Chapter 12: Diagnostic tests in acute and chronic hepatitis C

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Introduction

Common symptoms of hepatitis C like fatigue, muscle ache, loss of appetite or nausea are unspecific and, in many cases, mild or even not present. Consequently, hepatitis C is often diagnosed accidentally and, unfortunately, remains heavily under-diagnosed. It is estimated that only one out of four individuals infected with HCV is aware of their disease and so can not take advantage of treatment options and risk further transmission of the virus (McHutchison 2004). 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 (McHutchison 2004). 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, transmission 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 measurement in order to discriminate 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 is furthermore essential in the determination of treatment indication, duration and success (Terrault 2005). The latter has to be confirmed at clearly defined times during treatment to decide whether therapy should be continued or not. It should be repeated 24 weeks after treatment completion to assess whether a sustained virologic response (SVR) has been achieved. 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 over a wide range of copies and are essential in treatment monitoring. Qualitative and quantitative HCV RNA assays are now being 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.

After diagnosing hepatitis C, the HCV genotype should be determined by nucleic acid-based techniques in every patient considered for HCV therapy because the currently recommended treatment duration and ribavirin doses differ among the genotypes.

Morphological methods like immunohistochemistry, in situ-hybridization or PCR from liver specimens play only an accessory 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 labeled 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 completed by an antigen from the NS5 region and 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). The clinical specificity, however, is slightly decreased compared to the 2nd generation assays. 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 in general 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 rheuma-factors and in populations with a low hepatitis C prevalence, for example 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 the false-negatively-classifying of 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 haematological malignancies.

HCV core antigen assays

In principle, detection of the HCV core antigen could be a cheaper alternative to nucleic acid testing for the diagnosis and management of hepatitis C. However, the introduction of a reliable and sensitive HCV core antigen assay was burdened with a number of difficulties like the development of specific monoclonal antibodies recognizing all different HCV subtypes and the need for accumulation and dissociation of HCV particles from immune complexes to increase sensitivity. The first HCV core antigen detection system (trak-C‘, Ortho Clinical Diagnostics) became commercially available in the US and Europe several years ago. In this assay, HCV core proteins were bound to coated monoclonal antibodies in a microwell after dissociation of the HCV particles from immune complexes. Bound core antigen was incubated with an anti-core-specific Fab antibody fragment conjugated with horseradish peroxidase followed by quantitative detection performed by addition of o-phenylenediamine (OPD) / hydrogen peroxide and measurement of the optical density. The 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 which corresponds to an HCV RNA level of approximately 10,000–50,000 IU/ml. 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.

Most recently, another quantitative HCV core antigen assay (Architect HCV Ag, Abbott Diagnostics), a further development of the previous assay, was approved by the European Union. This assay comprises 5 different antibodies to detect HCV core antigen, is highly specific (99.8%) and shows equivalent sensitivity for determination of chronic hepatitis C as HCV RNA measurement (Morota 2009). The detection limit corresponds to HCV RNA levels of 600–1000 IU/ml. Further studies are ongoing to show the utility of this more sensitive HCV core antigen assay for diagnosis and management of patients with HCV infection.

Nucleic acid testing for HCV

Until 1997, HCV quantitative results derived 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 load determination for management of patients, 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 false-negative results due to polymerase inhibition, for example by drug interference, false-positive results due to sample contaminations because the reaction tubes had to be opened frequently, or due to under- and over-quantification of samples of certain HCV genotypes (Pawlotsky 2003; Morishima 2004). Currently, several HCV RNA assays are commercially available (Table 1).

Assay	Distributor	Technology	Approval status
Qualitative HCV RNA detection assays			
Amplicor™ HCV 2.0	Roche Molecular Systems	PCR	FDA, CE
Versant™ HCV	Siemens Medical Solutions Diagnostics	TMA	FDA, CE
Quantitative HCV RNA detection assays			
Amplicor™ HCV Monitor 2.0	Roche Molecular Systems	PCR	CE
HCV SuperQuant™	National Genetics Institute	PCR	
Versant™ HCV RNA 3.0	Siemens Medical Solutions Diagnostics	bDNA	FDA, CE
Cobas Ampliprep/ Cobas TaqMan	Roche Molecular Systems	Real-time PCR	FDA, CE
Abbott RealTime™ HCV	Abbott Diagnostics	Real-time PCR	CE

Table 1. Commercially available HCV RNA detection assays.

Qualitative assays for HCV RNA detection

Until recently qualitative assays for HCV RNA had substantially lower limits of detection in comparison with 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 (Roche Molecular Systems, USA) is an FDA- and CE-approved RT-PCR system for qualitative HCV RNA testing that allows detection of HCV RNA concentrations down to 50 IU/ml of all genotypes (Nolte 2001) (Table 1). 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.

Transcription-mediated amplification (TMA) of HCV RNA

TMA-based qualitative HCV RNA detection has a very high sensitivity (Sarrazin 2002; Hendricks 2003). TMA is performed in a single tube in three steps: target capture, target amplification and specific detection of target amplicons by a hybridization 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 500µl serum, the T7 promoter-containing primer hybridises with the viral RNA with the result of reverse transcriptase-mediated cDNA synthesis. The reverse transcriptase also provides an RNase activity which degrades the RNA of the resulting RNA/DNA hybrid strand. The second primer then binds to the cDNA already containing the T7 promoter sequence from the first primer, and a DNA/DNA double-strand is synthesised by the reverse transcriptase. Next, the RNA polymerase recognizes 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 labeled DNA probes. The unhybridised DNA probes are degraded during a selection step and the labeled DNA is detected by chemiluminescence.

A commercially available TMA assay is the Versant™ HCV RNA Qualitative Assay (formerly Bayer, now Siemens Medical Solutions Diagnostics, Germany). This system is accredited by the FDA and CE and provides an extremely high sensitivity, which is superior to RT-PCR-based qualitative HCV RNA detection assays (Sarrazin 2000; Sarrazin 2001; Hofmann 2005). The lower detection limit is 5-10 IU/ml with a sensitivity of 96-100%, and a specificity of more than 99.5%. These performance characteristics are independent of the HCV genotype.

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 from Roche Diagnostics is based on a competitive PCR technique whereas the Versant™ HCV RNA Assay (Siemens Medical Solutions Diagnostics) 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 summarized 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 Monitor 2.0 (Roche Diagnostics)

The Cobas Amplicor™ HCV Monitor 2.0 is 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 CE-labeled Amplicor™ HCV Monitor 2.0 assay is 500 to approximately 500,000 IU/ml

with a specificity of almost 100%, independent of the HCV genotype (Lee 2000; Konnick 2002). For higher HCV RNA concentrations pre-dilution of the original sample is required.

Branched DNA Hybridization Assay (Versant™ HCV RNA Assay 3.0, Siemens)

Branched DNAHybridization Assay is based on signal amplification technology. After reverse transcription of the HCV RNA, the resulting single-stranded complementary DNA strands bind to immobilised capture 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-labeled 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; Ross 2002). The bDNA assay only requires 50µl 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 of simultaneous amplification and detection of the target nucleic acid allowing direct monitoring of the PCR process (Higuchi 1992). RNA templates are first reverse-transcribed to generate complementary cDNA strands followed by a DNA polymerase-mediated cDNA amplification.

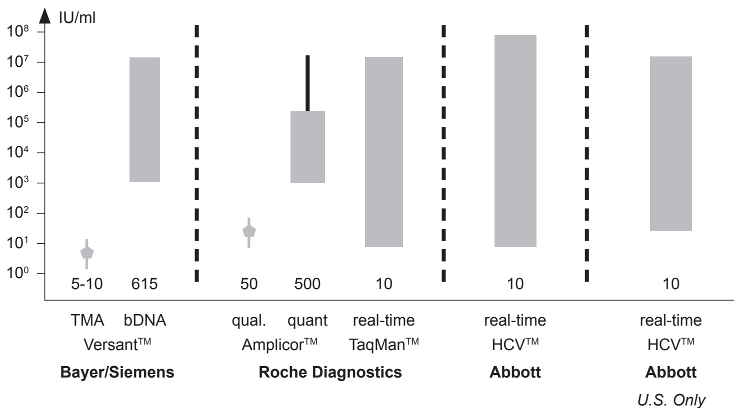

Figure 1. Detection limits and linear dynamic ranges of commercially available HCV RNA detection assays.

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 by Roche Molecular Systems (US) and Abbott Laboratories (US). For replacement of the qualitative TMA and the quantitative bDNA-based assays, Siemens Diagnostics has also developed a real-time based PCR, scheduled to be launched in 2010.

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 that in the case of the real-time PCR-based assays represent a slight under-quantification by one assay (real-time HCV) and a slight over-quantification by the other (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 (Roche Diagnostics)

The 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 fiber 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 recently released second version of this assay has now demonstrated equal quantification of all HCV genotypes (Colluci 2007). For the Cobas Ampliprep Cobas TaqMan (CAP/CTM) assay significant under-quantification of HCV genotype 4 samples has been shown (Konnick 2005; Gelderblom 2006; Colucci 2007; Sizmann 2007; Vermehren 2008) and a second version of this assay is in preparation. Taken together, with the exception of HCV genotype 4 samples with the CAP/CTM assay, 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.

RealTime™ HCV Test (Abbott Diagnostics)

The CE-accredited Abbott RealTime™ HCV Test uses reporter- and quencher-carrying oligonucleotides specific for the 5'UTR as well. HCV RNA concentrations are quantified by comparison with the amplification curves of a cDNA from the hydroxypyruvate reductase gene from the pumpkin plant Curcurbita 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 Abbott RealTime™ HCV Test provides a lower detection limit of 12 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; Schutten 2007; Vermehren 2008).

HCV genotyping

HCV is heterogeneous with an enormous genomic sequence variability, developed by the rapid replication cycle with the production of 10^{12} 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 characterized. 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 and ribavirin doses depend on the HCV genotype, HCV genotyping is mandatory in every patient who is considered for antiviral therapy (Bowden 2006).

Both direct sequence analysis and reverse hybridisation technology allows HCV genotyping. Initial assays were designed to analyse exclusively the 5' untranslated region (5'UTR), which is burdened with a high rate of misclassifications especially on the subtype level. Current assays were improved by additionally analyzing 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).

Genotyping by reverse hybridising assay (Versant™ HCV Genotype 2.0 System (LiPA), Siemens Medical Solutions Diagnostics)

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 (Siemens Medical Solutions Diagnostics) is suitable for indentifying genotypes 1-6 and more than 15 different subtypes and is currently the preferentially used assay for HCV genotyping. By simultaneous

analyses of the 5'UTR and core region, a high specificity is achieved especially 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).

Genotyping by direct sequence analysis (TRUGENE™ HCV 5'NC Genotyping Kit, Siemens)

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 because of the exclusive analyses of the 5'UTR. Currently, the TRUGENE™ NS5B HCV genotyping assay, which additionally analyzes the NS5B region, is under development (Pawlotsky 2003).

Genotyping by real-time PCR technology (Abbott RealTime™ HCV Genotype II assay)

The current Abbott 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 1 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 optimization (Vaghefi 2009).

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 newly developed 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 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 because its presence is common in both situations.

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 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 via 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 load determination before, during and after antiviral therapy. When HCV RNA has been detected, exact genotyping and HCV RNA load determination is necessary in every patient considered for antiviral therapy. Exact subtyping might gain increased importance for future STAT-C therapies because some subtypes behave differently regarding the development of resistance. Low HCV RNA concentrations (<600,000–800,000 IU/ml) is a positive predictor of SVR. Genotyping is mandatory before treatment initiation, as the dose of ribavirin and optimal treatment duration is determined specifically on the underlying HCV genotype (McHutchison 2004; Terrault 2005). For HCV genotype 1 (and 4) treatment can be shortened to 24 weeks in patients with low baseline viral load (<600,000–800,000 IU/ml) and rapid virologic response with undetectable HCV RNA at week 4 of treatment (RVR). In slow responders with a 2log decline but still detectable HCV RNA levels at week 12 and undetectable HCV RNA at week 24 treatment should be extended to 72 weeks, and in patients with complete early virologic response with undetectable HCV RNA at week 12 (cEVR) standard treatment is continued out to 48 weeks. Genotypes 5 and 6 are treated the same as genotype 1 infected patients due to the lack of adequate clinical trials whereas genotypes 2 and 3 generally allow treatment duration of 24 weeks, which may be shortened to 16 weeks (depending on RVR and [low] baseline viral load) or extended to 36-48 weeks depending on the initial viral decline (Layden-Almer 2006; Manns 2006). Independent of the HCV genotype, proof of HCV RNA load decrease is 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 should be discontinued if a decrease of less than 2log HCV RNA levels is observed (negative predictive value 88-100%). In a second step, HCV RNA should be tested with highly sensitive assays after 24 weeks of treatment because patients with detectable HCV RNA at this time point only have a 1-2% chance of achieving SVR. SVR, defined as the absence of detectable HCV RNA 24 weeks after treatment completion, should be assessed by an HCV RNA detection assay with a lower limit of 50 IU/ml or less to evaluate long-lasting treatment success (Layden-Almer 2006; Manns 2006).

Due to the differences in HCV RNA concentrations of up to a factor of 4 between the different commercially available assays, despite standardisation of the results to IU, and due to intra- and interassay variability of up to a factor of 2, it is recommended to always use the same assay in a given patient before, during and after treatment and to repeat HCV RNA measurements at baseline in cases with HCV RNA concentrations between 400,000 and 1,000,000 IU/ml.

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