic approach.

Therapeutic principles in PBC

There is currently no cure for PBC (Strassburg 2004). Ursodeoxycholic acid (UDCA) (15 mg/kg body weight per day) has been shown to improve serum biochemistry, histology and survival but has no effect on fatigue and osteoporosis. It has immunomodulatory properties, alters cell signal transduction and modifies hydrophilicity of the bile. UDCA should not be given in severe cholestasis and during the first trimester of pregnancy. Immunosuppression in PBC has shown disappointing results. Symptomatic therapy of the complications of PBC includes management of pruritus (cholestyramine, induction with rifampicin, opioid antagonists, serotonin antagonists), ascites (diuretics, beta blockers to control portal hypertension), osteoporosis (vitamin D and calcium supplementation, bisphosphonates in some), as well as endoscopic intervention for bleeding

oesophageal varices. Fat-soluble vitamin replacement is suggested. When liver cirrhosis-induced liver failure is progressive, liver transplantation remains a definitive therapeutic option. Ten-year survival rates are 75–80% and recurrence of PBC after transplant occurs in 10 to 40% of patients. Recurrence can be expected in 25 to 30% (Rowe 2008, Strassburg 2009). The number of PBC patients on the waiting lists has declined during the past decade. The risk for death on the waiting list in PBC patients with jaundice is significantly higher than in those patients with HCV infection or alcoholic liver cirrhosis.

Immunosuppression in PBC

Corticosteroids: Treatment with prednisolone can improve serum aminotransferase activities, alkaline phosphatase and elevated immunoglobulins. It does not lead to significant improvement of bilirubin, pruritus, or histology. In a placebo-controlled study with 36 asymptomatic patients for over one year osteopenia and cushingoid side effects were noted (Mitchison 1992).

Azathioprine: The classical immunosuppressant azathioprine, which has a pronounced effect in AIH, did not show significant effects in two different studies and is not used in PBC (Christensen 1985).

Cyclosporine A: In a large study of 346 patients with a median observation time of 2.5 years, this classical transplant immunosuppressant did not show significant effects on histological progression (Lombard 1993). Histology did improve in a small study with 20 patients who were treated for two years, but these results should be viewed with caution (Wiesner 1990). Because of the possibility of severe side effects, cyclosporin A is not a recommended therapeutic option.

D-penicillamine: Because PBC is characterised by copper accumulation in the bile ducts the chelator d-penicillamine was studied. D-penicillamine also has immunosuppressive and antifibrotic properties. It was tested on a total of 748 patients in six studies, without leading to a positive therapeutic effect while 30% of patients had severe side effects (Bodenheimer 1985). D-penicillamine in PBC is not recommended.

Colchicine: Because of its antifibrotic and anti-inflammatory properties colchicine was studied in the 1980s. Despite improvement of albumin, bilirubin, aminotransferases and alkaline phosphatase, an improvement of clinical symptoms and histology was not observed (Kaplan 1986, Warnes 1987, Bodenheimer 1988). Severe side effects were not reported but an effect on long-term prognosis was not seen.

Methotrexate: Despite its known hepatotoxicity, methotrexate was used as an immunosuppressant in PBC. In a placebo-controlled study with 60 patients, low-dose methotrexate (7.5 mg/week) led to an improvement of

biochemical parameters except for bilirubin but no effects were reported regarding necessity of liver transplantation or survival (Hendrickse 1999). Hepatotoxicity was not observed. Interstitial pneumonitis, which affects 3–5% of rheumatoid arthritis patients, was observed in 14% of PBC patients. Methotrexate cannot be recommended outside of scientific evaluations or studies.

In principle, other immunosuppressants (Table 7) such as mycophenolic acid (mycophenolate mofetil), tacrolimus (FK506) or even monoclonal antibodies against the interleukin 2 receptor may represent interesting candidate strategies. However, data is currently lacking.

Table 7. Effects of immunosuppressants in PBC

	Biochemical improvement	Histological improvement	Survival	Side effects/toxicity
Corticosteroids	++	++	-	++
Azathioprine	-	-	+	+
Cyclosporin A	++	-	++	++
D-penicillamine	-	-	-	++
Colchicine	++	-	+	-
Methotrexate	++	+	-	+

Ursodeoxycholic acid in PBC (UDCA)

In 1981, a positive effect of UDCA was observed on elevated liver parameters, the exact mechanism of which was unclear (Leuschner 1996). On one hand UDCA leads to a modification of the bile acid pool to a more hydrophilic environment with lower detergent-like properties, and it leads to increased bile flow. On the other hand an immunomodulatory activity is suggested regarding HLA antigens expressed on biliary epithelial cells and altered signal transduction (Paumgartner 2002). The optimal dose in PBC patients appears to be 13–15 mg/kg. In a meta-analysis of three studies that looked at 548 patients with this dose, biochemical improvement and a slower histological progression to fibrosis was observed (Poupon 1997). These effects were only evident when follow-up extended to four years. These data rely heavily on the positive effects of a single study and it is not surprising that a subsequent meta-analysis of eight studies with 1114 patients failed to find positive associations with UDCA therapy (Gouilis 1999).

There are a number of problems with this analysis. Doses varied and some protocols included patients with insufficient dosing, and follow up was less than two years in some studies. In a recently published analysis

of 367 patients from four clinical cohorts, initiation of UDCA therapy in early stages of PBC (stage I-II) and a treatment duration of two years led to a retardation of histological progression, which argues for an early initiation of UDCA therapy after diagnosis, even in the absence of fibrosis or cirrhosis. UDCA was also shown to improve biochemistry, delay portal hypertension and varices, and currently has no therapeutic alternative (Poupon 2003). No convincing effect was demonstrable on osteopenia and extrahepatic manifestations of PBC. A number of parameters have been studied to assess the prognosis of PBC measured by the observed biochemical response to UDCA therapy. Several criteria have been reported including the Corpechot, Parès, and Rotterdam criteria, which in summary describe the reduction of AST, AP, and bilirubin after one year of UDCA treatment. Currently the prognostic stratification is based upon the assessment of treatment response to UDCA after 12 months. A reduction of AST, AP and bilirubin indicates a favourable outcome of therapy and should be monitored during therapy (Corpechot 2011, Kuiper 2009). Additional predictive scores of UDCA-treated PBC patients are currently being developed and evaluated (Bowlus 2016).

A novel therapeutic strategy involves the use of obeticholic acid (OCA), which is an inducer of the farnesoid-X-receptor (FXR). The first studies have been favourable regarding biochemical response to therapy. Unfortunately OCA is associated with severe pruritus and drug discontinuations in a significant number of patients at doses above 10 mg (Hirschfeld 2014). OCA is also implicated to reduce portal hypertension (Verbeke 2014) and fibrosis (Verbeke 2016), and is a candidate for combined therapy with budesonide acting via the pregnane-X-receptor (PXR) in addition to FXR (Silveira 2014). A phase 3 trial assessing 5–10 mg OCA in combination with UDCA has recently been published demonstrating a decrease of baseline biochemical parameters believed to correlate with prognosis (Nevens 2016). OCA has been licensed for use as second line therapy in the US and Europe. The effects on fibrosis progression remain to be shown in long term observations.

Therapy in non-responders and combination strategies

Non-response is usually defined as a failure to lower cholestatic enzyme activities or to reach normalisation of these parameters (Kuiper 2009). In patients in whom alkaline phosphatase and gamma glutamyltransferase activities are not lowered by UDCA therapy, increased morbidity and progression is likely. Alternative therapeutic strategies can be considered.

Steroids and UDCA: The combination of immunosuppressants and UDCA was looked at in smaller studies and included the use of prednisolone (Leuschner 1996), azathioprine (Wolfhagen 1998) and budesonide

(Leuschner 1999, Angulo 2000) (Table 7). In a randomised, controlled study with 30 patients who received 10 mg prednisolone/day an improvement of inflammatory activity was reported (Leuschner 1996). A study with 9 mg budesonide/day in 39 patients showed not only biochemical but also histological improvement (Leuschner 1999). In an open study with 22 patients a deterioration of osteopenia was noted (Angulo 2000). The combination of budesonide and UDCA may have additional beneficial effects related to the activation of the anion exchanger AE2, which may serve to alter biliary composition and produce a more protective bicarbonate rich bile.

Sulindac and UDCA: In an open study with 23 patients and incomplete response to UDCA over 12 months treated with UDCA or UDCA and sulindac a trend towards histological improvement and biochemical improvement were reported in the combination group (Leuschner 2002).

Colchicine and UDCA: Three studies investigated the combination of colchicine and UDCA for 24 months in a total of 118 patients (Raedsch 1992, Ikeda 1996, Poupon 1996). Although mild biochemical improvement was noted, the effect of longer treatment remains unclear. Because of the biliary elimination of colchicine combinations with bile acids, there may be potentially toxic effects.

Methotrexate and UDCA: Several pilot studies and three randomised studies have looked at methotrexate in combination with UDCA. In one randomised placebo-controlled protocol with 60 patients a high rate of side effects without therapeutic benefit was reported (Van Steenberg 1996, Bach 2003).

Fibrates: An interesting therapeutic approach is the use of fibrates (bezafibrate or fenofibrate) to improve the response to UDCA in non- or partial responders. Fibrates act by induction peroxisome proliferator-activated receptor α (PPAR α)-UDP-glucuronosyltransferases (UGTs) signaling axis which is an important determinant of bile acid homeostasis. Beza- or fenofibrate have been studied in 25 studies (Floreani 2016). Results of a large trial with bezafibrate are pending.

Other strategies in future focus on the use of taurine conjugated UDCA (T-UDCA), norUDCA and synthetic PPAR δ agonists.

Primary sclerosing cholangitis

Diagnosis of primary sclerosing cholangitis (PSC)

PSC is classically characterised by the progressive destruction of large intra- as well as extrahepatic bile ducts and – contrasting with AIH and PBC – preferentially affects male patients with a maximum age of around

25 to 45 (Strassburg 1996). About 50 to 75% of the time, PSC is associated with ulcerative colitis. The aetiology of PSC remains elusive but genome-wide association studies have identified susceptibility loci, which share features between PSC and inflammatory bowel disease (Janse 2011, Liu 2013). PSC is clinically characterised by upper quadrant pain, pruritus, anorexia and fever, but up to 50% of patients lack symptoms (Weismüller 2008). The diagnosis is established by a typical biochemical profile of cholestasis with elevations of bilirubin, alkaline phosphatase and gamma glutamyl transferase, the characteristic findings upon cholangiography and a typical biopsy showing ring fibrosis around the bile ducts, which is not present in all patients. Serology regularly identifies atypical anti-neutrophil cytoplasmic autoantibodies (xANCA) in up to 80% of patients (Terjung 2000), although these are not disease-specific and can also occur in patients with ulcerative colitis without PSC. These autoantibodies also occur in bile of PSC patients and correlate with disease activity (Lenzen 2013). There is a significant association of PSC with cholangiocarcinoma (10–20%) and colorectal cancer (9% in 10 years). In a subgroup of patients, small bile duct PSC may be present (Broome 2002), which lacks typical strictures and pruning of the biliary tree upon cholangiography. In these cases the diagnosis can be established in the presence of the typical association with ulcerative colitis in male patients by performing a liver biopsy (Figure 3).

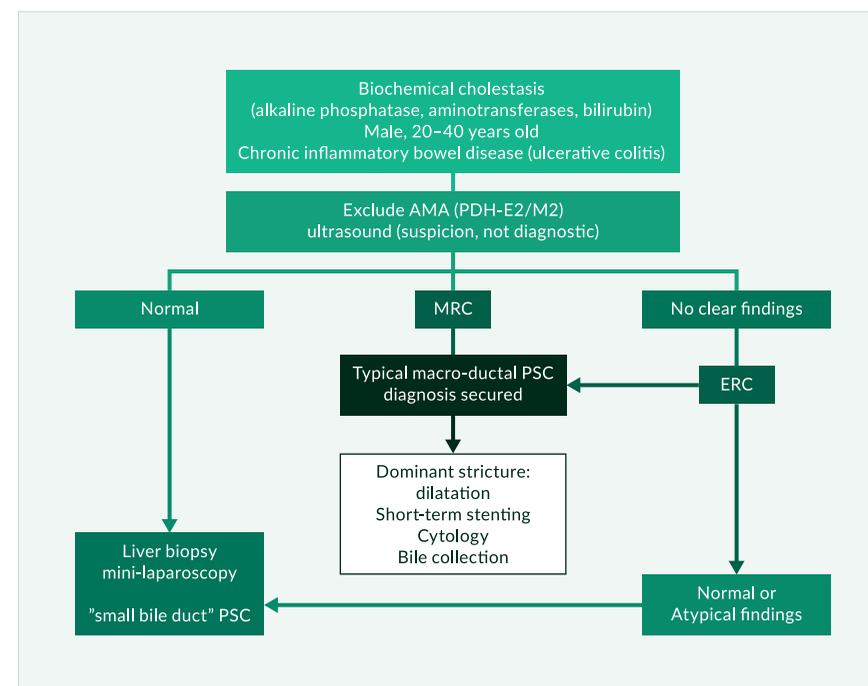


Figure 3. Diagnostic algorithm of PSC including clinical presentation

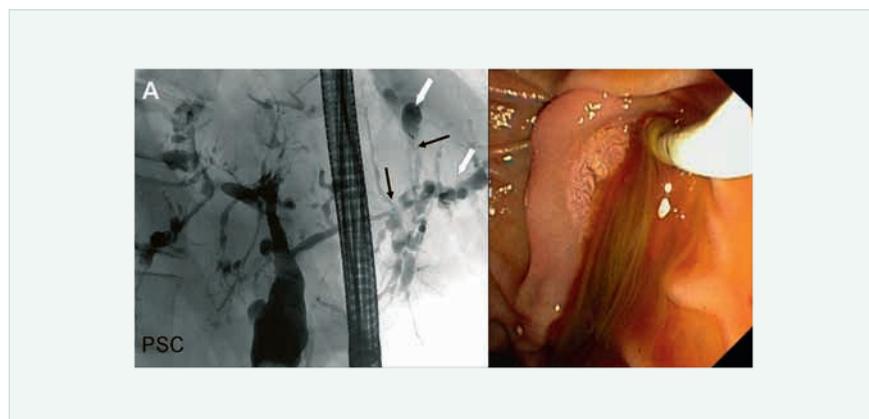
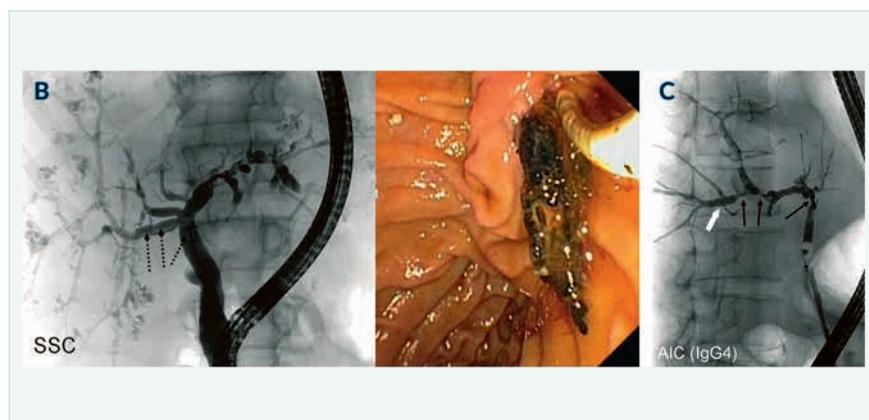


Figure 4a. Examples of different entities of sclerosing cholangitis. A) PSC showing multiple strictures with narrowing (black arrows) and prestenotic dilatation (white arrows) and an endoscopic aspect of purulent biliary infection at the biliary papilla



Figures 4b, 4c. Examples of different entities of sclerosing cholangitis. B) Secondary sclerosing cholangitis (SSC) with a similar intrahepatic picture but also biliary casts (dotted arrows) that can be extracted endoscopically (right panel). C) Cholangiogram of autoimmune (AIC) IgG4-associated cholangitis mimicking PSC. Black arrows show narrowing, white arrows show dilatations

Differential diagnosis: sclerosing cholangitis

The finding of macroductal sclerosing cholangitis can be brought about by a number of conditions, which include ischaemia, liver transplantation complications, and drugs. The dilemma is that PSC is primarily a visual diagnosis shared by many other entities leading to features of sclerosing cholangitis (Figure 5). Of note are two additional differential diagnoses that require attention (Figure 4): secondary sclerosing cholangitis (Gelbmann 2007, Esposito 2008, von Figura 2009, Al-Benna 2011) and IgG4-associated cholangitis (Webster 2009, Clendenon 2011, Takuma 2011, Zhang 2011).

Secondary sclerosing cholangitis is an entity with severe infection of the

biliary tree that develops in some patients following systemic infections and sepsis who are treated with aggressive intensive care unit management. IgG4-associated cholangitis is an immune-mediated systemic disease, which mainly affects the pancreas and bile ducts but also the lymph nodes, the kidneys, the thyroid and many other organs (Kamisawa 2014). It is characterised by often high plasma levels of IgG4 and IgG4 expression in plasma cells obtained upon brush or forceps biopsy. The latter can be treated with immunosuppression and should be diagnosed because of an available medical therapy (Kamisawa 2014).

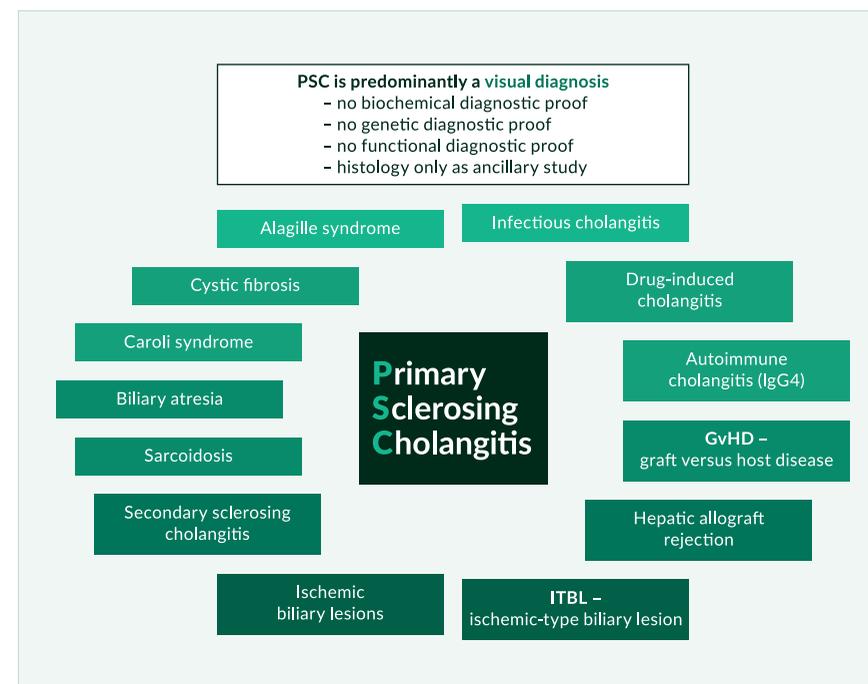


Figure 5. Diseases of the liver and those affecting the liver, which can lead to features of sclerosing cholangitis. The differential diagnostic considerations in visually apparent sclerosing cholangitis cover a diverse array of conditions apart from PSC.

Association of PSC with inflammatory bowel disease

A clinical hallmark of PSC is the high number of patients suffering from inflammatory bowel disease (IBD). In several studies with 605 PSC patients in the US (Mayo Clinic), UK (King's College) and in Sweden, IBD was found in 71%, 73% and 81% of PSC cases (Boberg 1998, Bergquist 2002). In our own experience it is found in 52% of cases (Tischendorf 2007). Ulcerative colitis is more often associated (UK 71%, Sweden 72%) than Crohn's disease. IBD is usually diagnosed before PSC but owing to the symptomatic latency of

both IBD and PSC it can also be diagnosed at the same time or later than PSC. Most commonly ulcerative colitis is diagnosed more than a year before PSC (67%). This is backed by genome wide association data (Janse 2011). In 22% the diagnoses occurred within one year of each other, and only in 11% the diagnosis of ulcerative colitis reached more than one year after PSC was established. IBD patients with elevated liver biochemistry are a risk group and require careful hepatological workup for PSC. About 5% of all patients with ulcerative colitis have PSC.

PSC as a risk factor for cancer

Apart from the risk of developing portal hypertension and cirrhosis, PSC is a severe risk factor for cancer, which distinguishes this disease from AIH and PBC (Table 8). The increased risk of cholangiocarcinoma is well described (Bergquist 2001, Boberg 2002). The numbers reported vary because explanted livers during liver transplantation, autopsies and *in vivo* diagnosed cases are taken into account in different analyses. The diagnosis of cholangiocarcinoma (CC) in PSC patients continues to represent a difficult task because stenoses upon cholangiography may be caused by inflammatory activity as well as tumour, and because biochemical tests and biopsy procedures have a low sensitivity and specificity. Imaging studies are equally complicated by a lack of sensitivity since tumours frequently grow intramurally and are diagnosed in late stages precluding curative therapeutic approaches. Studies from Sweden show that 54% of CC occurs within one year of the diagnosis of PSC and 27% are diagnosed at liver transplantation. Overall, 12.2% of Northern European PSC patients develop CC, which is corroborated by our data from Hannover (Boberg 2002, Tischendorf 2006). These patients suffer from jaundice, pruritus and abdominal pains and had a longer IBD history. Male gender and smoking are also risk factors (Tischendorf 2006, Weismüller 2008). In a Dutch study there were similar findings of 18 CC out of 174 patients (10%) (Ponsioen 2002). The CC risk of a PSC patient amounts to 1.5% per year and is 161-fold higher than in healthy controls. In the future, the option of proteomic analyses of bile (and urine) may be of importance to predict the risk of cancer (Metzger 2013).

It is also important to realise that the risk for colorectal cancer (CRC) is elevated 10-fold, in addition to a 14-fold risk of pancreatic cancer (Bergquist 2002). These data point to the need of annual colonoscopies and ultrasound studies after diagnosis of PSC to monitor the high potential for cancer development.

Table 8. Cancer association of PSC

Cholangiocarcinoma	10–20% of PSC patients Yearly risk 1.5% Frequent within 1 year of diagnosis Bilirubin, male gender, long-standing ulcerative colitis, abdominal symptoms, smoking
Colorectal cancer	10-fold risk (PSC and ulcerative colitis) Yearly colonoscopies in ulcerative colitis In ulcerative colitis and AP elevation: consider ERC
Pancreatic cancer	14-fold risk in PSC patients Abdominal ultrasound

Medical therapy of PSC

Present day data and clinical experience do not suggest that PSC can be curable by medical therapy (Zein 2010, Wiencke 2011). A cure would include the improvement or normalisation of abnormal cholestatic biochemical features but more importantly the improvement of sclerosing changes to the intra- and extrahepatic biliary tree, which ultimately lead to biliary cholangitis, to episodes of cholangitis, and, which carry the risk of cholangiocellular carcinoma. The only available drug that combines a favourable toxicity profile and can lead to a reduction of cholestatic serum parameters currently is ursodeoxycholic acid (UDCA). However, controversy surrounds the use of UDCA (Chapman 2010), which has recently led to guidelines that do not specifically recommend UDCA treatment in all adult patients (Guidelines 2009, Chapman 2010).

In two studies an improvement was documented using 20 mg/kg body weight, and 25–30 mg/kg body weight, respectively (Harnois 2001, Mitchell 2001). Both use UDCA doses, which are considerably higher than those common in the therapy of PBC (15 mg/kg body weight). From these data a higher dose appeared to be more beneficial in PSC. However, a study analysing UDCA in bile as a function of oral UDCA dose found that doses exceeding 25 mg/kg body weight are not likely to be useful since the maximum transport of UDCA into the bile leveled off at 25 mg/kg with no further increase (Rost 2004). After these and other initial reports, a meta-analysis was published in 2002 (Chen 2003), that concluded that UDCA therapy improved biochemical parameters but that overall benefit in patients with PSC, in particular survival benefit, was uncertain. A large study appeared to confirm this: 219 PSC patients in a placebo-controlled trial (Olsson 2005) received 17 to 23 mg/kg body weight of UDCA and a trend towards better survival and less need for transplantation was seen, but did not reach statistical significance. A difference in the incidence of cholangiocarcinoma was not observed. However, statistical analyses

reported in this study concluded that 346 patients would have been required to reach statistical significance.

Recent reports show that the withdrawal of UDCA – which would follow the US practice guidelines – leads to a biochemical deterioration in PSC patients (Wunsch 2014). As in PBC biochemical remission appears to be associated with a favourable prognosis also in PSC patients (Lindstrom 2013). Therefore, based on the body of literature available, a positive effect of UDCA at present cannot be excluded, withdrawal may not be in the best interest of the patient, and clearly larger placebo-controlled studies are required. This will only be possible in multicentre trials, which are not likely to be conducted in the near future.

The issue of a protective effect of UDCA on colonic neoplasia reported in the past has not been replicated (Lindstrom 2012).

The issue of immunosuppression in PSC is controversial and the majority of centres and publications do not recommend the routine administration of corticosteroids and other immunosuppressants (van Hoogstraten 2000, LaRusso 2006). In PSC one of the most feared and unpredictable complicating factors is bacterial cholangitis and cholangiosepsis (Negm 2011). Immunosuppression would be expected to aggravate this complication. In rare instances such as overlapping features of PSC and autoimmune hepatitis (AIH) (Boberg 2011), immunosuppression may be of benefit but this requires rigorous documentation of AIH, which includes biopsies, autoimmune serology and suggestive biochemistry (Boberg 1996, Beuers 2005).

A potential future drug is nor-UDCA, which is being evaluated in clinical studies. Nor-UDCA undergoes a different shunting route than UDCA and is not conjugated. In animal models nor-UDCA has led to significant effects on the development and progression of sclerosing cholangitis (Fickert 2006, Fickert 2013). Meanwhile a phase 2 trial has been completed and reported at international meetings but not yet published. Nor-UDCA was shown to reduce biochemical parameters in a dose-dependent fashion irrespective of prior treatment with UDCA or UDCA treatment failure. No safety issues were reported and a phase 3 study is planned.

Therapy of IBD in PSC

Many PSC patients suffer from a milder course of IBD. Ulcerative colitis is frequently characterised by pancolitis without severe symptoms, rectal sparing or backwash ileitis. Nevertheless the risk of dysplasia and CRC remains significantly higher in PSC patients with ulcerative colitis. Therapeutic intervention is no different than that for IBD without PSC.

Endoscopic therapy

The most important factor determining the course of PSC is the development of biliary strictures, which carry and increase the risk of septic cholangitis driving biliary fibrosis (Figures 4 and 5). Endoscopic dilatation can improve cholestasis, in some cases biliary stenting (Weismüller 2008), which is not recommended by all gastroenterologists. The international PSC study group is conducting a prospective study (DilStent Study) to evaluate stenting versus balloon dilatation therapy in PSC. The combination of endoscopic intervention and UDCA therapy appears to lead to a significant prolongation of transplant-free survival. UDCA alone does not lead to this effect.

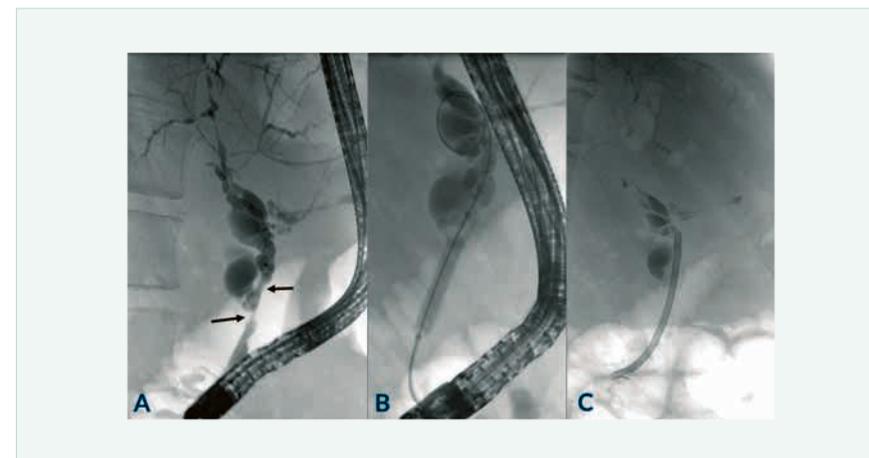


Figure 6. Management of PSC by dilatation of a dominant stricture of the common bile duct (arrows) and subsequent short-term stenting with a plastic stent. In this particular case it turned out that the biliary biopsy revealed cholangiocarcinoma

Liver transplantation in PSC (OLT)

In PSC patients survival has been shown to be reduced both in symptomatic and in asymptomatic patients (Kim 2000, LaRusso 2006), which is in part attributable to the inherent risk of cholangiocarcinoma affecting 10–20% of these patients, and renders decision-making for liver transplantation a formidable challenge. In addition, PSC patients with advanced destructive cholangiopathy frequently exhibit only mild signs of liver failure based upon coagulation abnormalities, hypoalbuminaemia, or complications of portal hypertension (Tischendorf 2007, Strassburg 2009). The course of deterioration to liver failure is often observed after long periods of clinical stability, and frequently proceeds rapidly following septic biliary complications. This is not well predicted by the aforementioned PSC

scores, which is also true for the model of end-stage liver disease (MELD), the measure for organ allocation in the US and the Eurotransplant member countries.

Two major problems define the challenges involved in the indication for liver transplantation in PSC. Firstly, timing is difficult (Wiesner 1992). PSC patients are young and preemptive liver transplantation carries a higher short-term risk of OLT itself than the most likely short-term natural course of the disease. On the other hand, patients that urgently require OLT because of advanced biliary destruction frequently do not meet priority criteria calculated by the MELD system. In Germany, allocation can proceed by stand exception priority if PSC patients fulfill the requirements. Secondly, the 161-fold increase of cholangiocarcinoma risk (Bergquist 2002) is a risk that may eliminate the option of liver transplantation altogether when evidence of cholangiocarcinoma is detected by diagnostic imaging procedures. The diagnosis of early cholangiocarcinoma is difficult and presently no single diagnostic procedure with high sensitivity and specificity is available (Tischendorf 2006). Moreover, the patients at risk cannot be reliably identified.

In terms of practical management the first point can only be addressed by careful clinical monitoring of PSC patients in experienced hepatological transplant centres, where the likelihood of early complication diagnosis and management, as well as the individualised timing of wait-listing for OLT is higher (Tischendorf 2007). The second point has been addressed in two centres by establishing specific protocols for the management of hilar cholangiocarcinoma and OLT (Sudan 2002, Rea 2005). A rigorous algorithm for non-resectable hilar cholangiocarcinoma patients who were carefully selected and capable of surviving chemotherapy, radiation therapy and surgery was reported. A multimodal approach including neoadjuvant chemo-/radiation therapy, brachytherapy, chemotherapy, laparotomy and OLT was employed resulting in a five-year survival of 82%, which did not differ from results in PSC patients without cholangiocarcinoma (Rea 2005). However, although attractive, these interdisciplinary strategies are best limited to studies and experienced hepatological transplant centres.

Overall the results of liver transplantation in PSC are good, leading to 10-year survival rates of around 70% (Graziadei 1999). In our centre, the median survival of PSC patients with cholangiocarcinoma was 12.7 months, and all PSC patients, irrespective of OLT, had a mean survival of 112 months (Tischendorf 2006). Recurrence after OLT is difficult to diagnose but appears to occur in up to 25% of patients (Graziadei 1999, Rowe 2008). Liver transplantation continues to represent the only curative option in PSC. Future developments will have to address the low sensitivity and specificity of early cholangiocarcinoma detection, the clinical prediction of the course of disease and consequently, specific allocation criteria for patients with PSC.

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25. Alcoholic hepatitis

Claus Niederau

Health and social problems due to alcohol overconsumption

Mortality due to alcohol overconsumption is high, in particular among young men (Mokdad 2000). Alcohol overconsumption not only increases the risk for liver disease but is also responsible for malignancies, accidents, violence, and social problems (Bellentani 1997, Vaillant 1995). Alcohol consumption in excess of 20–30 g for women and 40–60 g for men per day markedly increases the risk for liver disease (Becker 1996, Lucey 2008). However, liver cirrhosis is seen only in a minority of subjects with high alcohol consumption; less than 10% of subjects who drink more than 120 g of alcohol daily have cirrhosis (Bellentani 1997). In addition to the level of alcohol consumption, various other factors such as sex, other genetic characteristics and comorbidities contribute to the risk for liver disease (Nishigushi 1991, Becker 1996, Bellentani 1997, McCollough 1998, de Alwis 2007, Lucey 2009).

Alcoholic liver disease is the most prevalent cause of advanced liver disease in Europe (EASL 2012). Excessive alcohol use is also a major cause of preventable liver disease worldwide. However, despite its significant health burden, this is an area with limited research. Per capita alcohol consumption is closely associated with mortality from liver disease across countries (EASL 2012).

Excessive alcohol use and alcohol dependence may be seen as different sides of the same coin (EASL 2012). The WHO uses the terms hazardous and harmful alcohol use instead of alcohol abuse. A binge drinking pattern is becoming increasingly prevalent, mainly among young individuals, but little is known about its impact on liver disease (EASL 2012).

Quantity-frequency questionnaires and retrospective diaries can be used to calculate drinking habits. A good alternative to using quantity alcohol assessments are instruments to screen for risky drinking and alcohol dependence. Among these tools, the AUDIT (Alcohol Use Disorders Inventory Test) (Gual 2002) remains the 'gold standard' (EASL 2012). Brief motivational interventions should be routinely used in the medical management of alcohol use disorders (University of Sheffield 2009, Kaner 2009, EASL 2012).

Prevention of harmful alcohol use

- EASL guidelines make strong statements on preventive measures against excessive alcohol use (EASL 2012) which however still lack broader political and public support.
- Any evidence-based policy in Europe needs to implement preventive measures to reduce alcohol consumption at the population level.
- Excess alcohol consumption may need to be addressed and controlled using pricing-based policies (i.e., special taxes and tariffs, similar to cigarettes).
- Restrictions on the number of alcohol vendors should be used to control alcohol consumption.
- Advertising of alcohol either directly or indirectly should be banned.
- Primary care facilities for managing alcohol use disorders need to be made widely available.

Classification and natural history of alcoholic liver disease

Excessive alcohol consumption most often causes fat accumulation of hepatocytes, called hepatic steatosis (Figure 1). Alcohol-induced steatosis is in general reversible after alcohol abstinence. Continued alcohol overconsumption in the presence of steatosis markedly increases the risk for development of hepatitis, fibrosis and cirrhosis (Teli 1995, Cubero 2009). Patients with alcohol-induced cirrhosis have a significantly increased risk for hepatocellular carcinoma (McCollough 1998). Patients who only have fatty liver in the absence of inflammation and fibrosis have a much lower risk for development of cirrhosis than those with fatty liver plus presence of inflammation and fibrosis. The latter group of patients with alcoholic fatty liver, inflammation and fibrosis is defined as alcoholic steatohepatitis (ASH). The liver histology of patients with ASH is similar when compared to patients with non-alcoholic steatohepatitis (NASH) that is often associated with obesity and diabetes (Ludwig 1980, Brunt 1999).

The diagnosis of ASH by liver biopsy thus helps to define the risk for development of cirrhosis. The histological diagnosis of ASH however should not be confused with the term “alcoholic hepatitis” (also called “acute alcoholic hepatitis”) although its course can be a rather chronic one (Lucey 2009). This overview article concentrates on alcoholic hepatitis, which is a clinical diagnosis of a rather acute development of jaundice and liver

failure associated with a high short-term mortality. In contrast to alcoholic hepatitis, there is as yet no specific pharmacological therapy for alcoholic cirrhosis (EASL 2012).

The factor(s) that set off the development of severe alcohol hepatitis are not exactly known. In general, pathogenesis and individual predisposition are governed by gene-environment interactions in all types of alcoholic liver disease (Figure 1). Based on the second-hit or multiple-hits hypothesis, patients are predisposed to progressive alcoholic liver disease when a specific combination of gene and environmental interaction exists (Tsukamoto 2009). A loss or gain of function genetic model has become a popular experimental approach to test the role of a gene as a second hit. Significant interactions for progressive development of alcoholic liver disease have been proven in particular for female gender, obesity, various drugs, iron overload, and hepatitis B and C viral infections (Mueller 2009, Machado 2009, Cubero 2009). These factors may also interact in the development of hepatocellular carcinoma (HCC).

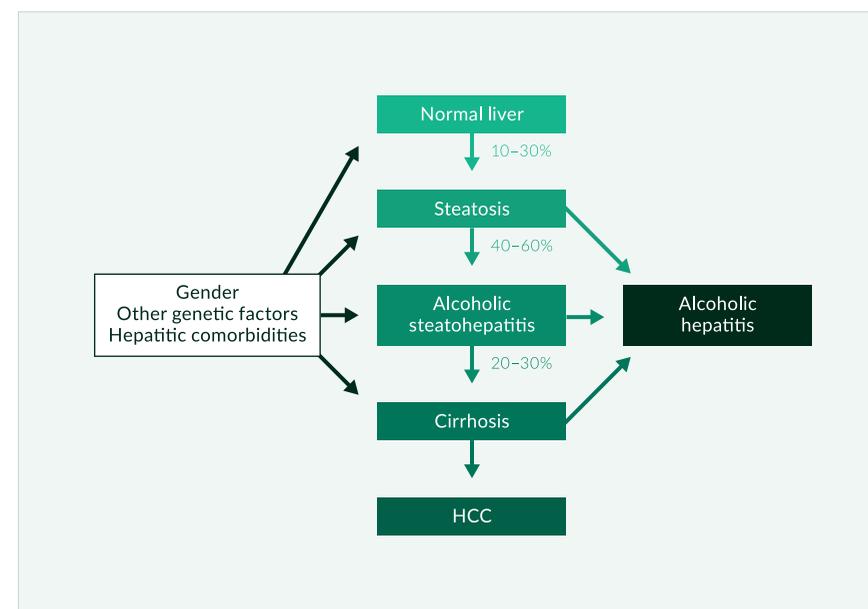


Figure 1. Effects of alcohol overconsumption on the liver

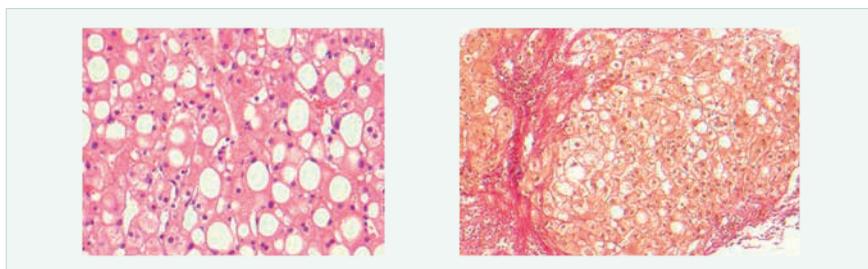
A liver biopsy in someone with alcoholic hepatitis is often similar to a histological feature of ASH. Most patients with histological features of ASH however will not develop alcoholic hepatitis. Alcohol overconsumption leads to a severe form of hepatitis and liver failure associated with a high short-term mortality only in some subjects. Such alcoholic hepatitis may be seen with or without preexisting cirrhosis.

Clinical features and diagnosis of alcoholic hepatitis

Alcoholic hepatitis is a clinical diagnosis characterised by the rapid development of jaundice and liver failure most often due to long-term alcohol overconsumption (Naveau 1997, McCollough 1998, Lucey 2009). Further characteristics include fever, ascites, and in some patients hepatic encephalopathy as well. Usually the liver is enlarged and tender. Women have a higher risk for alcoholic hepatitis than men assuming that both genders drink the same amount of alcohol. The type of alcohol is not associated with the risk. Prevalence was 20% in a cohort of 1604 patients who had a history of heavy alcohol consumption and underwent a liver biopsy (Naveau 1997).

Laboratory markers include increases in serum aspartate aminotransferase (AST) to approximately twice the upper limit of normal (ULN), while the increase in alanine aminotransferase (ALT) is less pronounced. The ratio of AST to ALT is typically >2 (Cohen 1979, Matloff 1980). Other laboratory abnormalities include increases in peripheral leukocytes, serum bilirubin and International Normalized Ratio (INR) (Mathurin 2002, Orrego 1979). In the presence of an increase in serum creatinine there is a high risk for development of an often lethal hepatorenal syndrome (Multimer 1993).

A liver biopsy usually shows large fat droplets and ballooning of hepatocytes that may also include alcoholic hyaline (also called Mallory bodies); these changes are accompanied by neutrophil infiltration and intrasinusoidal fibrosis (Figures 2 and 3) (MacSween 1986).



Figures 2 and 3. Liver biopsies of alcoholic hepatitis

The diagnosis of alcoholic steatohepatitis (ASH) requires the presence of fibrosis. The role of liver biopsy in defining prognosis and treatment of alcoholic hepatitis in the clinical setting remains unclear. Currently, prognosis is usually based on clinical scoring systems and not on liver biopsy (Lucey 2009).

Ultrasound is routinely done to look for hepatocellular carcinoma, biliary obstruction, ascites, splenomegaly, portal vein thrombosis, and signs of portal hypertension. Ascites should be checked for spontaneous bacterial peritonitis routinely.

Differential diagnosis of alcoholic hepatitis includes severe non-alcoholic steatohepatitis (NASH), acute or chronic viral hepatitis, drug-induced injury, autoimmune hepatitis, and Wilson's disease. NASH shares the histological features of ASH except for the rapid development of jaundice and liver failure.

After discontinuation of alcohol consumption the majority of patients will recover from alcoholic hepatitis, although jaundice, ascites and encephalopathy may persist for weeks or months (Alexander 1971). However, alcoholic hepatitis causes increased mortality in a considerable percentage of patients, despite adequate treatment and abstinence (Mathurin 2002, Orrego 1979).

Until recently, there was no histologic classification system to determine the prognosis of patients with alcoholic hepatitis. A recent study evaluated the histologic features associated with disease severity and proposed a histologic score to predict short-term (90-day) mortality (Altamirano 2014). The primary analysis included data from 121 patients admitted to a liver clinic in Barcelona, Spain. The system was updated in a test set of 96 patients from 5 academic centres in the United States and Europe, and a semiquantitative scoring system called the Alcoholic Hepatitis Histologic Score (AHHS) was developed (Alamirano 2014). The system was validated with an independent group of 109 patients. The degree of fibrosis and of neutrophil infiltration, type of bilirubinostasis, and presence of megamitochondria were independently associated with 90-day mortality. These four parameters are used for the AHHS to identify patients with a low (0–3 points), moderate (4–5 points), or high (6–9 points) risk of death within 90 days (3%, 19%, and 51%, respectively; $p < 0.0001$). The AHHS estimated 90-day mortality in the training and test sets with an area under the receiver operating characteristic analysis of 0.77 (95% confidence interval 0.71–0.83). The AHHS thus is likely to be useful in clinical decision-making.

Course and severity

Severe alcoholic hepatitis occurs in a small fraction of patients who have high alcohol consumption. In most cohorts, the 28-day mortality is high and ranges from 30% to 50% (Cohen 2009). Various scores have been used to predict the prognosis of alcoholic hepatitis. Maddrey's discriminant function (Maddrey 1978) and the Model for End-Stage Liver Disease (MELD) score may help to identify patients who can benefit from

corticosteroids. Most scores share some important characteristics such as serum bilirubin and prothrombin time (Srikureja 2005). Maddrey's discriminant function is calculated using the equation: $[4.6 \times (\text{prothrombin time} - \text{control prothrombin time, in seconds})] + \text{serum bilirubin (mg/dL)}$. A value of >32 indicates severe alcoholic hepatitis and consequently calls for the use of corticosteroids (Maddrey 1978). In two retrospective studies, the MELD score predicted short-term mortality in alcoholic hepatitis as well as, or better than, Maddrey's discriminant function (Dunn 2005, Srikureja 2005). A MELD score >21 was associated with a 90-day mortality of 20%. The Lille score is based on pretreatment data and on the response of serum bilirubin to a 7-day treatment with corticosteroids and has been used to determine whether corticosteroids should be discontinued after 7 days because of treatment failure (Forrest 2005, Dunn 2005, Louvet 2007). Patients with Maddrey's discriminant function of <32 usually have mild disease with a short-term survival of more than 90% and will not benefit from corticosteroid treatment.

Investigators reported the results of a stepwise logistic-regression identifying variables related to survival 1–4 months after hospital admission in patients with alcoholic hepatitis (Forrest 2005); by using this data the Glasgow alcoholic hepatitis score was developed (not to be confused with the Glasgow coma score). The score, which includes age, peripheral leukocytes, urea nitrogen, bilirubin, and prothrombin time, may help to identify high-risk patients who should receive corticosteroids. Patients with a Maddrey's discriminant function >32 and a Glasgow alcoholic hepatitis score of >9 who were treated with corticosteroids had an 84-day survival of 59%, while untreated patients had a 38% survival (Forrest 2007). In one study, the Glasgow score indicated which subgroup of patients with a high score of Maddrey's discriminant function would benefit from corticosteroid therapy (Forrest 2007).

Child-Pugh (CP) and MELD scores have been widely used to predict survival in cirrhotic patients. Recent studies have suggested that the addition of serum sodium to MELD (MELD-Na score) may improve its prognostic accuracy. Another recent study compared the performance of CP, MELD, and MELD-Na scores in predicting 6-month mortality in patients with alcoholic cirrhosis, and developed a new prognostic score (Demy 2009). In this study, two French centres randomised 520 patients (mean age 56.4 ± 10.2 years) with alcoholic cirrhosis into two groups. MELD, MELD-Na1, and MELD-Na2 were calculated according to UNOS recommendations. Frequencies of CP classes were: A – 29.6%, B – 25.8%, C – 44.6%. Of the 520 patients, 93 died during the 6-month follow-up. In the whole population, the values of CP, MELD, MELD-Na1, and MELD-Na2 for prediction of 6-month mortality were similar. Multivariate analysis identified age, bilirubin, urea, prothrombin time, sodium, and alkaline phosphatase as independent predictors of

6-month mortality. The score combining these six variables was named the Prognostic Score for Alcoholic Cirrhosis (PSAC) and compared to the four other scores. The predictive value of PSAC was better than all other scores except for MELD-Na2. By stepwise multivariate analysis, PSAC was identified as independently associated with 6-month mortality at the first step, and CP at the second. The new PSAC score may improve the prognostic accuracy to predict the 6-month outcome (Demy 2009).

Another study analysed the outcome of 79 patients who were admitted to an Intensive Care Unit (ICU) because of alcoholic liver disease (Rye 2009). The value of various scores was analysed for predicting mortality including the Acute Physiology, Age and Chronic Health Evaluation (APACHE II), Sequential Organ Failure Assessment (SOFA), CP, and MELD scores. The major reason for admission was sepsis (44%). The observed mortality in the ICU was 49% and hospital mortality 68%. Compared to survivors, non-survivors had a significantly higher serum bilirubin, creatinine and prothrombin time, and lower GCS and length of ICU stay. Survival was affected by cardiac arrest pre-admission (mortality 75%) and number of organs supported (mortality 8% with no organ support, 79% ≥ 2 organs, 100% ≥ 3 organs). Renal replacement therapy was associated with 100% mortality. Mortality due to GI bleeding was only 33%. Thus, cirrhotic patients who are admitted to ICU with cardiac arrest pre-admission, the need for renal replacement therapy, or multiple organ support, have a poor prognosis. The predictive accuracy of SOFA and MELD scores were superior to APACHE II and Child-Pugh scores in cirrhotic patients (Rye 2009).

A further study analysed the mortality of 105 patients presenting with alcoholic hepatitis (Hussain 2009). Patients were evaluated by the modified discriminant function (mDF) for alcoholic liver disease, CP score, and Glasgow alcoholic hepatitis score (GAHS). Mean survival for those alive at the end of the study ($n=36$) was 34.6 ± 17.8 months. Mean survival for those who died ($n=50$) was 13.2 ± 14.4 months. The mDF, CP and GAHS scores were significant predictors of mortality in this population. Prothrombin time was also a significant predictor of mortality (Hussain 2009).

Mechanisms of alcohol-related liver injury

Alcoholic liver disease is initiated by different cell types in the liver and a number of different factors including products derived from alcohol-induced inflammation, ethanol metabolites, and indirect reactions from those metabolites, as well as genetic predisposition (Colmenero 2007). Ethanol oxidation results in the production of metabolites that have been shown to bind and form protein adducts, and to increase inflammatory, fibrotic and cirrhotic responses. Lipopolysaccharide (LPS) has many deleterious effects

and plays a significant role in a number of disease processes by increasing inflammatory cytokine release. In alcoholic liver disease, LPS is thought to come from a breakdown in the intestinal wall enabling LPS from resident gut bacterial cell walls to leak into the blood stream. The ability of adducts and LPS to independently stimulate various cells of the liver provides for a two-hit mechanism by which various biological responses are induced and result in liver injury.

Alcohol (ethanol) can be oxidised by various enzymatic and non-enzymatic pathways (Figure 4). In hepatocytes, the most important pathway is oxidation of ethanol via alcohol dehydrogenase (ADH) to acetaldehyde (Figure 4). In mitochondria, acetaldehyde is converted to acetate and in turn acetate is converted to acetyl CoA, which leads the two-carbon molecule into the TCA (tricarboxylic acid cycle).

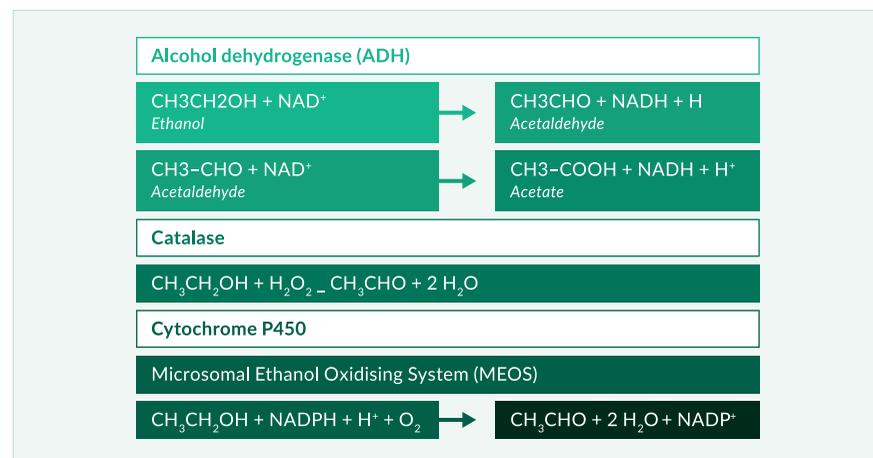


Figure 4. Oxidation of ethanol to acetaldehyde by enzymatic pathways

This oxidation generates reducing equivalents, primarily reduced nicotinamide adenine dinucleotide (NAD), i.e., NADH. The changes in the NADH–NAD⁺ potential in the liver inhibit both fatty acid oxidation and the TAC and may thereby increase lipogenesis (You 2004a). Ethanol has also been shown to increase lipid metabolism by inhibiting peroxisome-proliferator-activated receptor α (PPAR α) and AMP kinase as well as by stimulation of sterol regulatory element-binding protein (Fischer 2003, You 2004b, Ji 2006). All these mechanisms lead to hepatic steatosis. Further enzymatic pathways of ethanol oxidation include catalase and the “Microsomal Ethanol Oxidising System” (MEOS), a cytochrome P450 component. Oxidation of ethanol to acetaldehyde may also be due to non-enzymatic free radical pathways (Figure 5). These pathways include strong oxidising intermediates such as the hydroxyl radical which can abstract a

hydrogen atom from ethanol, preferentially producing the 1-hydroxyethyl radical; hypervalent iron complexes may also catalyse this reaction without involvement of $\cdot\text{OH}$ (Reinke 1994, Welch 2002, Qian 1999). Hydroxyethyl radicals may then react with oxygen to form a peroxy radical intermediate which can rearrange to release acetaldehyde and superoxide. Hydroxyethyl radicals can also react with proteins to produce antigenic adducts or induce mitochondrial permeability transition (Clot 1995, Sakurai 2000).

There are probably various other mechanisms by which ethanol may cause or contribute to liver disease. Ethanol increases the translocation of lipopolysaccharide (LPS) from the small and large intestines to the portal vein and on to the liver. In Kupffer cells, LPS can bind to CD14, which combines with toll-like receptor 4 (TLR4) thereby activating multiple cytokine genes (Schaffert 2009). In addition, NADPH oxidase may release reactive oxygen species (ROS) that activate cytokine genes within Kupffer cells, hepatocytes, and hepatic stellate cells. These cytokines including TNF- α may cause fever, anorexia, and weight loss. Interleukin-8 and monocyte chemotactic protein 1 (MCP-1) have been shown to attract neutrophils and macrophages. Platelet-derived growth factor (PDGF) and transforming growth factor b (TGF-b) contribute to the activation, migration, and multiplication of hepatic stellate cells, thereby promoting liver fibrosis.

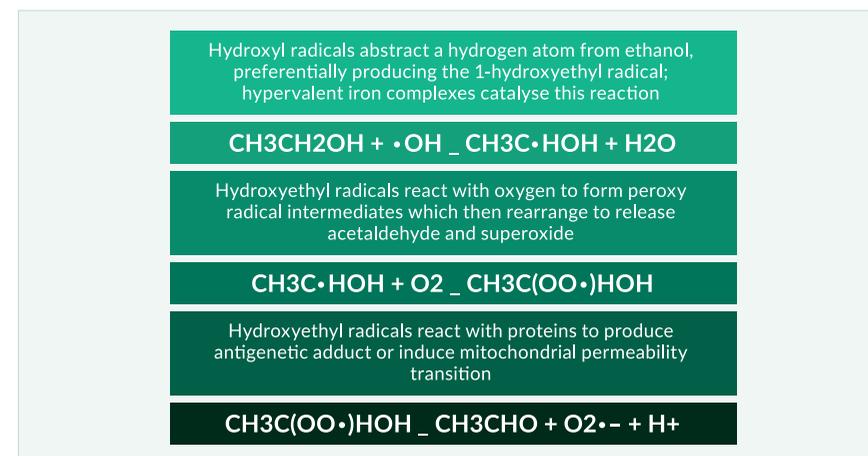


Figure 5. Oxidation of ethanol to acetaldehyde by non-enzymatic free radical pathways

In the hepatocyte, ethanol is converted to acetaldehyde by the cytosolic enzyme alcohol dehydrogenase (ADH) and the microsomal enzyme cytochrome P450 2E1 (CYP2E1). Acetaldehyde is converted to acetate. These reactions produce NADH and inhibit the oxidation of triglycerides and fatty acids. ROS released by CYP2E1 and mitochondria cause lipid peroxidation. Inhibition of proteasomes due to ethanol disturbs protein catabolism and

may be partly responsible for the formation of Mallory bodies. Reduction in enzymes that convert homocysteine to methionine may increase homocysteine, thereby injuring the endoplasmic reticulum. Decrease in binding of peroxisome proliferator-activated receptor alpha (PPAR- α) to DNA reduces the expression of genes involved in fatty acid oxidation.

Glutathione transport from the cytosol into the mitochondria is reduced by ethanol. Ethanol may also activate Fas and TNF receptor 1 (TNF-R1) thereby activating caspase 8, causing mitochondrial injury and opening the mitochondrial transition pore (MTP), releasing cytochrome c, and activating caspases; all these processes contribute to apoptosis. Activation of TNF-R1 leads to nuclear factor kappa B (NFkB) activation (Schaffert 2009).

Gut permeability and the circulating LPS endotoxin levels of the outer wall of gram negative bacteria are increased in patients with alcoholic liver injury (Uesugi 2002, Bjarnason 1984, Urbaschel 2001). In various animal studies, alcohol exposure promoted the transfer of LPS endotoxins from the intestine into portal blood (West 2005). Oral treatment with antibiotics reduced such increases in LPS endotoxins and ameliorated alcoholic liver injury in animals (Uesugi 2001, Nanji 1994, Adachi 1995). Activation of Kupffer cells by LPS endotoxins involves CD14, toll-like receptor 4 (TLR4), and MD2 (Uesugi 2001, Akira 2001, Yin 2001). The downstream pathways of TLR4 signalling include activation of early growth response 1 (EGR1), NFkB, and the TLR4 adapter also called toll-interleukin-1 receptor domain-containing adapter inducing interferon- β (TRIF) (McCuillen 2005, Zhao 2008). TRIF-dependent signaling may contribute to alcohol-induced liver damage mediated by TLR4 (Hritz 2008).

Many animal studies have also shown that alcohol increases various markers of oxidative stress (Meagher 1999, Wu 2009). Studies in rats and mice suggest that activated macrophages (Kupffer cells) and hepatocytes are the main sources of alcohol-induced free radicals (Bailey 1998, Kamimura 1992). Oxidative stress may mediate alcohol-induced liver injury, e.g., via cytochrome P450 2E1 (Mansuri 1999, Lu 2008), leading to mitochondrial damage, activation of endoplasmic reticulum-dependent apoptosis, and up-regulation of lipid synthesis (Ji 2003, Yin 2001). Activated Kupffer cells will also release TNF- α ; this cytokine plays an important role in the pathogenesis of alcoholic hepatitis. Circulating TNF- α concentrations are higher in patients with alcoholic hepatitis than in heavy drinkers with inactive cirrhosis, heavy drinkers who do not have liver disease and persons who do not drink alcohol and who do not have liver disease (Adachi 1994, Bird 1990). Circulating TNF- α concentrations are associated with high mortality (Bird 1990). In animal studies, knockouts of the TNF receptor 1 and administration of the anti-TNF agent thalidomide both ameliorated alcohol-induced liver injury (Yin 1999, Imuro 1997, Enomoto 2002). Ethanol was also shown to release mitochondrial cytochrome c and to induce

expression of the Fas ligand that may then cause apoptosis via the caspase-3 activation pathway (Zhou 2001). Both TNF- and Fas-mediated signals may increase the vulnerability of hepatocytes (Minagawa 2004).

Role of PNPLA3 polymorphisms and other genetic factors in the progression of alcoholic liver disease

The genetic determinants of the pathogenesis and disease progression of alcoholic and non-alcoholic fatty liver disease remained obscure until recently. In 2008, two genome-wide association (GWAS) studies linked the rs738409 polymorphism (I148M) of patatin-like phospholipase domain containing 3 (PNPLA3) with hepatic fat content and ALT levels (Romeo 2008, Yuan 2008). Later several further studies corroborated such association between the I148M polymorphism and NAFLD in almost all ethnic and age groups (Shang 2014, DiStefano 2014, Baclig 2014, Trepo 2014; for further literature see chapter on non-alcoholic fatty liver disease). The I148M polymorphism also predisposed to cirrhosis (Shen 2014) and hepatocellular carcinoma (Burza 2012, Valenti 2013, Trepo 2014). Other recent data suggest that the I148M PNPLA3 polymorphism also accelerates fibrosis progression and HCC incidence in alcoholic liver disease (Trepo 2012, Nault 2014, Buch 2015, Stickel 2015, Falleti 2015). A recent genome-wide association study (GWAS) confirmed PNPLA3 and identified TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis (Buch 2015). All three loci are known to have a role in lipid processing, suggesting that lipid turnover is important in the pathogenesis of alcohol-related cirrhosis. Another recent study assessed the interaction between PNPLA3 rs738409 and TM6SF2 rs58542926 variants for HCC development (Falleti 2015). The results showed that TM6SF2 C/T or T/T in conjunction with PNPLA3 G/G variants may be potential genetic risk factors for developing HCC in alcohol-related cirrhosis (Falleti 2015). Details and mechanisms are discussed in a recent review about the genetics of alcoholic liver disease (Anstee 2015).

Treatment

Abstinence from alcohol

Continued alcohol use after diagnosis of alcoholic liver disease is the most important risk factor for complications and death (EASL 2012, Bell 2004). In such patients the development of new episodes of ASH are associated with

a bad prognosis. Nicotin use has also been shown to be associated with mortality in patients with alcoholic liver disease (Pessione 2003). Other comorbid diseases further increase the risk of both cirrhosis-related and non-cirrhosis-related death (Jepsen 2008). Thus, after recovery from liver failure all patients with alcoholic hepatitis need to have psychological and social support to assure continued abstinence (Saitz 2007).

Supportive therapy

There is still a lack of specific therapy for patients with alcoholic hepatitis although prednisolone and pentoxifylline may have beneficial effects in severe disease. It is, however, generally accepted that all complications and risks such as ascites, encephalopathy, hepatorenal syndrome, and infections should be treated like other decompensated liver diseases (Kosten 2003, Sanyal 2008, Lim 2008). Daily protein intake should be at least 1.5 g/kg. Vitamin B1 and other vitamins should be administered according to recommended references (Barr 2006).

Corticosteroids

Various studies and meta-analyses show controversial results for the use of corticosteroids in alcoholic hepatitis (Imperiale 1990, Christensen 1999, Imperiale 1999, Rambaldi 2008). In general, corticosteroids have not been shown to increase survival, in particular during longer follow-up (Rambaldi 2008). However, there is evidence that corticosteroids do reduce mortality in a subgroup of patients with a Maddrey's discriminant function >32 or in those presenting with hepatic encephalopathy (Rambaldi 2008). A meta-analysis of three studies corroborated that corticosteroids given for 28 days increase 1-month survival by 20% in severe alcoholic hepatitis (Maddrey's discriminant function >32) (Mathurin 2002). In these studies Maddrey's discriminant function >32 resembled a MELD score of >21 . Prednisolone was usually given at 40 mg a day for 28 days. In some studies prednisolone was stopped completely at 28 days (Mathurin 2003), while the dose was gradually reduced in other studies (Imperiale 1990). Corticoids should not be given in the presence of sepsis, severe infection, hepatorenal syndrome, chronic hepatitis B, or gastrointestinal bleeding (O'Shea 2006).

The mechanisms by which corticosteroids improve short-term survival in severe alcoholic hepatitis are not fully understood. In general, corticosteroids inhibit various inflammatory processes by acting on activator protein 1 and NF κ B (Barnes 1997). In patients with alcoholic hepatitis, some studies reported that corticosteroids were associated with

a decrease in circulating levels of proinflammatory cytokines such as interleukin-8, TNF- α and others (Taieb 2000, Spahr 2001).

Recent reviews and recommendations conclude that corticosteroids should not be given to patients with a Maddrey's discriminant function <32 or a MELD score <21 until further data can identify patients with a high short-term risk (Lucey 2009). Corticosteroids are thus ineffective in a large group of patients with alcoholic hepatitis and probably do not affect the long-term outcome. A randomised controlled clinical trial has shown that prednisolone 30 mg daily is superior to a broad antioxidant cocktail in the treatment of severe alcoholic hepatitis (Phillips 2006).

There is also evidence that corticosteroids can be discontinued after 7 days if there is no obvious improvement in clinical signs and symptoms and in serum bilirubin (Maddrey 1978, Dunn 2005, Forrest 2005, Louvet 2007).

The most recent Cochrane meta-analysis reported that corticosteroids significantly reduce mortality in trials that enrolled patients with a Maddrey score of at least 32 or with hepatic encephalopathy (Rambaldi 2008). Recent EASL and US guidelines thus also recommend corticosteroids for initial treatment of severe alcoholic hepatitis in the absence of sepsis and infections (O'Shea 2010, EASL 2012).

Pentoxifylline

In a randomised, controlled trial, pentoxifylline (400 mg TID for 28 days) reduced short-term mortality in severe alcoholic hepatitis (Maddrey's discriminant function >32); mortality was 24% in the pentoxifylline group and 46% in the placebo group ($p<0.01$) (Akrivadis 2000). This study did not include a group on corticosteroid treatment. Although the phosphodiesterase inhibitor pentoxifylline has been suggested to act as an anti-TNF agent, TNF- α concentrations did not differ significantly between the two groups. Thus, the mechanisms by which pentoxifylline may improve the prognosis in alcoholic hepatitis remains unknown. Interestingly, almost all deaths (22 of 24; 92%) in the placebo group were associated with hepatorenal syndrome while hepatorenal syndrome was considered the cause of death in only 6 of 12 patients (50%) in the pentoxifylline group. Pentoxifylline might therefore exert its beneficial effects by preventing the development of hepatorenal syndrome. Another study (De BK 2009) compared the efficacy of pentoxifylline and prednisolone in the treatment of severe alcoholic hepatitis. This randomised double-blind controlled study enrolled 68 patients with severe alcoholic hepatitis (Maddrey score >32) who received either pentoxifylline (400 mg TID for 28 days) ($n=34$) or prednisolone (40 mg QD for 28 days) ($n=34$) for 28 days, with a subsequent open-label study (with a tapering dose of prednisolone) for a total of 3 months, and follow-up

over 12 months. Twelve patients in the corticosteroid group died by the end of month 3 in contrast to five patients in the pentoxifylline group (mortality 35.3% vs 14.7%, $p=0.04$). Six patients in the corticosteroid group but none in the pentoxifylline group developed hepatorenal syndrome. Pentoxifylline was associated with a significantly lower MELD score at the end of 28 days of therapy when compared to corticosteroids (15.5 ± 3.6 vs 17.8 ± 4.6 ; $p=0.04$). Reduced mortality, improved risk: benefit profile and renoprotective effects of pentoxifylline compared with prednisolone suggest that pentoxifylline is superior to prednisolone for treatment of severe alcoholic hepatitis. Interestingly, long-term pentoxifylline therapy had also been shown to effectively achieve sustained biochemical improvement and even histological improvement in non-alcoholic steatohepatitis (Satapathy 2007). In a prospective, randomised study pentoxifylline (400 mg orally, three times a day for 4 weeks) for 4 weeks in patients with severe alcoholic hepatitis (Sidhu 2012) reduced mortality at 4 weeks compared to placebo (20% versus 40%; $p=0.216$). Renal failure was the cause of mortality in 20% of patients in the pentoxifylline group and in 70% of controls ($p=0.11$). Significant reduction in urea, creatinine, Maddrey score and TNF was noted in pentoxifylline group. This study showed that pentoxifylline improved renal and hepatic function with a trend towards decreased short-term mortality (Sidhu 2012). Pentoxifylline treatment is still recommended for severe alcoholic hepatitis by current EASL and US guidelines, in particular when the use of corticosteroids is risky for infection and sepsis (O'Shea 2010, EASL 2012).

Comparison and combination of corticosteroids and pentoxifylline

A multicentre, randomised, double-blind study evaluated whether the addition of pentoxifylline to prednisolone is more effective than prednisolone alone in patients with severe biopsy-proven alcoholic hepatitis (Mathurin 2013). The study included 270 French and Belgian heavy-drinkers with a recent onset of jaundice in the previous 3 months and a Maddrey score of at least 32. Patients were randomly assigned to receive either a combination of 40 mg of prednisolone once a day and 400 mg of pentoxifylline 3 times a day for 28 days, or 40 mg of prednisolone and matching placebo for 28 days. In an intention-to-treat analysis, 6-month survival was not different in the pentoxifylline-prednisolone vs. placebo-prednisolone groups (69.9% versus 69.2%; $p=0.91$). By multivariate analysis, only the Lille model and the Model for End-Stage Liver Disease (MELD) scores were independently associated with 6-month survival. Also, the 7-day response and the incidence of hepatorenal syndrome at 6 months were not significantly different in the pentoxifylline-prednisolone and the

placebo-prednisolone groups (Mathurin 2013).

Another study compared the efficacy of corticosteroids plus pentoxifylline with that of corticosteroids alone in patients with severe alcoholic hepatitis (Sidhu 2012). Four-week and six-month survival were not significantly different in the two groups (72.2% and 73.5%, respectively; $p=1.00$; 30.6% and 23.5%, respectively; $p=0.417$) (Sidhu 2012).

There is therefore no evidence that a combination of corticosteroids and pentoxifylline has an advantage compared with corticosteroids or pentoxifylline alone.

Although guidelines recommend the use of corticosteroids and/or pentoxifylline in severe alcoholic hepatitis (O'Shea 2010, EASL 2012), many studies reporting a benefit with these agents have methodological limitations. The STOPAH study, a multicentre, double-blind, factorial (2×2) trial, randomised 1,200 patients with severe alcoholic hepatitis in order to provide sufficient power to determine whether either of the two interventions is effective. Patients were randomised to one of four groups: Group A: placebo / placebo; Group B: placebo / prednisolone; Group C: pentoxifylline / placebo; Group D: pentoxifylline / prednisolone (Forrest 2013). The primary endpoint of the study is mortality at 28 days, with secondary endpoints being mortality at 90 days and 1 year (Forrest 2013). Preliminary results showed that steroids reduced mortality by 39% at day 28 without further sustained effects, whereas pentoxifylline did not have any beneficial effects. The final published results corroborated that pentoxifylline did not improve survival in patients with alcoholic hepatitis. Prednisolone was associated with a reduction in 28-day mortality that did not reach significance and with no improvement in outcomes at 90 days or 1 year. The authors conclude that pentoxifylline should not be used any longer for treatment of alcoholic hepatitis (Thurz 2015).

Recent reviews however still recommend to consider the use of pentoxifylline when prednisolone is contraindicated (Dugum 2015, Rahimi 2015, Liang 2015).

N-acetyl cysteine

A multicentre, randomised, controlled trial (Nguyen-Khac 2011) analysed treatment of severe acute alcoholic hepatitis via corticoids plus N-acetyl cysteine (C+NAC) versus corticoids (C) alone. The background to this approach was the hypothesis that the glutathione precursor NAC may rebuild antioxidant stocks in the hepatocyte. Deaths were significantly lower in the C+NAC group than in the C group at month 1 ($n=7/85$ (8.2%) vs. $21/89$ (23.6%), $p=0.005$) and at month 2 ($13/85$ (15.3%) vs. $29/89$ (32.6%), $p=0.007$) but not at month 3 ($19/85$ (22.4%) vs. $30/89$ (33.7%), $p=0.095$) or

at month 6 (23/85 (27.1%) vs. 34/89 (38.2%)). NAC may improve short-term survival. This improvement, however, is lost by month 3.

Anti-TNF- α therapy

Some smaller studies have shown beneficial results using the TNF- α receptor antagonists infliximab and etanercept in patients with acute alcoholic hepatitis (Spahr 2007, Mookerjee 2003, Tilg 2003, Menin 2004). A larger randomised, controlled clinical trial compared the effects of infliximab plus prednisolone vs placebo plus prednisolone in patients with severe alcoholic hepatitis (Maddrey's discriminant function >32) (Naveau 2004). The trial was stopped early by the safety monitoring board because of a significant increase in severe infections and a (nonsignificant) increase in deaths in the infliximab group. Similarly, etanercept reduced 6-month survival when compared with placebo in a randomised, placebo-controlled trial (Boetticher 2008). Thus, TNF- α receptor antagonists should not be used for clinical therapy of alcoholic hepatitis (Lucey 2009).

Therapy with granulocyte colony-stimulating factor (G-CSF)

A recent randomised study evaluated the hypothesis that treating patients with alcoholic hepatitis with granulocyte colony-stimulating factor (G-CSF) might mobilise bone marrow-derived stem cells and promote hepatic regeneration and thereby improve survival (Singh 2014). One group received standard medical therapy and the other G-CSF at a dose of 5 $\mu\text{g}/\text{kg}$ subcutaneously every 12 h for 5 consecutive days. There was a statistically significant increase in the number of CD34 (+) cells in the peripheral blood in the G-CSF group as compared with the standard therapy group after 5 days of therapy. There was also a significant reduction of survival and in Child-Pugh and MELD scores at 1, 2, and 3 months between the groups favouring G-CSF (Singh 2014). Further studies have to evaluate whether G-CSF is safe and effective in improving liver function and survival in patients with severe alcoholic hepatitis.

Nutritional support

Many patients with alcoholic hepatitis have signs of malnutrition associated with high mortality (Mendenhall 1984, Mendenhall 1986, Stickel 2003). Parenteral and enteral nutrition have been shown to improve malnutrition in alcoholic hepatitis but has not improved survival

(Mendenhall 1984). A randomised, controlled clinical trial looked at the effects of enteral nutrition of 2000 kcal/day via tube feeding versus treatment with 40 mg/day prednisolone for 28 days in severe alcoholic hepatitis. Survival in both groups was similar after one month and one year. It may be concluded that nutritional support is as effective as corticosteroids in some patients (Cabre 2000). However, corticoids in many studies failed to improve long-term survival.

A randomised controlled trial comparing enteral nutrition versus corticosteroids did not show any difference in 28-day mortality rate (Cabre 2003). Indeed, deaths occurred earlier with enteral nutrition whereas steroid therapy was associated with a higher mortality rate in the weeks following the treatment period. Enteral nutrition probably deserves to be tested in combination with corticosteroids (EASL 2012). As yet, only one pilot study suggests that enteral nutrition associated with a short course of steroids may be a good therapeutic strategy for severe alcoholic hepatitis (Alvaraz 2004).

A recent randomised controlled trial determined whether the combination of corticosteroid and intensive enteral nutrition therapy is more effective than corticosteroid therapy alone in patients with severe AH (Moreno 2016). The study enrolled 136 heavy alcohol consumers with recent onset of jaundice and biopsy-proven severe AH; they were assigned randomly (1:1) to groups that received either intensive enteral nutrition plus methylprednisolone or conventional nutrition plus methylprednisolone (controls). In the intensive enteral nutrition group, enteral nutrition was given via feeding tube for 14 days. The primary end point was patient survival for 6 months. In the intention-to-treat analysis, there was no significant difference between groups in 6-month cumulative mortality: 44.4% in the enteral nutrition group vs. 52.1% in the controls ($P = 0.406$). Intensive enteral nutrition was difficult to implement and did not improve survival. However, further analysis showed that low daily energy intake was associated with greater mortality, so adequate nutritional intake should be a main goal for treatment.

Other pharmacologic treatments

The anabolic steroid oxandrolone failed to improve survival in patients with alcoholic hepatitis (Mendenhall 1984). Numerous studies have shown that alcoholic hepatitis is accompanied by oxidative stress. So far, all studies with antioxidants such as vitamin E, silymarin (milk thistle) and others have failed to improve survival in alcoholic hepatitis (Pares 1998, Mezey 2004). Older studies did show that colchicine, propylthiouracil, insulin and glucagon failed to improve survival in alcoholic hepatitis (Lucey 2009).

Liver transplantation

Alcoholic liver disease is still one of the most common indications for liver transplantation in Europe and in the US (Burra 2005, European Liver Transplant Registry 2011, Neuberger 1998, US Transplant Org 2011). In guidelines for liver transplantation, patients need to have at least a 6-month period of alcohol abstinence before they can be evaluated for transplantation, thus alcoholic hepatitis is usually a contraindication for liver transplantation (Lucey 1997, Everhardt 1997, Lucey 2007).

A substantial number of patients with severe alcoholic hepatitis fail to recover despite abstinence and medical therapy (Nakano 1982), and their chances for spontaneous recovery may be poor (Worner 1985). The classical opinion of European and North American experts considering acute alcoholic hepatitis as a contraindication for transplantation (EASL 2012) has recently been challenged by a case-control study showing an unequivocal improvement of survival in patients who received early transplantation (Mathurin 2011). Despite the fact that early liver transplantation for severe alcoholic hepatitis may improve survival in those patients who fail medical therapy, in many countries regulatory rules do not allow such transplants without documentation of six months of abstinence. Future evaluation of liver transplantation in carefully selected patients with severe alcoholic hepatitis who do not respond to standard medical therapy may be supported (Mathurin 2011).

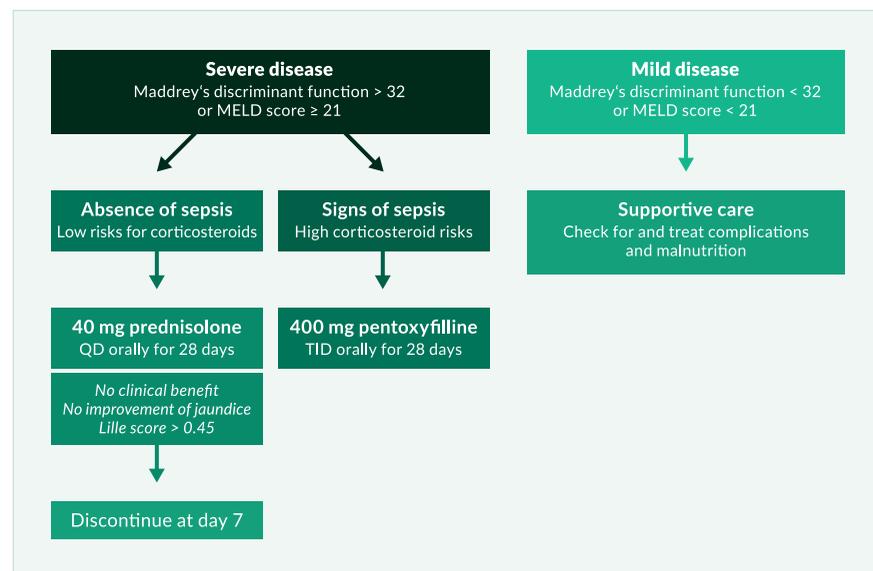


Figure 6. Treatment algorithm in alcoholic hepatitis. The use of pentoxifylline has recently been challenged by a large randomised trial (Thurz 2014); thus, its use is questionable

Summary

Alcoholic hepatitis is a clinical diagnosis based on a history of heavy alcohol consumption, jaundice, other signs of liver failure, and the absence of other causes of hepatitis. A liver biopsy may be helpful but is not required either to determine the diagnosis or prognosis. Abstinence from alcohol is the prerequisite for recovery. Patients with signs of malnutrition should have adequate nutritional support. Subjects with severe alcoholic hepatitis (Maddrey's discriminant function >32 or MELD score >21) who do not have sepsis or other corticosteroid contraindications may receive 40 mg prednisolone daily for 28 days (McCullough 1998, Lucey 2009). A treatment algorithm based on current literature and EASL and US guidelines (O'Shea 2010, EASL 2012) is shown in Figure 6. After 7 days of corticosteroid treatment, patients without obvious clinical benefit, without significant improvement of jaundice and with a Lille score >0.45 may have disease that will not respond to continued treatment with corticosteroids or an early switch to pentoxifylline (Louvet 2008). In situations where administration of corticosteroids appears to be risky, pentoxifylline may be tried (Lucey 2009, O'Shea 2010, EASL 2012); this drug may decrease the risk of hepatorenal syndrome that is often lethal in alcoholic hepatitis. Patients with less severe alcoholic hepatitis have a good short-term survival of $>90\%$ and should not be treated with corticosteroids or pentoxifylline (Mathurin 2002).

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26. Vascular liver disease

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“It is impossible to explain or to understand the morbid appearances of the liver, without referring to its intimate structure, and as some points relating to this have been only lately made out, I shall commence with a short account of it.”

Georg Budd, Diseases of the Liver, 1853

Vascular liver diseases comprise a heterogeneous group of mostly rare hepatic disorders – some of them exceedingly rare.

Every single part of the hepatic vasculature may be affected, i.e., hepatic sinusoids, portal vein, hepatic artery and liver veins. The clinical presentation varies widely depending on the type of disease but also within the individual disease entities. Vascular liver diseases may present as acute disorders or chronic liver disease, as hepatocellular necrosis or cholestasis, as tumour-like lesions or portal hypertension.

The spectrum of underlying causes is wide, and in many cases multiple risk factors will result in the development of clinically significant disease (Table 1).

Table 1. Classification of predisposing factors for vascular liver disease

Hereditary disorders	<ul style="list-style-type: none">• Inherited thrombophilia, e.g., factor V Leiden mutation, mutations of prothrombin, protein C, protein S, antithrombin III• Hereditary hemorrhagic teleangiectasia• SP110-associated sinusoidal obstruction syndrome
Congenital or acquired malformations	<ul style="list-style-type: none">• Webs, shunts, aneurysms
Acquired cellular defects	<ul style="list-style-type: none">• Myeloproliferative neoplasms• Paroxysmal nocturnal hemoglobinuria• Malignancy
Inflammatory disease, immune-mediated disorders	<ul style="list-style-type: none">• Focal inflammatory lesions, e.g., pancreatitis, diverticulitis, appendicitis, cholecystitis, abscesses, inflammatory bowel disease• Vasculitis, e.g., polyarteritis nodosa, Behçet’s disease• Rheumatic disease
Toxicity, radiation, trauma	

Disorders of the hepatic sinusoid

Hepatic sinusoidal disease may present as luminal obstruction (i.e., sinusoidal obstruction syndrome), as luminal enlargement (i.e., peliosis hepatis) or as perisinusoidal fibrosis. Whether the latter represents a separate disease entity is debatable, as perisinusoidal fibrosis is also observed in common diseases such as steatohepatitis. Both sinusoidal obstruction syndrome as well as peliosis hepatis are not strictly confined to the hepatic sinusoids but may extend to the hepatic venous system.

Sinusoidal obstruction syndrome (Hepatic veno-occlusive disease)

Sinusoidal obstruction syndrome (SOS), also referred to as hepatic veno-occlusive disease (VOD), is a circulatory disorder primarily affecting the hepatic sinusoids. Involvement of the hepatic central veins may occur, but studies after conditioning for hematopoietic cell transplantation have demonstrated that in more than 40% of patients with SOS the hepatic venous system is not involved. The proportion of sole sinusoidal affection falls to 25% in patients with progression to severe SOS (DeLeve 2009).

Pathophysiology

Sinusoidal obstruction syndrome may be triggered by a variety of factors (Valla 2016). By far the most common cause in the Western world are myeloablative regimens in preparation for hematopoietic stem cell transplantation (HSCTx), particularly when the transplant is for a malignancy. Historically, the proportion of patients with SOS after HSCTx varied from the single-digit percentage range up to 50% if highly toxic regimens were chosen. Currently, rates between 8% and 14% are reported (Mohty 2015, Richardson 2013). Apart from conditioning regimens for HSCTx (high-dose chemotherapy plus total body irradiation), other drugs have been implicated in the development of SOS (Table 2). Among others and in addition to the intensity of the chemotherapy applied, additional risk factors appear to increase the risk for SOS: genetics, Karnofsky score, exposure to estroprogestatives in women, autologous or allogeneic type of HSCTx, prior myeloablative transplantation or preexistent liver disease (DeLeve 2009, Mohty 2016).

Originally, the syndrome was described in conjunction with the ingestion of herbal teas or foods containing pyrrolizidine alkaloids. Rarely, SOS is caused by hereditary SP110 defects also leading to immunodeficiency syndrome, VODI (Cliffe 2012). Whether immunodeficiency may give rise

to infections causing secondary SOS is under debate. In addition, MTHFR mutations are suggested as a risk factor for SOS (Efrati 2014).

Both the histopathological changes and the clinical picture of SOS were experimentally studied in a rat model using monocrotaline, a pyrrolizidine alkaloid that is directly toxic to sinusoidal endothelial cells. These experiments have confirmed the primary sinusoidal damage infrequently followed by central venous involvement (DeLeeve 1996, Mohty 2015). In addition, chemotherapy might disturb sinusoidal repair by inhibiting mobilisation of bone marrow progenitors of endothelial cells (Vion 2015).

Table 2. Conditions associated with sinusoidal obstruction syndrome

<ul style="list-style-type: none"> • Pyrrolizidine alkaloid-containing herbs, e.g. comfrey, groundsel, rattlebox, traditional Chinese medicine preparations • Radiation exposure • Pregnancy 	<ul style="list-style-type: none"> • Hereditary SP110 defects • MTHFR mutations • ABCB11 mutations
DRUGS	
<ul style="list-style-type: none"> • 6-mercaptopurine • 6-thioguanine • Actinomycin D (Dactinomycin) • Azathioprine** • Busulfan* • Cytosine arabinoside • Cyclophosphamide* • Dacarbazine • Doxorubicin (Adriamycin) 	<ul style="list-style-type: none"> • Gemtuzumab ozogamicin • Irinotecan • Melphalan* • Mitomycin • Oxaliplatin, Carboplatin • Urethane • Vinblastine • Sirolimus • Isavuconazole

*Exclusively reported with conditioning regimens for HSCTx

**Reports for azathioprine-associated SOS included concurrent potential causes of SOS (modified according to DeLeve 2009, Thatishetty 2013, Tewari 2017)

Clinical presentation and diagnosis

SOS characteristically presents with weight gain (associated or not with ascites), hepatomegaly with right upper quadrant pain, and jaundice. The onset of symptoms usually occurs between day 10 and day 20 after cyclophosphamide-containing regimens but can be delayed up to 1 month after conditioning therapy if other therapies are used.

Primarily, SOS is a clinical diagnosis with the following characteristics: (1) hepatotoxic conditioning regimen for HSCTx with an appropriate temporal relation to the development of clinical signs and symptoms, (2) weight gain & hepatic pain & jaundice and, (3) negative work-up for other causes (Dignan 2013, Bajwa 2017). In patients meeting these criteria, diagnosis can be made with reasonable certainty and solely based on clinical judgement. Differential diagnoses comprise cholestatic jaundice

due to sepsis, drug-induced cholestasis, fluid overload due to renal failure or congestive heart failure, liver involvement by viral or fungal infections, and acute graft-versus-host disease.

However, in up to 20% of patients the diagnosis of SOS cannot reliably be made on clinical grounds (McDonald 1993 & 2004). This has promoted the development of scoring systems such as the Seattle or the Baltimore Criteria (Jones 1987; McDonald 1993) (Table 3). However, up to 50% of patients not meeting the Baltimore criteria may exhibit histological features of SOS (Shulman 1994). Measurement of various biomarkers was suggested as indicator and follow-up marker of SOS (e.g. von Willebrand factor, thrombomodulin, E-selectin, sICAM1, PAI-1). Their use, however, is still regarded as experimental (Dignan 2013, Bajwa 2017). In 2016 the European Society for Blood and Marrow Transplantation revised the criteria for diagnosis and severity (Table 4). Taking into account that the paediatric population significantly differs from adults, separate criteria were recently established for children (Corbacioglu 2018).

Table 3. Diagnosis of sinusoidal obstruction syndrome after HSCTx

Seattle criteria (McDonald 1993)	Baltimore criteria (Jones 1987)
<p>At least two of the following findings within 20 days of transplantation*:</p> <ul style="list-style-type: none"> • Bilirubin >34.2 µmol/L (2 mg/dL) • Hepatomegaly or right upper quadrant pain of liver origin • ≥2% weight gain due to fluid accumulation 	<p>Hyperbilirubinaemia >34.2 µmol/L (2 mg/dL) plus ≥2 additional criteria</p> <ul style="list-style-type: none"> • Usually painful hepatomegaly • ≥5% weight gain • Ascites

*The 20-day rule applies to cyclophosphamide-containing regimens and should be adjusted according to the regimen actually used

Table 4. Revised EBMT criteria for diagnosis of sinusoidal obstruction syndrome in adults* (Mohty 2016)

Classical SOS In the first 21 days after HSCT	Late onset SOS >21 Days after HSCT
<p>Bilirubin >34 µmol/L (2 mg/dL) and two of the following criteria must be present:</p> <ul style="list-style-type: none"> • Painful hepatomegaly • Weight gain >5% • Ascites 	<ul style="list-style-type: none"> • Classical SOS beyond day 21 <p>OR</p> <ul style="list-style-type: none"> • Histologically proven SOS <p>OR</p> <ul style="list-style-type: none"> • Two or more of the following criteria: <ul style="list-style-type: none"> – Bilirubin >34 µmol/L (2 mg/dL) – Painful hepatomegaly – Weight gain >5% – Ascites <p>AND</p> <ul style="list-style-type: none"> • Hemodynamical +/- ultrasound evidence of SOS

*Symptoms/signs should not be attributable to other causes

The gold standard to confirm SOS is based on the combination of hepatic histology plus measurement of the wedged hepatic venous pressure gradient (HVPG >10 mmHg, specificity >90%, PPV >85%). Both can be achieved during a single procedure via the transvenous route, especially as increased bleeding risk often precludes percutaneous liver biopsy. However, histology may be negative due to the sometimes patchy character of the disease.

Imaging techniques are used to confirm hepatomegaly or ascites and will help to rule out differential diagnoses such as biliary obstruction. A more specific sign is the finding of hepatic inflow blockage with reduced or reversed portal flow in colour Doppler ultrasound (Figure 1). In addition, attenuation of hepatic venous flow or gallbladder wall edema may be detected. Some authors suggest the use of composite ultrasound imaging scores (Lassau 2002). Though less specific, CT imaging (i.e. heterogeneous hypoattenuation and patchy enhancement in the portal venous or equilibrium phase) may be suggestive for SOS (Yang 2018).

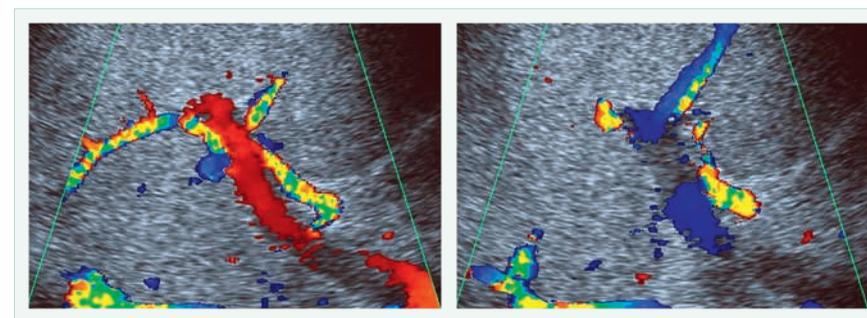


Figure 1. Doppler ultrasound in sinusoidal obstruction syndrome. Exemplary case showing undulating portal venous flow in a jaundiced patient after HSCTx

Severity of SOS varies from mild forms to rapidly progressing and eventually life-threatening disease (McDonald 1993). In patients without need for treatment of fluid excess or hepatic pain, SOS is considered mild and is associated with a self-limited course. Treatment associated with a complete remission within 100 days is considered moderate disease. If SOS does not resolve by day 100, it is categorised as severe. This classification, however, is retrospective and does not support clinical decision-making. The EBMT has proposed a modified classification system (Mohty 2016) (Table 5).

Table 5. EBMT criteria for severity of sinusoidal obstruction syndrome in adults (Mohty 2016)

	Mild ^a	Moderate ^a	Severe	Very severe
Time since first clinical symptoms of SOS ^c	>7 Days	5–7 Days	≤4 Days	Any time
Bilirubin (μmol/L)	≥34 and <51	≥51 and <85	≥85 and <136	≥136
Bilirubin kinetics			Doubling within 48 h	
Transaminases	≤2 x normal	>2 and ≤5 x normal	>5 and ≤8 x normal	>8 x normal
Weight increase	<5%	≥5% and <10%	≥5% and <10%	≥10%
Renal function	<1.2 x baseline at transplant	≥1.2 and <1.5 x baseline at transplant	≥1.5 and <2 x baseline at transplant	≥2 x baseline at transplant or signs of MOD/MOF ^b

^a In two or more risk factors for SOS, patients should be in the upper grade

^b Multi-organ dysfunction must be classified as very severe

^c Time between first signs/symptoms and fulfillment of SOS diagnostic criteria

Management and prognosis

Taking into account that SOS is probably under-diagnosed by solely employing clinical criteria, case fatality rates of detected SOS vary between 15 and 20% (DeLeve 2009). Apart from deep jaundice, additional signs of liver failure such as coagulopathy or hepatic encephalopathy may be missing. In contrast, systemic complications leading to multiple organ failure (renal, pulmonary) are the main reasons for death in these patients (Mohty 2015). This underlines the necessity of a closely supervised management concept. Highly toxic conditioning regimens should possibly be avoided. Recently, SOS prophylaxis using ursodeoxycholic acid was recommended (Cheuk 2015). In high-risk patients, defibrotide may be used (Dignan 2013, Mohty 2015).

Several treatments have been suggested for established SOS, e.g., thrombolysis using tPA, defibrotide or methylprednisolone (DeLeve 2009, Dignan 2013, Richardson 2013). In addition, invasive strategies such as TIPS or liver transplantation have been evaluated. Primarily, fluid management should aim to control fluid overload (using diuretics, paracentesis, hemofiltration/hemodialysis) and adequate oxygenation should be provided (Mahadeo 2017, Ovchinsky 2018). Thrombolysis has not proved successful and was associated with severe complications. Defibrotide, a mixture of single-stranded oligodeoxyribonucleotides derived from porcine intestinal mucosa, works as an endothelial protective agent (Palomo 2016). Defibrotide was successfully tested in phase II and III trials both in paediatric and

adult settings (Richardson 2010, Corbacioglu 2012, Richardson 2016). This compound can also be used in multiple organ failure without substantially increasing the bleeding risk. However, current data support defibrotide use as soon as SOS is diagnosed. Methylprednisolone may be considered as additional therapy (Dignan 2013).

Unlike Budd–Chiari syndrome, decompression of portal hypertension using TIPS does not improve SOS. For patients with favourable prognosis of the underlying hematopoietic disorder after HSCTx, liver transplant might possibly be considered.

Peliosis hepatis

Peliosis hepatis is a rare and potentially reversible disorder characterised by single or multiple blood-filled cystic cavities within the hepatic tissue. Whether it is related to nonobstructive sinusoidal dilatation is currently unclear (Marzano 2015). Prevalence of peliosis hepatis may vary between 0.03% in HIV infection, 0.2% in pulmonary tuberculosis and up to 20% after renal transplantation. There is no favoured localisation of the peliotic lesions. It may occur at all ages, including a fetal form. The size ranges from submillimetres to centimetres but rarely exceeds 3 cm. The histopathological appearance may show a missing endothelial cell lining with hepatocytes directly serving as boundary (parenchymal type). Alternatively, the endothelium may be preserved but the hepatic sinusoids appear dilated. The aneurysmal dilation may extend to the central vein (phlebotatic type) (Yanoff 1964, Tsokos 2005).

Pathophysiology

Several risk factors have been suggested as promoters of peliosis hepatis, e.g., infections, drugs or malignant disorders (Table 6). However, the exact pathogenesis of peliosis is still unclear. Histology suggests endothelial damage leading to destruction of the endothelial lining. Other hypotheses favour an increased sinusoidal pressure resulting in the widening of the sinusoidal lumen with consecutive destruction of the sinusoidal endothelium or primary hepatocellular necrosis replaced by blood-filled cystic lesions. Fibrotic changes and even liver cirrhosis as well as regenerative nodules may be found, but it is unclear whether these features are directly linked to peliosis hepatis or whether they are just coincidental.

Table 6. Risk factors reported with peliosis hepatis

Infections	<ul style="list-style-type: none"> • Human immunodeficiency virus • Bartonella spp. (bacillary angiomatosis) • Tuberculosis
Drugs, toxins	<ul style="list-style-type: none"> • Azathioprine, cyclosporine • Anabolic steroids, glucocorticoids, oral contraceptives, tamoxifen • Vinyl chloride, arsenic, thorium oxide
Malignant and benign tumours	<ul style="list-style-type: none"> • Multiple myeloma, Waldenström disease • Hodgkin disease • Hepatocellular adenoma
Inflammatory disease	<ul style="list-style-type: none"> • Celiac disease • Systemic lupus erythematoses
Miscellaneous	<ul style="list-style-type: none"> • Renal or heart transplantation • Diabetes mellitus • Hereditary hemorrhagic telangiectasia • Pregnancy • No underlying disorder in up to 50%

Clinical presentation and diagnosis

Peliosis hepatis is mostly asymptomatic and incidentally detected by hepatic imaging. Rarely, the peliotic cysts may rupture leading to intrahepatic or intraabdominal hemorrhage. Individual cases with overt liver disease have been reported, characterised by hepatomegaly, jaundice, ascites, portal hypertension and liver failure. Extrahepatic manifestations may be found in organs of the mononuclear phagocytic system (e.g., spleen, lymph nodes, bone marrow) but also in the lungs, kidneys, parathyroid or adrenal glands, or other parts of the gastrointestinal tract.

Usually, peliosis hepatis is easily detected by imaging techniques (Ronot 2016). However, discrimination between peliosis and other benign or malignant lesions may turn difficult. Peliotic lesions miss a mass effect on the adjacent hepatic vasculature. Blood flow within the lesion is slow, resulting in a hypodense appearance after contrast application in CT. However, in some patients a ring-like accumulation of contrast media may be present. Using MRI, low intensity is seen in T1-weighted images while T2-weighted images show a high signal (Iannaccone 2006). In contrast-enhanced ultrasound (CEUS) both centrifugal as well centripetal contrast filling might be detected, in some cases even tumour-like behaviour occurs (Schuldes 2011). Though imaging techniques may assist the diagnosis of peliosis hepatis, a liver biopsy is often needed for final confirmation. Wedged hepatic venography may also be diagnostic, but its use needs strong suspicion.

Management and prognosis

Typically, peliosis hepatis will not progress to symptomatic disease. In these patients management has to concentrate on the identification and, if required, treatment of the underlying disease. Causal treatment is the therapeutic mainstay mostly leading to regression of the peliotic lesions. Individual cases may require surgery if the risk of cyst rupture and consecutive bleeding is estimated to be high. If liver failure or portal hypertension dominate the clinical picture liver transplantation might be considered provided aetiology does not pose a contraindication.

Disorders of the hepatic artery

Pathologies involving the hepatic artery may lead to different clinical pictures (Table 7, Figure 2).

Occlusion of the arterial lumen results in ischaemia of the supplied tissue. Though gross hepatocellular necrosis may follow, such as in ischemic hepatitis, preserved portal venous oxygen supply often prevents the most devastating damage. In contrast to the hepatic parenchyma, the biliary system is exclusively supplied arterially and, therefore, more susceptible to ischemic damage. Clinically, this may present as an elevation of cholestasis-associated liver enzymes (i.e., gamma GT, alkaline phosphatase). In more severe cases, structural damage to bile ducts may be irreversible (i.e., ischemic cholangiopathy). Especially after orthotopic liver transplantation ischaemia type biliary lesions (ITBL) still pose a major challenge for clinical management.

Table 7. Aetiology of hepatic artery disease

Obstruction or destruction of the hepatic artery	<ul style="list-style-type: none"> • Hepatic artery embolism or thrombosis • Vasculitis • Sickle cell disease • Thrombotic microangiopathy (e.g., hemolytic uremic syndrome, thrombotic thrombocytopenic purpura, HELLP syndrome) • Chronic transplant rejection • Trauma
Aneurysms	<ul style="list-style-type: none"> • Congenital malformations • Polyarteritis nodosa (PAN) • Focal inflammation, trauma
Shunts	<ul style="list-style-type: none"> • Congenital malformations • Hereditary hemorrhagic telangiectasia

Apart from sequelae due to hepatic ischaemia, hepatic artery disease may present either as an aneurysm or as a shunt. Aneurysms of the hepatic artery are often detected incidentally by imaging. In the majority, they are asymptomatic but abdominal pain or – in rare cases – obstructive jaundice may develop. In about 20% of cases multiple aneurysms are present. Males are more often affected than women. The risk of rupture and subsequent hemorrhage is high and may reach up to 80% depending on the size of the aneurysm. Therefore, either radiological intervention or surgery needs to be evaluated (Hulsberg 2011, Christie 2011).

In contrast to aneurysms, shunts involving the hepatic artery are predominantly symptomatic. The spectrum of symptoms is wide including abdominal pain, portal hypertension or signs of high-output heart failure. The therapeutic approach has to be individualised including radiological interventions or surgical procedures.



Figure 2. Spontaneous arterioportal shunt. Angiography in a patient with non-cirrhotic portal hypertension. A small arterioportal shunt is detected by superselective catheterisation

Hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu syndrome)

Hereditary hemorrhagic telangiectasia (HHT) is a highly penetrant, autosomal dominant disease showing a heterozygous prevalence between 1:5,000 and 1:8,000. It is characterised by progressive and multivisceral development of arteriovenous malformations (Govani 2009, Garg 2014, Arthur 2015).

Mutations in several genes interacting with transforming growth factor (TGF)- β receptor have been identified in HHT. According to the genes involved, different subtypes can be discriminated:

- HHT 1 (ENG coding for endoglin, chromosome 9q33-q34.1),
- HHT 2 (ACVRL1 coding for activin A receptor type II-like kinase ALK-1, chromosome 12q11-q14),
- HHT 3 (gene not yet identified, chromosome 5q31.3-q32),
- HHT 4 (gene not yet identified, chromosome 7p14),
- HHT 5 (HHT5 coding for GDF-2, also known as BMP-9, chromosome 10q11.22),
- Juvenile polyposis/HHT (SMAD4, chromosome 18q21.1).

Liver involvement may be found in all subtypes but appears to be more frequent in HHT 2. Though hereditary, HHT is characterised by marked intrafamilial variation. Recently, the first case of tissue-specific mosaicism was reported (McDonald 2018).

Clinical presentation and diagnosis

HHT is a multivisceral disease. Apart from the nasopharynx and the gastrointestinal tract, central nervous (~10%), pulmonary (~50%) and hepatic involvement occur at high frequency. Accordingly, the spectrum of clinical disease is wide, e.g., anaemia, seizures, subarachnoid hemorrhage, paraplegia, transient ischemic attacks/stroke, dyspnea, cyanosis, polycythaemia, abdominal pain and hepatic abscesses. Symptoms develop progressively throughout life. Telangiectasias appear before the age of 20 in half, before 40 in two-thirds of the patients. Thereafter it takes one or two decades for the development of significant bleeding or symptomatic visceral involvement (Plauchu 1989, Govani 2009, Arthur 2015). Overall, life expectancy of patients suffering from HHT is two decades less than in the general population (Droege 2018).

The proportion of hepatic involvement in HHT reaches up to 75%. Hepatic malformations appear more common in females. However, less than 20% of patients with hepatic involvement are symptomatic (Singh 2014). The clinical picture of liver involvement in HHT depends on the predominant type of malformation (i.e., arterioportal vs. arteriovenous shunts). Arteriovenous malformations increase cardiac output. In individual cases up to 20 L/min may be reached. These patients suffer from high output cardiac failure. In addition, symptoms of a mesenteric steal syndrome (e.g., postprandial abdominal pain) and complications of biliary ischaemia (e.g., biliary abscesses) may occur. As a consequence of ischaemia, nodular regeneration of the liver develops (HHT-associated pseudocirrhosis). Arterioportal malformations will cause portal hypertension (Buscarini 2006, Garcia-Tsao 2000).

Diagnosis of HHT is made using the Curaçao criteria, 3 of 4 of which need to be fulfilled (Shovlin 2000, Faughnan 2011):

- recurrent spontaneous epistaxis,
- telangiectasias, multiple and in typical localisation,
- positive family history,
- visceral arteriovenous malformations (lung, liver, brain, spine).

Table 8. Ultrasound criteria for hepatic involvement in HHT*

Major criteria	<ul style="list-style-type: none"> • Dilated common hepatic artery >7 mm (inner diameter) • Intrahepatic arterial hypervascularisation
Minor criteria	<ul style="list-style-type: none"> • V_{max} of the proper hepatic artery >110 cm/s • RI of the proper hepatic artery <0.60 • V_{max} of the portal vein >25 cm/s • Tortuous course of the extrahepatic hepatic artery
Facultative findings	<ul style="list-style-type: none"> • Dilated portal vein >13 mm • Dilated liver veins >11 mm • Hepatomegaly >15 cm in midclavicular line • Nodular liver margin

*Two major criteria: definitive hepatic involvement in HHT, one major criterion plus minor criteria: probable hepatic involvement (modified according to Caselitz 2003)

Current guidelines do not endorse routine screening for hepatic vascular malformations. Recently, a diagnostic score involving age, gender, hemoglobin and alkaline phosphatase was presented to identify patients at risk for significant liver disease (Singh 2014). However, using Doppler ultrasound, screening is performed with high sensitivity and specificity (Table 8) (Caselitz 2003). If hepatic involvement is confirmed, cardiac output should be estimated (e.g., via echocardiography). Furthermore, screening at regular intervals is advised to detect complications such as development of portal hypertension or biliary lesions.

Management of hepatic involvement in HHT

Currently, no established medical therapy for HHT exists. In chronic GI bleeding the use of hormonal therapy (estrogen-progesterone preparations, danocrine), antifibrinolytics (aminocaproic acid, tranexamic acid) and other experimental drugs (tamoxifen, interferon, thalidomide, sirolimus) were suggested (Ardelean 2015, Faughnan 2011). However, no data supports the use of these drugs to treat hepatic vascular malformations. A phase 2 trial evaluated bevacizumab to treat liver involvement in HHT (Dupuis-Girod 2012). Significant improvements in cardiac output, epistaxis and SF-36 scores were achieved. However, long-term effects, dosing and necessity of maintenance therapy are still unclear (Ardelean 2015, Chavan 2017). Registry data comparing thalidomide and bevacizumab show positive effects on transfusion dependency, GI bleeding and epistaxis for both drugs while only

bevacizumab was helpful in treating vascular malformations (Buscarini 2019). Single cases using kinase inhibition (i.e., sunitinib, nintedanib) were reported, but still have to be regarded experimental.

Limited data exist for the use of hepatic artery embolisation and liver transplantation (Buscarini 2006, Chavan 2013, Felli 2017). Due to the invasiveness and complication rates of these approaches only patients with moderate to severe symptoms should be regarded as candidates for interventional therapy. Hepatic artery embolisation can be used to reduce shunt flow in patients with arteriovenous hepatic shunts leading to significant reduction of cardiac output and improvement of associated symptoms. However, complications such as hepatic and biliary necrosis or acute cholecystitis have been described. Success of hepatic artery embolisation very much depends on adequate patient selection. Current guidelines do not endorse general use of embolisation outside experienced centres but do favour liver transplantation in advanced hepatic involvement of HHT.

Disorders of the portal vein

Portal vein thrombosis is a common disease located within the main portal vein and its larger branches. Additionally, rare affections of the medium-sized and preterminal portal vein branches have been identified. The nomenclature for the latter has been inconsistent (e.g., obliterative portal venopathy, hepatoportal sclerosis, idiopathic portal hypertension, nodular regenerative hyperplasia). Recently, the term idiopathic non-cirrhotic portal hypertension was established replacing and incorporating the different previously described subtypes (EASL 2016).

Portal vein thrombosis

Portal vein thrombosis (PVT) is the most frequent disorder affecting the hepatic vasculature. Autopsy studies report a prevalence range between 0.05% and 0.5%. In compensated cirrhosis PVT may be found in 1% of cases, while prevalence between 8% and 26% are reported for decompensated cirrhosis.

PVT is of heterogeneous aetiology. It is promoted by both local and systemic risk factors (Tables 9 & 10). In about 20 to 30% of patients a local risk factor can be identified. Systemic risk factors are found in 50-70% (DeLeve 2009, Plessier 2010). Recently, central obesity was identified as a major risk factor for idiopathic PVT (Bureau 2016).

Table 9. Local risk factors for portal vein thrombosis

Malignancy	<ul style="list-style-type: none"> • Primary hepatic or abdominal cancer • Metastatic disease
Focal inflammation	<ul style="list-style-type: none"> • Neonatal omphalitis, umbilical vein catheterisation • Pancreatitis, duodenal ulcer, cholecystitis • Diverticulitis, appendicitis, inflammatory bowel disease • Tuberculosis, CMV hepatitis
Portal venous injury	<ul style="list-style-type: none"> • Cholecystectomy, splenectomy, colectomy, gastrectomy • Surgical portosystemic shunting, TIPS • Oesophageal sclerotherapy • Liver transplantation, hepatobiliary surgery • Abdominal trauma, exercise
Vascular haemodynamics	<ul style="list-style-type: none"> • Cirrhosis with impaired hepatic inflow • Budd-Chiari syndrome • Constrictive pericarditis

Clinical presentation and diagnosis

Portal vein thrombosis may present as acute or chronic disease, representing successive stages of disease progression. As management depends on the PVT subtype, non-cirrhotic, non-malignant PVT needs to be regarded separately from (a) thrombi resulting from slowed portal venous flow in liver cirrhosis, (b) malignant thrombi resulting from tumours invading the portal venous circulation, and (c) septic thrombi also known as acute pylephlebitis (DeLeve 2009, Plessier 2010). A classification focusing on anatomico-functional aspects has found wide acceptance (Table 10) (Sarin 2016).

Table 10. Sarin classification of portal vein thrombosis (Sarin 2016)

Site of PVT	Type 1: trunk only Type 2: branch only: 2a, one branch; 2b, both branches Type 3: trunk and branches
Extent of PV system occlusion	S: splenic vein M: mesenteric vein SM: both
Degree of portal venous system occlusion	O: occlusive, no visible flow in PV lumen on imaging/Doppler study NO: non-occlusive, flow visible in PV lumen on imaging/Doppler study
Duration and presentation	R: recent (previously patent PV, hyperdense thrombus, absent/limited collaterals, dilated PV at the site of occlusion) Ch: chronic (previously diagnosed PVT, no hyperdense thrombus, portal cavernoma, portal hypertension) As: asymptomatic S: symptomatic
Type of underlying liver disease	Cirrhotic Non-cirrhotic Hepatobiliary malignancy Local malignancy Post-transplant Associated conditions

The typical clinical presentation of acute PVT includes abdominal or lumbar pain of sudden onset or progressing over a few days. Depending on the extent of the thrombosis the pain may be severe and colicky. The diminished mesenteric outflow leads to intestinal congestion. Paralytic ileus may develop. Moderate distension of the abdomen is common. However, peritoneal signs are usually absent unless intestinal infarction develops. Fever and a marked systemic inflammatory response may develop even without systemic infection. This is accompanied by elevated laboratory markers of inflammation. In contrast, liver function – apart from intermittent elevation of aminotransferases – is usually not substantially affected by acute PVT unless significant liver damage pre-exists. Clinical features should improve within 5-7 days. Otherwise transmural intestinal ischaemia has to be suspected.

Pylephlebitis often develops secondary to a primary site of infection (e.g. pancreatitis, diverticulitis). It is characterised by high, spiking fever with chills, a painful liver, and sometimes shock. Blood cultures should be taken (often *Bacteroides* spp., *E. coli* ± other enteric species). Infected thrombi give rise to hepatic microabscesses (Kanellopoulou 2010, Choudhry 2016).

Cases without resolution of acute portal vein thrombosis progress to the chronic stage. The obstructed portal vein is replaced by collateral veins bridging the thrombotic part, known as portal cavernoma (also addressed as Extra Hepatic Portal Venous Obstruction, EHPVO). There is wide variation in the clinical picture of portal cavernoma. It may rarely lead to obstruction

of the extrahepatic bile ducts (i.e., portal cholangiopathy/biliopathy, portal cavernoma cholangiopathy), which may be associated with marked jaundice (Dhiman 2014, Khuroo 2016). However, the leading symptom of chronic PVT are the facets of portal hypertension (e.g., portosystemic collaterals such as gastric or oesophageal varices). As liver function is usually not impaired, complications such as hepatic encephalopathy or ascites are substantially less frequent than in liver cirrhosis. Hepatopulmonary syndrome may be found in up to 10% of patients.

PVT is a common complication of liver cirrhosis with an increasing prevalence in more severe disease stages. It needs to be discriminated from portal venous obstruction caused by hepatocellular carcinoma. Pathophysiologically, PVT in cirrhosis arises as a consequence of reduced hepatic inflow leading to diminished flow velocity and eventually stasis within the portal vein. Therefore, thrombi are often partial and development of portal cavernoma is rather unusual. The use of non-selective beta-blockers (NSBB) in cirrhosis may increase the risk of PVT development by more than 4-fold (Xu 2019). In patients with cirrhosis, a newly developed ascites or significant worsening of existing ascites should trigger the search for PVT.

Both acute PVT and portal cavernoma are easily detected using sonography, CT or MR imaging. Acute PVT presents as intraluminal hyperechoic material in ultrasound, while Doppler imaging demonstrates a lack of blood flow (Figure 3). Using contrast-enhanced ultrasound (CEUS), vascularisation of the thrombus may be used to identify malignant thrombi. As PVT may extend to the mesenteric or splenic veins, thorough assessment of the splanchnic tributaries is mandatory. For detailed assessment of thrombus extension, CT or MR angiography are more sensitive than Doppler sonography. Portal cavernoma presents as serpiginous vessel structures, while the main portal vein or its branches are not visible. As a compensatory mechanism hepatic arteries are usually enlarged. Depending on the individual location and appearance of portal cavernoma it may be mistaken as part of the surrounding organs or as tumour.

Management and prognosis

In acute PVT, recanalisation of the obstructed veins should be aspired. Causal factors require correction where possible. Spontaneous recanalisation without anticoagulation occurs infrequently (<10%). Therefore, anticoagulation is the most commonly used therapeutic strategy to reopen the obstructed portal vein. Though no controlled studies exist, prospective data suggest success rates between 25% and 80%. Response increases if neither the splenic vein is involved nor ascites is detectable. Anticoagulation should be initiated as early as possible – delay might be associated with treatment failure. Major complications are reported in

less than 5% of treated patients (DeLeve 2009, Plessier 2010, Hall 2011). Direct oral anticoagulants are not used as routine anticoagulation yet, but may represent a more convenient anticoagulation alternative for PVT management in future practice (Wu 2019). Depending on whether a transient or a persistent risk factor has facilitated PVT development, anticoagulation should be maintained for 6 months or long-term, respectively (EASL 2016).

Experience with other treatment modalities is limited (e.g., systemic/local thrombolysis, surgical thrombectomy, transjugular intrahepatic portosystemic stent [TIPS]). Systemic thrombolysis appears largely ineffective. Although performed successfully in some centres, major procedure-related complications and even death have been reported for local thrombolysis. A recent meta-analysis attested that TIPS placement is technically highly feasible, effective and safe (Rodrigues 2018). Emergency surgical intervention is indicated in suspected intestinal infarction. In these cases, surgical thrombectomy can be performed.

If treatment is initiated early in acute PVT the outcome is favourable. Symptoms may sometimes disappear within hours after start of therapy and portal hypertension rarely develops. Overall mortality is well below 10% (DeLeve 2009, Plessier 2010).

In patients with portal cavernoma, prevention of gastrointestinal bleeding due to portal hypertension is a main focus of therapy (Chaudhary 2013). The use of non-selective beta-blockers is incompletely evaluated in portal cavernoma. However, an approach similar to portal hypertension in liver cirrhosis is supported by current guidelines and appears to improve prognosis (DeLeve 2009). Due to the variable genesis of PVT, individual assessment for risk of recurrence of thrombosis and risk of bleeding should be performed. Although data is scarce, anticoagulation seems to be favourable for most patients.

The therapeutic approach in patients with PVT associated with liver cirrhosis has to be regarded separately. Whether PVT increases mortality in patients with cirrhosis remains unclear (Berry 2015, Cool 2019). On the other hand, anticoagulation is safe both in the prophylactic as well as in the therapeutic setting (Villa 2012, Delgado 2012). Use of enoxaparin as primary prophylaxis completely prevented the development of PVT. In subacute PVT, anticoagulation (using either vitamin K antagonists or LMWH) achieved complete recanalisation in nearly half of the patients, while at least partial response was seen in 2/3 of cases. Similar results are achieved using DOAC (De Gottardi 2017). Interventional therapy using TIPS appears even more effective showing complete response in 57% and at least partial response in all patients (Luca 2011, Rössle 2014). Preliminary data suggest that systemic thrombolysis is feasible (De Santis 2010). As PVT does not change the clinical course of liver cirrhosis, primary treatment candidates are patients on the waiting list for liver transplantation.

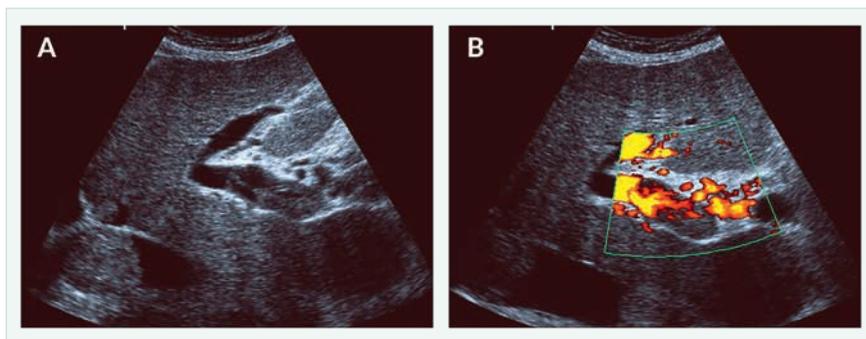


Figure 3. Acute portal vein thrombosis. Ultrasound of patient with acute PVT. (A) Hyperechoic material is located within the main portal vein. (B) Using the power mode for flow detection, blood flow is limited to those parts of the portal vein without hyperechoic material

Malignant PVT resulting from hepatocellular carcinoma should not lead to therapeutic nihilism. While systemic therapy (e.g. sorafenib) is the recommended strategy in Western countries, experience from Asia favours resection to TACE or conservative treatment (Lu 2019, Zhang 2019). Portal vein stenting has been reported for malignant PVT, however, the effect on relevant end-points is unclear.

If pyelephlebitis is suspected antibiotic therapy must be commenced immediately. Additional anticoagulation appears to improve outcomes in this setting (Naymagon 2020).

Idiopathic non-cirrhotic portal hypertension (INCPH)

The term INCPH was introduced to replace an ambiguous nomenclature including hepatoportal sclerosis, non-cirrhotic portal fibrosis, idiopathic portal hypertension, incomplete septal cirrhosis, nodular regenerative hyperplasia and obliterative portal venopathy (EASL 2016). Most recently, an even more comprehensive nomenclature was proposed, i.e. porto-sinusoidal vascular disease (De Gottardi 2019).

The histopathological correlate is an affection of the medium-sized and preterminal portal venous branches generating different morphological features (Guido 2019):

(a) Occlusion of the portal venous branches induces hypotrophy of the supplied tissue. As a compensatory reaction, growth of appropriately perfused liver tissue gives rise to the development of regenerative nodules. This combination of hypotrophic and hypertrophic liver tissue without signs of fibrosis is the equivalent of nodular regenerative hyperplasia (Wanless 1990).

(b) As a second type of reaction, portal veins are not just destroyed but replaced by filiform fibrotic strands penetrating the hepatic tissue. These

fibrotic strands are strictly confined to the portal tracts and do not form fibrotic septae (Aggarwal 2013, Nakanuma 2001). This feature is equivalent to hepatoportal sclerosis.

Both histological features may exist side by side.

Nodular regenerative hyperplasia is found in 14-27% of cases with non-cirrhotic portal hypertension (Naber 1991, Nakanuma 1996). In autopsy studies the prevalence is 3.1/100,000, one third of which are associated with portal hypertension (Colina 1989). Hepatoportal sclerosis less frequently described in the Western world but is more common in Asia (e.g., India, Japan).

A number of associated pathologies have been suggested to promote INCPH: Immune and hematologic disorders, e.g., rheumatoid arthritis, Felty's syndrome, other connective tissue disorders, CVID, HIV infection, myeloproliferative and lymphoproliferative disease. INCPH has been described in infective endocarditis, inflammatory bowel disease and after kidney transplantation. Furthermore, it may occur in conjunction with chemotherapy, HAART, other drugs and after toxin exposure (e.g., arsenic, vinyl chloride). Also, a hereditary component is discussed (Albuquerque 2013, Ghabril 2014, Hartleb 2011, Matsumoto 2000, Sarin 2007, Schouten 2011, Schouten 2015, Vilarinho 2016).

Clinically, INCPH presents with complications of portal hypertension. Liver function is usually not significantly impaired, although individual cases with liver failure and liver transplantation have been described. The prognosis depends on the underlying disorder and on the control of portal hypertension (Ataide 2013, Blendis 1978, Dumortier 2001, Naber 1990, Sarin 2007, Schouten 2015, Siramolpiwat 2014). TIPS has proven an effective measure in INCPH (Bissonnette 2016).

EASL guidelines have suggested all of the following 5 criteria to be fulfilled for the diagnosis of INCPH: a) Clinical signs of portal hypertension, b) exclusion of cirrhosis on liver biopsy, c) exclusion of chronic liver disease causing cirrhosis or noncirrhotic portal hypertension (chronic viral hepatitis B/C, NASH/ASH, autoimmune hepatitis, hereditary haemochromatosis, Wilson's disease, primary biliary cholangitis), 4) exclusion of conditions causing non-cirrhotic portal hypertension (congenital liver fibrosis, sarcoidosis, schistosomiasis), 5) patent portal and hepatic veins.

The above criteria point to the importance of liver biopsy for the diagnosis of INCPH. However, interobserver agreement in histology evaluation is variable (Jharab 2015). Even more, histological features of INCPH may be found in up to 10% of the general population (Zuo 2017). In imaging studies, differentiation between nodular regenerative hyperplasia and cirrhosis

may be impossible. In ultrasound, “atoll-like lesions” have been described as a characteristic imaging feature (Caturelli 2011). A recent paper pointed to value of non-cirrhotic transient elastography results for the diagnosis of INCPH (Seijo 2012).

Disorders of the hepatic veins

Budd-Chiari syndrome is the only defined entity of hepatic venous disease. However, other disorders such as the sinusoidal obstruction syndrome or peliosis hepatis may also affect the hepatic venous system. Furthermore, hepatic congestion due to cardiac or pericardial disease shares clinical similarities with Budd-Chiari syndrome.

Budd-Chiari syndrome

Budd-Chiari syndrome (BCS) is defined as hepatic venous outflow obstruction at any level from the small hepatic veins to the junction of the inferior vena cava (IVC) and the right atrium, regardless of the cause of obstruction (Janssen 2003). Excluded by definition are obstructions caused by sinusoidal obstruction syndrome and cardiac or pericardial disorders. BCS is a rare disorder with an estimated incidence of 1 per million and a prevalence of 11 per million (Li 2019).

Pathophysiology

Obstruction of the hepatic outflow may arise from endoluminal lesions, e.g., thrombosis, webs, endophlebitis (primary BCS) or from outside the venous system by luminal invasion or by extrinsic compression, e.g., tumour, abscess, cysts (secondary BCS) (Janssen 2003).

On rare occasions, BCS originates from congenital malformations, e.g., webs or stenotic vessels (Ciesek 2010, Darwish Murad 2009). However, outflow obstruction is usually caused by thrombosis. Prevalence of thrombophilic risk factors is shown in Table 11. However, the underlying etiologies may vary in different parts of the world (Qi 2016). Thrombi are exclusively located within the hepatic veins in 49% of patients, exclusively within IVC in 2%, and as combined thrombosis of hepatic veins and IVC in 49%. In about 18% a concomitant portal vein thrombosis is identified (Darwish Murad 2009).

Obstruction of hepatic outflow leads to congestion of the drained tissue. Over time this will induce hypertrophy of affected and consecutive regenerative growth of non-affected parts of the liver. A typical area of

hypertrophy is liver segment I (caudate lobe), favoured by its separate venous drainage into the IVC. Regenerative nodules may occasionally progress to hepatocellular carcinoma. In addition, intrahepatic collaterals may develop.

Table 11. Prevalence of thrombophilic risk factors in acute and chronic portal vein thrombosis and in primary Budd-Chiari syndrome*

Risk factor	Portal vein thrombosis	Budd-Chiari syndrome
Myeloproliferative neoplasms	21% – 40%	40% – 50%
Atypical	14%	25% – 35%
Classical	17%	10% – 25%
Paroxysmal nocturnal hemoglobinuria	0% – 2%	0% – 19%
Antiphospholipid syndrome	6% – 19%	4% – 25%
Factor V Leiden mutation	3% – 32%	6% – 32%
Factor II (prothrombin) mutation	14% – 40%	3% – 7%
Protein C deficiency	0% – 26%	4% – 30%
Protein S deficiency	2% – 30%	3% – 20%
Antithrombin deficiency	0% – 26%	0% – 23%
Plasminogen deficiency	0% – 6%	0% – 4%
Hyperhomocysteinaemia	11% – 22%	22% – 37%
TT677 MTHFR genotype	11% – 50%	12% – 22%
Recent pregnancy	6% – 40%	6% – 12%
Recent oral contraceptive use	12% – 44%	6% – 60%
Behçet's disease	0% – 31%	0% – 33%
Connective tissue disease	4%	10%

*Adult patients without malignancy or cirrhosis (according to DeLeve 2009, Darwish Murad 2009, Plessier 2010)

Clinical presentation and diagnosis

Depending on the location of outflow obstruction, the number of vessels involved and the temporal dynamics of BCS, the clinical presentation varies between subclinical disease to light symptoms, and dramatic acute complaints which may progress to acute liver failure. The disease might present with a progressively relapsing course successively involving different hepatic veins.

Symptoms of hepatic congestion are ascites (>80% of patients), abdominal pain (>60%) and oesophageal varices (>50%). Significant disturbance of liver function is rather rare, e.g., hepatic encephalopathy (<10%), as is involvement of extrahepatic organs, e.g., hepatorenal syndrome (<10%) (Darwish Murad

2009).

In the majority of cases, diagnosis of BCS can be obtained using Doppler ultrasound. If technical difficulties obviate sonographic diagnosis, MRI is the imaging method of choice. Only in rare cases is liver biopsy or hepatic venography required to confirm the diagnosis (Janssen 2003). Ultrasound characteristics of BCS are clearly defined (Boozari 2008). They comprise specific signs such as direct visualisation of thrombi, stenosis, webs, replacement of hepatic veins by fibrotic strands or reversed flow in hepatic veins or IVC. Suggestive signs are hepatic collaterals that may be interposed between hepatic veins or may be located on the hepatic capsule. Widening of the caudate vein (>3 mm) is also regarded as suggestive for BCS. These signs serve in the diagnosis of BCS and may be accompanied by a myriad of non-specific changes (e.g., ascites, regenerative nodules, splenomegaly).

As regeneration nodules in BCS may progress to hepatocellular carcinoma, thorough imaging is mandatory. However, identification of malignant transformation may be difficult (Van Wettere 2019).

Management and prognosis

Treatment of BCS has to be adjusted to the aetiology and the severity of the clinical picture. If BCS is caused by congenital malformations such as webs, radiological interventions using balloon catheter-assisted dilation may succeed.

In case of a primary thrombotic event, anticoagulation is the mainstay of therapy (Janssen 2003, DeLeve 2009, Darwish Murad 2009, Seijo 2013, EASL 2015). However, in long-term follow-up less than half of patients will be solely treated with anticoagulation and remain free of further interventions (Seijo 2013). Therefore, interventional techniques (e.g., TIPS, recanalisation) should be evaluated early, especially in patients with moderate to severe symptoms. With the advent of TIPS, the necessity for liver transplantation in BCS has declined sharply. Success rates of TIPS – both in the short-term and in the long-term – are high (Seijo 2013, Zhang 2015). Thus, surgical procedures (e.g., surgical shunt, liver transplantation) are only rarely performed. With this approach, current data show that survival in BCS is above 70% after 5 years (Seijo 2013).

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27. Acute liver failure

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Introduction and definition

Acute liver failure (ALF) is a devastating clinical syndrome, occurring in previously healthy individuals, which is characterised by hepatocellular death and dysfunction (O'Grady 2005). ALF is characterised by onset of coagulopathy (International Normalized Ratio, INR ≥ 1.5) and hepatic encephalopathy within 26 weeks of symptom appearance in a previously healthy subject (Larson 2010). Exclusion of an underlying liver disease (alcoholic hepatitis, chronic hepatitis B (HBV) and hepatitis C (HCV), autoimmune hepatitis) is mandatory, as management of acute-on-chronic liver failure differs from ALF treatment. The most common causes of ALF in Europe and the US are acetaminophen intoxication, acute HBV infection and non-acetaminophen drug-induced liver injury (Bernal 2010). With progressive loss of hepatic function, ALF leads to hepatic encephalopathy, coagulopathy and multiorgan failure within a short period of time. Established specific therapy regimens and the introduction of liver transplantation improve the prognosis for some etiologies. However, the overall mortality rate remains high (Bernal 2010). ALF accounts for approximately six to eight percent of liver transplantation procedures in the US and Europe (Lee 2008). The accurate and timely diagnosis of ALF, rapid identification of the underlying cause, transfer of the patient to a specialised transplant centre and, if applicable, initiation of a specific therapy and evaluation for liver transplantation are crucial in current ALF management. Therefore, we focus here on epidemiology, pathophysiology, diagnosis and treatment of ALF, including a brief overview of different aetiologies and specific treatment options as well as novel tools to predict prognosis.

Epidemiology and aetiologies

ALF is a rare disease based on multiple causes and varying clinical courses, and exact epidemiologic data is scarce. The overall incidence of ALF is assumed as one to six cases per million people each year (Bernal 2010). Data from the US (Ostapowicz 2002), the UK (Bernal 2004), Sweden (Wei 2007), and Germany (Canbay 2009) reveal drug toxicity as the main cause of ALF, followed by viral hepatitis, followed by unknown aetiology. In contrast, in the Mediterranean, Asia and Africa, viral hepatitis is the main cause of ALF (Escorsell 2007, Koskinas 2008, Mudawi 2007, Oketani 2011).

Table 1. Aetiologies of ALF

Intoxication	Direct, idiosyncratic, paracetamol, ecstasy, amanita, phenprocoumon, tetracycline, halothane, isoniazid, anabolic drugs
Viral hepatitis	HBV, HAV, HEV, HBV+HDV, CMV, EBV, HSV
Immunologic	Autoimmune, GVHD
Metabolic	Wilson's disease, alpha-1 antitrypsin deficiency, hemochromatosis
Vascular	Budd-Chiari syndrome, ischemic, veno-occlusive disease
Pregnancy-induced	HELLP syndrome

Intoxication

Drug-induced liver injury

Drug toxicity is the main cause of ALF in high-income societies. Although the incidence of drug-induced liver injury (DILI) in the general population was estimated at 1–2 cases per 100,000 person years (de Abajo 2004), DILI in Germany accounts for approximately 40% of patients with ALF (Hadem 2012). As a structured medical history may be difficult in some cases, a standardised clinical management to identify the cause of DILI and optimise specific treatment has been proposed (Fontana 2010). This includes assessment of clinical and laboratory features, determining the type of liver injury (hepatocellular vs. cholestatic), the clinical course after cessation of the suspected drug, assessment of risk factors (age, sex, alcohol consumption, obesity), exclusion of underlying liver diseases, previous episodes of DILI, liver biopsy and in some cases re-challenge to identify the drug. Furthermore, identifying a cause involves distinguishing between a direct (intrinsic; dose-dependent) and an idiosyncratic (immune-mediated hypersensitivity or metabolic injury) type of liver injury (Larson 2010). Acetaminophen intoxication, as discussed in detail below, is the prototype of a direct, dose-dependent intoxication with acute hepatocellular necrosis. However, most cases of DILI are due to idiosyncratic reactions with a latency period of up to one year after initiation of treatment. Drugs that induce idiosyncratic DILI include narcotics (halothane), antibiotics (amoxicillin/clavulanate; macrolides, nitrofurantoin, isoniazid), antihypertensive drugs (methyldopa) and anticonvulsants and antipsychotic drugs (valproic acid, chlorpromazine) and many others, including herbal medicines. Demonstrating the need for new algorithms and biomarkers of liver injury, Hy Zimmerman's observation that elevation of transaminase levels above three times the upper limit of normal indicates early DILI is still in use to assess the risk of DILI in drugs in development, since the 1970s (Reuben 2004).

Acetaminophen intoxication

In a recent study, more than seventy percent of the patients with acetaminophen-induced ALF were reported as suicidal intents, the rest as accidents (Canbay 2009). The presence of any ALF risk in the recommended dose range of acetaminophen is controversial. However, the presence of risk factors, particularly obesity and alcohol abuse seem to increase the risk of ALF in patients that use acetaminophen (Canbay 2005, Krahenbuhl 2007). Acetaminophen serum concentration above 300 µg/mL four hours after ingestion is a predictor for severe hepatic necrosis. With high doses of acetaminophen, its metabolite N-acetyl-p-benzoquinone imine (NAPQI) accumulates in hepatocytes and induces hepatocellular necrosis (McGill 2012). In the presence of glutathione, NAPQI is rapidly metabolised to non-toxic products and excreted via the bile (Bessems 2001). In acetaminophen intoxication, the glutathione pool is rapidly diminished, but could easily be restored by N-acetylcysteine therapy (see below).

Table 2. Clinical determination of the cause of ALF

Aetiology	Subtype	Investigation
Intoxication	Drug	Drug concentrations in serum
	Amanita	History
	Idiosyncratic drug toxicity	Drug concentrations in serum/ eosinophil count
Viral hepatitis	HAV	IgM HAV
	HBV	HBsAg, IgM anti-core, HBV DNA
	HBV/HDV	HBsAg, IgM HDV, HDV RNA
	(HCV)	Anti-HCV, HCV RNA
	HEV	Anti-HEV, HEV RNA
Immunologic	Autoimmune	ANA, LKM, SLA, ASMA, IgG
	GVHD	Biopsy
Metabolic	Wilson's disease	Urinary copper, ceruloplasmin in serum, slit-lamp examination
	AT deficiency	AT level in serum, AT genotyping
	Haemochromatosis	Ferritin in serum, transferrin saturation
Vascular	Budd-Chiari syndrome	Ultrasound (Doppler)
	Ischemic	Ultrasound (Doppler), echocardiography (ECO)
	Veno-occlusive disease	Ultrasound (Doppler)
Pregnancy-induced	HELLP syndrome	Hematocrit test, peripheral blood smear, platelet count

ANA, anti-nuclear antibody; ASMA, anti-smooth muscle antibody; IgM, immunoglobulin M; IgG, immunoglobulin G; HBsAg, hepatitis B surface antigen

Amanita intoxication

The spectrum of mushroom poisoning varies from acute gastroenteritis to ALF. Even though the mortality rate of all mushroom poisoning cases is low, the mortality rate of those patients who develop ALF is extremely high, despite the improvement in intensive care management (Broussard 2001). Deadly mushroom poisoning is attributed to *Amanita phalloides*, the wild mushroom, and occurs mostly in spring and early summer. *Amanita* toxin has a dose-dependent, direct hepatotoxic effect and disrupts hepatocyte mRNA synthesis (Kaufmann 2007).

Viral hepatitis

Historically the most common cause of ALF in Europe and still today the most prevalent aetiology in developing countries is fulminant viral hepatitis (Hadem 2012). Hepatitis A and E (HAV and HEV), both transmitted via the fecal-oral route are endemic in countries with poor sanitation, tropical and subtropical countries. HEV was determined as the main cause of ALF in some Asian countries. The clinical presentation of HAV is more severe in adults than in children, and HEV is more common in pregnant women, especially in the third trimester (Dalton 2008). Current data indicates that HEV infection might be responsible for up to 10% of ALF in unknown or ambiguous cases in Europe (Manka 2015). Therefore, HEV should be considered as possible cause in unclear ALF cases. Fulminant HBV, transmitted vertically or by infected blood and body fluids, is the most predominant viral cause of ALF in Western countries (Bernal 2010, Canbay 2009). The incidence of fulminant HBV is decreasing with the implementation of routine vaccination. Superinfection with hepatitis D (HDV) in HBV infection is associated with higher risk to develop ALF. HBV infection and treatment is discussed in detail elsewhere. Acute cytomegalovirus, Epstein-Barr virus, parvovirus B19, and herpes simplex virus-1 and -2 are less frequently associated with ALF.

Immunologic etiologies

Autoimmune hepatitis

In rare cases autoimmune hepatitis (AIH) may induce ALF. The acute onset of ALF and its potentially rapid progression causes a diagnostic dilemma since exclusion of other liver diseases might be too time-consuming in patients with ALF secondary to AIH. Thus, IgG elevation

and positive ANA titre, combined with typical histological features may be sufficient to induce specific therapy in this instance (Suzuki 2011). However, as DILI might perfectly mimic AIH, a detailed history is key to adequate therapy in all ALF patients with features of AIH (Bjornsson 2010).

Graft-versus-host disease

With the development of new options of donor leukocyte infusion, non-myeloablative methods and umbilical cord blood transplantation, the indications of allogeneic haematopoietic stem cell transplantation have been expanding in recent years (Ferrara 2009). Therefore, any hepatopathy in patients who have undergone bone marrow transplant is suspicious for graft-versus-host disease (GVHD). On the other hand, chemotherapy and myeloablation themselves are hepatotoxic and might induce reactivation of HBV, leading to fulminant liver failure.

Wilson's Disease

Wilson's Disease (WD), the autosomal recessive disorder of copper metabolism, is a rare cause of ALF. The prognosis of WD patients presenting with ALF is devastating, and almost all die without liver transplantation (Lee 2008). Very high serum bilirubin and low alkaline phosphatase, ALT and AST are typical laboratory readings, and renal failure is a common clinical feature in WD (Eisenbach 2007).

Vascular disorders

Acute systemic hypotension secondary to heart failure or systemic shock syndromes may induce acute liver injury (Herzer 2012). Occlusion of at least two liver veins in Budd-Chiari syndrome or veno-occlusive disease is a rare cause of ALF. Anticoagulatory or lysis therapy is the management of choice; in severe cases, emergency TIPSS or surgical shunt placement may be indicated, as well as a thorough workup to identify any underlying prothrombotic conditions (Fox 2011).

Pregnancy-induced liver injury

Besides acute fatty liver of pregnancy (AFLP), which usually occurs in the third trimester of pregnancy, HELLP syndrome (haemolysis, elevated liver enzymes, low platelet level) is a rare complication of pregnancy and

presents with ALF. HELLP syndrome typically presents with LDH, ALT and bilirubin elevation and thrombocytopenia. Hepatopathy usually completely reverses after termination of pregnancy. Patients are at increased risk for complications in future pregnancies (Hay 2008, Westbrook 2010).

Undetermined

Despite dramatic improvements in diagnostic tests in approximately twenty percent of patients with ALF, the aetiology remains undetermined (Canbay 2009, Hadem 2008, Hadem 2012).

Molecular mechanisms and clinical presentation

As mentioned above, ALF occurs on the basis of acute hepatocellular injury caused by toxic, viral or metabolic stress or hypotension. However, regardless of the initial type of liver injury, ALF propels a series of events inducing hepatocellular necrosis and apoptosis, reducing the regeneration capacity of the liver. Massive loss of hepatocytes reduces the functional capacity of the liver for glucose, lipid and protein metabolism, biotransformation, synthesis of coagulation factors, leading to encephalopathy, coagulopathy, hyperglycaemia, infections, renal and multi-organ failure. In fact, even the pattern of hepatic cell death might be of clinical importance, as necrosis or apoptosis seem to be specific for different causes and are associated with clinical outcome (Bechmann 2008, Volkmann 2008).

Apoptosis, programmed cell death, occurs when ATP-dependent processes lead to activation of caspases that induce a cascade of events, ending in the breakdown of the nucleus into chromatin bodies, interruption of membrane integrity and finally total breakdown of the cell into small vesicles, called apoptotic bodies. Upon massive cell injury, ATP depletion leads to necrosis with typical swelling of the cytoplasm, disruption of the cell membrane, imbalance of electrolyte homeostasis and karyolysis. Necrosis typically leads to local inflammation, induction of cytokine expression and migration of inflammatory cells (Jaeschke 2007). However, apoptosis itself might induce mechanisms that lead to necrosis and the ratio of apoptosis vs. necrosis seems to play an important role in liver injury rather than the individual events (Canbay 2004). This hypothesis is supported by observations that a death receptor agonist triggers massive necrosis secondary to the induction of apoptosis (Rodriguez 1996).

The rates of apoptosis or necrosis in ongoing ALF processes seem to be different according to the underlying aetiologies (Bechmann 2010, Herzer

2012). The degree of apoptosis and necrosis, assessed by specific ELISA assays were significantly increased in amanita intoxication compared to other causes. Apoptosis is the predominant type of cell death in HBV and amanita-related ALF, vs. necrosis in acetaminophen and congestive heart failure. Furthermore, entecavir treatment of fulminant HBV significantly reduces serum cell death markers and improves clinical outcome (Jochum 2009).

The regenerative capacity of the liver depends on the patient's gender, age, weight and previous history of liver diseases. Important mediators of liver regeneration include cytokines, growth factors and metabolic pathways for energy supply. In the adult liver, most hepatocytes are in the G₀ phase of the cell cycle and non-proliferating. Upon stimulation with the proinflammatory cytokines tumour necrosis factor- α (TNF- α) and interleukin-6 (IL-6), growth factors like transforming-growth factor α (TGF α), epidermal growth factor (EGF) and hepatocyte growth factor (HGF) are able to induce hepatocyte proliferation. TNF and IL-6 also induce downstream pathways related to NF κ B and STAT3 signalling. Both transcription factors are mandatory for coordination of the inflammatory response to liver injury and hepatocyte proliferation (Dierssen 2008). Emerging data supports an important role for hepatic progenitor and oval cells as well as vascular endothelial growth factor (VEGF)-mediated angiogenesis in liver regeneration (Ding 2010, Dolle 2010).

TNF- α , IL-1 and IL-6 are also important mediators of the hyperdynamic circulation by alterations of nitric oxide synthesis in ALF (Larson 2010). Renal failure, hepatic encephalopathy and brain oedema are the results of these pathophysiologic changes. Hyperammonaemia correlates with brain oedema and survival (Clemmesen 1999). Decreased hepatic urea synthesis, renal insufficiency, the catabolic state of the musculoskeletal system and impaired blood-brain barrier leads to ammonia accumulation and alterations in local perfusion, which induces brain oedema in ALF. Interestingly, brain oedema is a presentation of ALF rather than cirrhosis, and the risk of brain oedema increases with the grade of hepatic encephalopathy. After acute and massive hepatic cell death, the release of proinflammatory cytokines and intracellular material result in low systemic blood pressure leading to impairment of splanchnic circulation. Indeed, renal failure in ALF patients is common, up to 70% (Larsen 2011). Reduced qualitative and quantitative functions of platelets and inadequate synthesis of prothrombotic factors are the causes of coagulopathy. Leukopaenia and impaired synthesis of complement factors in ALF patients increases the risk for infections, which might result in sepsis. Infections increase the duration of ICU stays and the mortality rate in ALF dramatically. With the impairment of hepatic gluconeogenesis, hypoglycaemia is a frequent feature of patients with ALF (Canbay 2011).

Recent data indicates that high-density lipoprotein (HDL) could be a marker for the severity of ALF (Etogo-Asse 2012). Data in ALF patients regarding lipid-associated parameters is limited, but HDL and cholesterol seem to be important for liver cell regeneration. In patients with ALF, HDL was suppressed, correlated with serum ALT levels, and was lower in patients without spontaneous remission (i.e., deceased or requiring transplantation) (Manka 2014). However, further studies are required to confirm which mechanisms play a role and what effects can be expected. More recently it was shown that liver biopsy by laparoscopy can assist in prognosis of ALF course and outcome, as immunohistochemical assessment of regeneration (i.e., KI67) and cell death (M30) become available (Dechêne 2014).

Table 3. Grade of hepatic encephalopathy (West Haven criteria)

Grade	Clinical findings	Asterixis	EEG
I	Changes in behavior, euphoria, depression, mild confusion	+/-	Triphasic waves
II	Inappropriate behavior, lethargy, moderate confusion	+	Triphasic waves
III	Marked confusion, somnolence	+	Triphasic waves
IV	Coma	-	Delta waves

Prognosis

With persistently high, although variable, mortality rates from ten to ninety percent, accurate prediction of the clinical course is crucial for accurate management and decision-making. Most importantly, identification of the underlying aetiology improves prognosis and opens the door for specific treatment. The degree of hepatic encephalopathy is traditionally considered an important indicator of prognosis (O'Grady 1989). Cerebral oedema and renal failure worsen the prognosis dramatically. In some studies, the INR was determined as the strongest single parameter in predicting the prognosis of ALF. Another interesting point is that the presence of hepatic encephalopathy means a poor prognosis for acetaminophen-induced ALF, which in contrast has little meaning for amanita mushroom poisoning. Liver transplantation is the last treatment option in patients with ALF, when conservative treatment options fail and a lethal outcome is imminent. Therefore, assessment of likelihood of the individual patient to undergo a fatal course is important for timely listing of the patient. Standardised prognosis scores based on reproducible criteria are important in times of donor organ shortage and to avoid liver transplantation in patients that

might fully recover without liver transplantation (Canbay 2011).

King's College criteria (KCC) were established in the 1990s based on findings from a cohort of 588 patients with ALF (O'Grady 1989). The authors also introduced a classification based on the onset of encephalopathy after an initial rise in bilirubin levels into hyperacute (<7 days), acute (8–28 days) and subacute (5–12 weeks) liver failure (O'Grady 1993). KCC includes assessment of encephalopathy, coagulopathy (INR), acid homeostasis (pH), bilirubin and age. For patients with acetaminophen-induced ALF, a KCC formula was implied, deviating from that in patients with non-acetaminophen-induced liver injury. Clichy criteria were introduced for patients with fulminant HBV infection and include the degree of encephalopathy and factor V fraction as a measure for hepatic synthesis (Bernuau 1986). The model for end stage liver disease (MELD) was designed to predict the likelihood of survival after transjugular portacaval shunt (TIPS) in cirrhotic patients. However, it has recently been established as an allocation tool for liver transplantation in patients with cirrhosis in the US and Europe. It was tested as a model for prediction of ALF and was found to be superior to KCC and Clichy criteria in independent studies (Schmidt 2007, Yantorno 2007). Novel approaches that include mechanistic characteristics of ALF like the CK-18 modified MELD, which includes novel markers for hepatocellular death or lactate are promising, but need validation in prospective cohorts (Bechmann 2010, Hadem 2008, Rutherford 2012). In a recent, large, prospective study, a prognostic model was developed using dynamic changes of four independent variables (atrial ammonia, INR, serum bilirubin, hepatic encephalopathy) over three days, to predict mortality (Kumar 2012). Recently an association of thyroid hormone status and outcome of ALF has been demonstrated. Since thyroid hormones are involved in hepatocellular regeneration, thyroid status might be useful as early indicator for severity of ALF (Anastasiou 2015).

Table 4. Scoring systems in patients with ALF for emergency liver transplantation

Scoring System		Prognostic factors
King's College Criteria (KCC)	Paracetamol intoxication	Arterial pH <7.3 or INR >6.5 and creatinine >300 μmol/L and hepatic encephalopathy grade 3–4
	Non-paracetamol	INR >6.5 and hepatic encephalopathy or INR >3.5 and any of these three: bilirubin >300 μmol/L, age >40 years, unfavourable aetiology (undetermined or drug-induced)
Clichy Criteria	HBV	Hepatic encephalopathy grade 3–4 and factor V <20% (for <30 years old); <30% (for >30 years old)
MELD		$10 \times [0.957 \times \ln(\text{serum creatinine}) + 0.378 \times \ln(\text{total bilirubin}) + 1.12 \times \ln(\text{INR} + 0.643)]$
CK-18 modified MELD		$10 \times [0.957 \times \ln(\text{serum creatinine}) + 0.378 \times \ln(\text{CK18/M65}) + 1.12 \times \ln(\text{INR} + 0.643)]$
Bilirubin-lactate-aetiology score (BILE score)		Bilirubin (μmol/L)/100 + Lactate (mmol/L) + 4 (for cryptogenic ALF, Budd-Chiari or Phenprocoumon induced) –2 (for acetaminophen-induced) +0 (for other causes)
ALFSG Index		Coma grade, bilirubin, INR, phosphorus, \log_{10} M30
ALFED Model		Dynamic of variables over 3 days: HE 0–2 points; INR 0–1 point; arterial ammonia 0–2 points; serum bilirubin 0–1 point

Adapted from Canbay 2011; INR, International Normalized Ratio; MELD, model of end stage liver disease

Treatment

General management

Given the high risk of deterioration and development of hepatic coma, immediate transfer of the patient presenting with ALF to the ICU is mandatory. Early referral or at least consultation of an experienced transplant centre is indicated in any ALF patient, since liver transplantation is the ultimate treatment for ALF in case conservative therapy fails. The cause of ALF should be determined as soon as possible. Besides specific detailed history taking, laboratory and radiologic tests need to be done in order to establish the diagnosis of ALF and identify the underlying cause. Diagnostic studies include, but are not limited to, arterial blood gas analysis, glucose, electrolytes, bilirubin, ammonia, lactate, protein, albumin, C-reactive protein (CRP), procalcitonin (PCT), urine electrolytes, urinalysis, and chest X-ray, cranial computed tomography (CT) in patients with advanced hepatic encephalopathy as well as assessment of intracranial pressure (ICP) in some cases. Beyond specific diagnostic studies (HBV

serology, coaguloplasmin, urine copper concentration, etc.), transjugular or laparoscopic liver biopsy might be indicated to identify the underlying disease (Canbay 2011).

Hepatic encephalopathy

In general in patients with hepatic encephalopathy, sedative agents should be avoided and if necessary restricted to short-acting benzodiazepines or propofol, as it might decrease intracranial pressure (Wijdicks 2002). Some studies favour utilisation of ICP monitoring, especially in patients with hepatic encephalopathy grade III/IV, and clinical signs of brain oedema. Mannitol therapy (0.5–1 g/kg) might be beneficial in some patients. Head elevation, induction of hypothermia and hyperventilation are recommended by some experts in patients with increased ICP. With worsening of brain oedema, patients present with systemic hypertension and bradycardia (Cushing reflex), dilated and fixed pupils, and in the end respiratory arrest. The target ICP should remain below 20 mmHg, with cerebral perfusion pressure above 70 mmHg and jugular venous saturation of 55 to 80%. Phenytoin is the drug of choice for treatment of seizures and hypertonic sodium chloride might be beneficial on ICP (Larsen 2011). Symptomatic treatment of encephalopathy includes bowel decontamination with neomycin or rifaximin, induction of diarrhoea and reduction of colonic pH and thus reduction of ammonia absorption by lactulose as well as treatment with branched-chain aminoacids to improve peripheral ammonia metabolism, although large, randomised clinical trials have failed to show clinical improvement (Larson 2010, Nguyen 2011).

Coagulopathy

In general, without clinical signs of bleeding coagulation factor treatment is not indicated. To exclude vitamin K deficiency, vitamin K challenge should be performed. Platelets and recombinant activated factor VII are indicated in case of bleeding or before invasive procedures. Interestingly, in ALF patients with impaired coagulation according to conventional testing (INR) may not be at risk for bleeding in laparoscopic procedures (Dechêne 2014).

Liver transplantation

Liver transplantation is the therapy of choice for ALF in those individuals with insufficient regeneration capacity and an otherwise fatal

prognosis. In patients without contraindications to liver transplantation, the one-year survival rate is as high as 80–90% with a five-year survival of 55%. As mentioned above, with liver transplantation available as the most favourable therapy, the accurate assessment of the patient's prognosis is crucial to initiate evaluation of the patient for liver transplantation and decision making in this clinical setting. The underlying disease, the clinical condition and the status of the graft influence the patient's prognosis after the transplant. In times of general organ shortage, the graft pool might be extended by using living-donor transplants, split liver surgery or transplantation of livers in reduced conditions (Canbay 2011).

Extracorporeal liver support systems

Extracorporeal systems include support devices or bioreactors, which provide individual or a combination of functions that are insufficiently performed by the diseased liver. The scientific and clinical aim of the introduction of these novel techniques is to stabilise the patient until a donor organ is available or ideally until the liver completely recovers. However, adequately powered, randomised studies to establish these techniques in the treatment of ALF are either lacking or have failed to show any benefit over conventional therapy. Thus, treatment with these devices most likely remains a part of a bridging-to-transplantation strategy within an academic setting. The same accounts for novel stem cell and adult hepatocyte transplant approaches (Canbay 2011).

Specific treatment options

Acetaminophen poisoning

Activated oral charcoal (1 g/kg) might be indicated if administered up to four hours after acetaminophen ingestion. N-acetyl cysteine infusion to restore glutathione should be administered until as late as 24 to 36 hours after ingestion, and continued for 20 hours or longer. Monitoring of blood acetaminophen levels might help in decision-making regarding the duration or initiation of treatment. N-acetyl cysteine should be started as soon as possible, even in patients with a low probability of acetaminophen overdose or even in patients with non-paracetamol drug-induced ALF (Lee 2009). Steroid and ursodeoxycholic acid combination seems to be effective in drug-induced severe liver injury (Wree 2011).

Mushroom poisoning

Silibinin, with its cytoprotective effects against amanita toxin is used despite a lack of the controlled trials (Broussard 2001, Ganzert 2008).

Acute HBV infection

Antiviral therapy with lamivudine or entecavir has proven efficient and safe in fulminant HBV infection (Tillmann 2006). Moreover, with initiation of entecavir within the first days of admission, HBsAg concentrations and cell death were significantly reduced (Jochum 2009).

Pregnancy related

Immediate delivery and abortion are the available causal treatments. With early delivery, the rates of foetal death remain high; however the mortality rate of the mother decreases significantly (Westbrook 2010).

Autoimmune hepatitis

Steroid treatment should be initiated and if started in time might help to avoid the need for liver transplantation. With improvement of liver function, prednisone might be tapered and azathioprine treatment added to the regimens. Recent studies identified the topical steroid budesonide as a potential substitute for systemic prednisone therapy (Schramm 2010).

Table 5. Specific treatments for the causes of ALF

Causes	Medication	Doses
Acetaminophen	Activated oral charcoal	1 g/kg
	N-acetyl cysteine (oral/IV)	150 mg/kg loading dose, 50 mg/kg for 4h, 100 mg/kg for 20h
Mushroom	Silibinin	20–50 mg/kg/day
Acute HBV	Lamivudine	100–300 mg/day
	Entecavir	0.5–1 mg/day
	Tenofovir	245 mg/day
Pregnancy	Delivery	
Autoimmune	Prednisolone	1–2 mg/kg/day
Budd-Chiari syndrome	TIPS/surgical shunt	
HSV	Acyclovir	3 x 10 mg/kg/day

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