

Chapter 20: Assessment of hepatic fibrosis in chronic viral hepatitis

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Introduction

Non-invasive methods for the assessment of liver fibrosis versus invasive liver biopsy are increasingly being used thanks to patient acceptance and the low but ever-present morbidity of biopsies. Yet, despite recent advances in the use of surrogate markers and the development of new technical developments such as elastography, liver histology remains the gold standard for fibrosis staging (Goodman 2007). Most experts agree that non-invasive techniques will not replace liver biopsies completely but will help reduce the number of biopsies required (Leroy 2007; Pinzani 2005; Sebastiani 2006).

Non-invasive tests should be able to discriminate between non-significant (stages F0-F1) and significant (stages \geq F2) fibrosis, to help either delay or initiate antiviral treatment. Non-invasive markers should be able to reliably predict liver cirrhosis, and dose adjustments or monitoring can occur while on antiviral treatment.

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 components (Friedman 2008). Endstage liver fibrosis (cirrhosis) may include insufficient detoxification, hepatocellular carcinoma, portal hypertension, renal and pulmonary failure, and is associated with excess mortality. In chronic viral hepatitis, fibrosis develops as a consequence of the host immunological response. This immunological response activates antiviral defence mechanisms that aim to clear infected hepatocytes. The mechanisms underlying fibrogenesis in HBV or HCV are complex (Friedman 2007).

A key feature of hepatic fibrosis is the activation and proliferation of hepatic stellate cells. Quiescent hepatic stellate cells store vitamin A and reside in the subendothelial space of Disse. Chronic liver injury leads to activation of these cells, which become contractile, produce extracellular matrix components and secrete pro-inflammatory cytokines and chemokines such as transforming growth factor β . 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 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. In liver fibrogenesis, TIMP-1 is also produced by activated hepatic stellate cells.

Liver histology, by helping visualise the fibrosis, is regarded as the gold standard for the assessment and progression of fibrosis. However, the disadvantages of this method have motivated researchers and clinicians to test non-invasive strategies. These strategies are based either on single serum surrogate markers, compositional scores derived from combinations of different surrogate markers, or modifications of imaging techniques.

Liver biopsy – the gold standard for staging of liver fibrosis

In the majority of liver centres worldwide, liver biopsy is performed as a “blind” or ultrasound-guided puncture, as either an out- or in-patient procedure. Liver punctures are considered to be relatively safe procedures with complication rates ranging from 0.75% up to 13.6% (Myers 2008; Piccinino 1986; van der Poorten 2006). The most frequent complications are minor bleeding or pain. After efficient substitution with clotting factors, percutaneous liver biopsy is also possible in patients with inherited bleeding disorders with no obvious increase of complication rates (DiMichele 2003; Schwarz 2008). Procedure-related mortality rates are reported to range from 0.001 to 0.003% (Piccinino 1986). Of note, excess rates with severe bleedings and biopsy related deaths have been reported after percutaneous biopsy in populations with advanced fibrosis, cirrhosis, or hepatic tumors (Terjung 2003). Thus, liver biopsies in these patients should always be performed as in-patient procedures, as >90% of complications are detected within the first 24 hours (Piccinino 1986).

Transjugular puncture of the liver via cannulation of an hepatic vein is an alternative, that can be performed in patients with severe coagulation deficiencies. It is resource-intensive and carries a risk of intrahepatic haemorrhage or capsule perforation with intra-abdominal bleeding. Complication rates are lower as compared to percutaneous biopsies and range from 2.5% (Mammen 2008) to 6.5% with a reported mortality rate of up to 0.09% in high-risk groups (Kalambokis 2007). However, the quality of specimens from transjugular biopsies may be lower because of the higher fragmentation of specimens and the lower numbers of portal fields in transjugular biopsies (Cholongitas 2006).

Laparoscopy and mini-laparoscopy are even more invasive procedures for obtaining liver biopsies. A recent randomized trial showed a higher detection rate of liver cirrhosis as compared to percutaneous biopsies with lower complication rates for laparoscopy (Denzer 2007). No data is available for detection in lower fibrosis stages. Thus, we recommend this procedure only in selected cases if the results might have an impact on the clinical management of the patient (Helmreich-Becker 2003).

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 20-25 mm long and more than 11 portal tracts should be visible (Cholongitas 2006; Rousselet 2005; Bedossa 2003). 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). Recent studies reported up to one stage difference between specimens from the right and the left lobe in up to 38% of biopsies (Regev 2002; Siddique 2003). Discrepancies of more than one stage are rare (Regev 2002; Siddique 2003; Skripenova 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). Standardized automatic staging via image analysis may improve inter-observer variability (Hui 2003).

All staging systems for liver fibrosis are based on the definition of categorical stages of liver fibrosis that describe the increase of deposition of collagen and the progressive destruction of liver architecture ranging from no fibrosis to cirrhosis with a variable number of intermediate stages (Table 1). The use of categories decreases inter-observer variation, but also results in a loss of information that may be covered by more detailed scoring systems (Standish 2006).

Whereas the METAVIR score is considered best in HCV fibrosis, there is a wide variability in the use of other staging systems in patients with chronic viral hepatitis. In Germany, current guidelines recommend the staging system defined by Desmet & Scheuer (Table 1) (The French METAVIR Cooperative Study Group 1994; Knodell 1981; Ishak 1995; Desmet 1994; Schirmacher 2004).

Staging System	Fibrosis stages	Remark	
METAVIR Score	F0, F1, F2, F3, F4	Best evaluated in HCV fibrosis	(The French METAVIR Cooperative Study Group 1994)
Knodell Score	F0, F1, F3, F4	No intermediate stage	(Knodell 1981)
Desmet & Scheuer	Analogous to METAVIR	Recommended by German guidelines for the assessment of liver fibrosis	(Desmet 1994; Schirmacher 2004)
Batts & Ludwig	Similar to METAVIR		(Batts 1995)
Ishak Score	F0, F1, F2, F3, F4, F5, F6		(Ishak 1995)

Table 1. Commonly used liver fibrosis staging scores.

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 a more precise grading system. Surrogate markers can be subdivided into direct and indirect markers. Direct markers reflect changes in the content of extracellular matrix proteins (such as collagen) in the liver. In contrast, indirect markers reflect alterations in hepatic function, increase in portal hypertension with subsequent splenic enlargement, and/or grade of hepatic inflammation that may correlate with liver fibrosis stage (Table 2). Direct and indirect markers may be used alone or - more commonly - in combination ("composite scores"). The calculation of such scores can be simple or based on complicated formulas (e.g., Fibrotest / Fibrosure) (Table 2). Most studies of non-invasive markers were performed in HCV patients, while studies in HBV or co-infected cohorts are sparse (Pinzani 2008). Primary endpoints of the studies that evaluated surrogate markers vary from discrimination of no fibrosis and cirrhosis to the determination of fibrosis stages. However, for the clinical management of patients with chronic viral hepatitis both are needed: whereas the former is needed to identify patients in need of urgent treatment, the latter may separate those patients with an indication for antiviral treatment due to significant fibrosis from those with no or minor fibrosis in whom treatment may be postponed.

Index	Markers	Calculation	Interpretation	PPV/NPV (%)
Direct surrogate markers				
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	NPV=95 PPV=66 NPV=75 PPV=91
ELF	PIIINP, HA	Proprietary	>0.102 Scheuer 3-4 <0.102 Scheuer 0-2	PPV = 35 NPV = 92
Indirect surrogate markers				
Forns	Age, plt, γGT, cholesterol	$7.811 - 3.131 \text{ xln}(\text{plt}) + 0.781 \text{ xln}(\gamma\text{GT}) + 3.467 \text{ xln}(\text{age}) - 0.014 (\text{cholesterol})$	>6.9 ≈Scheuer 2-4 <4.2 ≈Scheuer 0-1	PPV = 66 NPV = 96
APRI	AST, plt	$([\text{AST}/\text{ULN}]/\text{plt} [\text{x}109/\text{l}]) \text{ x}100$	>1.5 ≈Ishak 3-6 ≤0.5 ≈Ishak 0-2	PPV = 91 NPV = 90
Fibrotect Fibrosure	Haptoglobin, α2-MC, apo-A1, γGT, bilirubin, γ-globulin	Proprietary	0.75-1.00 ≈F4 0.73-0.74 ≈F3-F4 0.59-0.72 ≈F3 0.49-0.58 ≈F2 0.32-0.48 ≈F1-F2 0.28-0.31 ≈F1 0.22-0.27 ≈F0-F1 0.00-0.21 ≈F0	PPV = 78 PPV = 76 PPV = 76 PPV = 67 PPV/NPV = 61/85 NPV = 91 NPV = 92 NPV = 94
Fibroindex	Plt, AST, γ GT	$1.738 - 0.064 (\text{plt} [\text{×}104/\text{mm}3]) + 0.005 (\text{AST} [\text{I}/\text{U}]) + 0.463 \text{ x} (\gamma\text{GT}(\text{g}/\text{dl}))$	≤ 1.25 ≈F0-F1 ≥ 2.25 ≈F2-F3	NPV = 61.7 PPV = 90
Testa	Plt, spleen diameter	Plt count/spleen diameter	>1750 ≈Ishak ≤2 ≤1750 ≈Ishak >2	NPV = 79 PPV = 78.9c
Fibrosis probability index	AST, cholesterol, past alcohol intake, HOMA, age	$E^{*1} + e^{*}$, where $* = -10.929 + (1.827 \text{ xln}(\text{AST})) + (0.081 \text{ xage}) + (0.768 \text{ x} [\text{past alcohol use graded as } 0-2]) + (0.385 \text{ x HOMA})$	<0.2 ≈F0-F1 ≥0.8 ≈F2-F4	NPV = 77.4 PPV = 87
FIB-4	Plt, AST, ALT, age	$(\text{Age x AST})/(\text{plt count x ALT}^2)$	<1.45 ≈Ishak <4-6 >3.25 ≈Ishak ≥4-6	NPV = 90 PPV = 65
Bonancini	ALT, AST, INR, plt	Sum (range 0-11) of (plt score) + (ALT/AST score) + (INR score) plt (x109/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	PPV = 92.9
Pohi	AST, ALT, plt	Positive if: AST/ALT ≥1 and platelet count <150 x109/l	Positive ≈F3-F4	PPV = 93
Shet Park	AST, ALT AST, ALT	AST/ALT AST/ALT	≥1 = Scheuer 4 ≥1≈ Scheuer 4	PPV = 100 PPV = 73.7
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 (x109/l): ≥225 = 0; 200-224 = 1; 175-199 = 2; 150-174 = 3; 125-149 = 4; <125 = 5	≥6 ≈F2-F4	PPV = 96
Combined direct and indirect surrogate markers				
SHASTA	HA, AST, albumin	$-3.84 + 1.70 (1 \text{ if HA } 41-85 \text{ ng/ml, } 0 \text{ otherwise}) + 3.28 (1 \text{ if HA } >85 \text{ ng/ml, } 0 \text{ otherwise}) + 1.58 (1 \text{ if albumin } <3.5 \text{ g/dl, } 0 \text{ otherwise}) + 1.78 (1 \text{ if AST} >60 \text{ IU/l, } 0 \text{ otherwise})$	>0.8 ≈Ishak ≥3 <0.3 ≈Ishak ≤2	PPV = 100 NPV = 94
FM	plt, PI, AST, HA, α2-MC, gender, age	$-0.007 \text{ plt (G/L)} - 0.049 \text{ PI (\%)} + 0.012 \text{ AST (IU/l)} + 0.005 \alpha\text{-2-MC (mg/dl)} + 0.021 \text{ HA (g/l)} - 0.270 \text{ urea (mmol/l)} + 0.027 \text{ age (years)} + 3.718$	≥F2	PPV = 86.3/96.6
Hepascore	HA, α2-MC, γGT, age, gender	$y/1 + y$, where $y = \exp [-4.185818 - (0.0249 \text{ x age}) + 0.7464 \text{ x sex}] + (1.0039 \text{ x } \alpha\text{-2-MC}) + (0.0302 \text{ x HA}) + (0.0691 \text{ x bilirubin}) - (0.0012 \text{ x } \gamma\text{GT})]$	≥0.5 ≈F2-F4 <0.5 ≈F0-F1	PPV = 88 NPV = 98
FSII	HA, α2-MC, TIMP-1	Proprietary	≥42 ≈F2-F4 <40 ≈F0-F1	PPV = 77.4 NPV = 78

Table 2. Summary of surrogate markers of liver fibrosis modified according to Pinzani.

From the whole range of surrogate markers only a few are in clinical use. The simple APRI score has been widely studied in HCV and HBV as well as in coinfecting patients (Cacoub 2008; Vallet-Pichard 2008; Wai 2006; Lebensztejn 2005). A recent 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 1.0. However, the authors conclude that using this marker alone, only about one third of all biopsies can be avoided. Importantly, the test performance varied with the quantity of advanced fibrosis in the different patient groups (Shaheen 2007a; Shaheen 2007b). Fibrotest has also achieved some clinical significance. However, this test may not be available for all patients. Recent meta-analyses of the predictive performance of Fibrotest summarize that the reliability for the detection of advanced fibrosis or cirrhosis is adequate for clinical practice and a cut-off of 0.6 is suggested (Shaheen 2007b; Poynard 2007). Of note, the reliability for the detection of earlier fibrosis stages appears to be relatively low (Shaheen 2007b; Poynard 2007) and the most positive conclusions concerning the Fibrotest come from authors who are directly involved in the commercial distribution of this test (Shaheen 2007b; Poynard 2007).

In summary, surrogate markers may support the clinical decision making process, but a single surrogate marker or score can not replace the 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 punctures (Table 2).

Transient elastography

Transient elastography (TE) is a non-invasive technique to assess liver fibrosis that was first described in the medical literature in 1999 (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 actual 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. 10 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 by echosense (FibroScan®). Liver stiffness is expressed in kilo Pascal (kPa). The method is easy to learn, quick, results are available immediately, and a technical assistant may perform the procedure. TE displays robust intra- and inter-observer variability (Fraquelli 2007) and may be applied in children and adults (de Ledinghen 2007).

Evaluation of liver stiffness in subjects without apparent liver disease shows that liver stiffness is influenced by gender and body mass index (BMI). In general, liver stiffness is higher in men than in women (5.81 ± 1.54 vs. 5.23 ± 1.59 kPa) (Roulot 2008). It is important to note that the applicability of TE is limited to relatively lean patients ($BMI \leq 28$ kg/m²), patients without ascites, and “cooperative” patients. In addition, TE is hampered in those with acute liver injury such as acute viral or alcoholic hepatitis, or chronic viral hepatitis flares, that may lead to an overestimation of liver fibrosis (Arena 2008; Coco 2007; Sagir 2008). 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 unpublished data).

In patients with chronic liver disease eligible for TE, liver stiffness values correlate well with the stage of fibrosis, irrespective of the underlying disease aetiology. TE has been evaluated in patients with chronic viral hepatitis, PBC, PSC, and NASH. 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 (Yoneda 2007). 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 HIV/HCV-coinfected patients and in patients with HCV re-occurrence post-transplantation (de Ledinghen 2006; Maida 2007; de Ledinghen 2006; Carrion 2006). In chronic viral hepatitis, it is unknown whether there is a difference in TE results between patients with chronic HBV, HCV and/or HIV/HCV-coinfected patients.

In some clinical situations, e.g., older patients or patients with risk factors for therapy, a positive decision for treatment of chronic hepatitis B and C is guided by the diagnosis of significant fibrosis. The presence of F2 fibrosis indicates significant liver fibrosis, which justifies treatment according to treatment guidelines for chronic hepatitis B, C and co-infected patients (e.g., German Guidelines for the Management of Patients with Chronic Hepatitis C Viral Infection 2009).

Study	Population	Cut-off (kPa)				
Castera 2006	HCV N = 183	F = 0	F ≥1	F ≥2	F ≥3	F = 4
		n.d	n.d	7.1 Se: 0.67 Sp: 0.95 PPV: .95 NPV: .48	9.5 Se: 0.73 Sp: 0.91 PPV: .87 NPV: .81	12.5 Se: 0.87 Sp: 0.91 PPV: .77 NPV: .97
Ziol 2005	HCV N = 327	n.d	n.d	8.8	9.6	14.6
		n.d	n.d	Se: 0.56 Sp: 0.91 PPV: .88 NPV: .56	Se: 0.86 Sp: 0.85 PPV: .71 NPV: .93	Se: 0.86 Sp: 0.96 PPV: .78 NPV: .97
Foucher 2006	HCV / HBV N = 711	n.d	n.d	7.2 Se: 0.64 Sp: 0.85 PPV: .90 NPV: .52	12.5 Se: 0.65 Sp: 0.95 PPV: .90 NPV: .80	17.6 Se: 0.77 Sp: 0.97 PPV: .91 NPV: .92
Ogawa 2007	HCV / HBV N = 229	3.5	6.4	9.5 Se: 0.67 Sp: 0.95 PPV: .95 NPV: .48	11.4 Se: 0.67 Sp: 0.95 PPV: .95 NPV: .48	15.4 Se: 0.67 Sp: 0.95 PPV: .95 NPV: .48
		6.3	6.7	9.1 Se: 0.67 Sp: 0.95 PPV: .95 NPV: .48	13.7 Se: 0.67 Sp: 0.95 PPV: .95 NPV: .48	26.4 Se: 0.67 Sp: 0.95 PPV: .95 NPV: .48
Arena 2008	HCV N = 150			7.8 Se: 0.83 Sp: 0.82 PPV: .83 NPV: .79	10.8 Se: 0.91 Sp: 0.94 PPV: .89 NPV: .95	14.8 Se: 0.94 Sp: 0.92 PPV: .73 NPV: .98
de Ledinghen 2006	HIV/HCV N = 72	n.d	n.d	4.5 Se: 0.93 Sp: 0.18 PPV: n.d. NPV: n.d.	n.d	11.8 Se: 1.0 Sp: 0.93 PPV: n.d. NPV: n.d.

Table 3. Cut-off values for transient elastography in different study populations.

Recent 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 ≥ 2 compared to more advanced stages of liver fibrosis (sensitivity 56-67%), resulting in moderate negative predictive values. Thus, 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 may not be necessary. One drawback in clinical practice is that the different TE studies suggest slightly different cut-off values (Table 3). A recent 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 (Friedrich-Rust 2008). This cut-off proved to be robust especially in patients with chronic HCV infection.

In addition to the assessment of liver fibrosis stages, TE may also be used to predict the presence of portal hypertension and thus the need to evaluate the patient for the presence of oesophageal varices (Rockey 2008). Whether TE is reliable enough to predict the stage of cirrhosis is still debatable and needs further study (Foucher 2006).

Other imaging techniques

A number of different imaging techniques such as conventional ultrasound, real-time elastography, NMR imaging and CT have been applied for the assessment of liver fibrosis. None of these methods has yet achieved an overall clinical acceptance regarding the detection of early forms of liver fibrosis, either due to low sensitivity and/or specificity, or high costs.

Clinical decision algorithms

Until now, no non-invasive marker for staging of liver fibrosis has been able to replace the liver histology as the gold standard. This is largely due to the fact that outcome studies with clear endpoints like mortality have not been performed. These will probably not be available in the near future. 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, thereby using liver biopsy only in case of doubt. However, current algorithms vary greatly. Whereas some authors have calculated a reduction of liver biopsies of 30%, others have estimated reductions of up to 80% (Leroy 2007; Sebastiani 2007; Sebastiani 2004). Interestingly, the performance of such algorithms and their components depend on underlying diseases (HCV, HBV or coinfections). Thus to date, no widely applicable algorithm is available. However, Figure 1 shows a concept used in our daily practice.

Summary

Non-invasive tests will not replace liver biopsies, but smart combinations of both options may save many patients from the more invasive procedure. Whatever the current standard of care, the patient should be informed about non-invasive tests, their applicability and their limitations. The decision to biopsy should ultimately be made together with the informed patient.

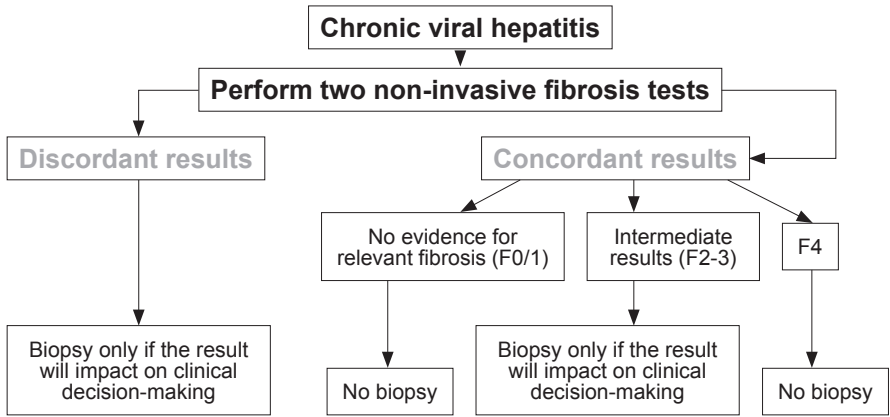


Figure 1. Potential clinical decision algorithm for safer liver biopsies in patients with chronic viral hepatitis.

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