Tools for the diagnosis of hepatitis C virus infection and hepatic fibrosis staging

Verónica Saludes, Victoria González, Ramon Planas, Lurdes Matas, Vicente Ausina, Elisa Martró

Verónica Saludes, Victoria González, Lurdes Matas, Vicente Ausina, Elisa Martró, Microbiology Service, Fundació Institut d’Investigació en Ciències de la Salut Germans Trias i Pujol, Hospital Universitari Germans Trias i Pujol (Universitat Autònoma de Barcelona), 08916 Badalona, Spain
Verónica Saludes, Victoria González, Lurdes Matas, Elisa Martró, CIBER Epidemiología y Salud Pública (CIBERESP), 08003 Barcelona, Spain
Victoria González, Centre for Epidemiological Studies on HIV/STI in Catalonia (CEEISCAT)-ICO, 08916 Badalona, Spain
Ramon Planas, Liver Unit, Hospital Universitari Germans Trias i Pujol, 08916 Badalona, Spain
Ramon Planas, CIBER Enfermedades Hepáticas y Digestivas (CIBEREHID), 08036 Barcelona, Spain
Vicente Ausina, CIBER Enfermedades Respiratorias (CIBERSER), 07110 Bunyola, Spain

Author contributions: Martró E conceived the topic; Saludes V, González V and Martró E reviewed the literature and wrote the manuscript; Planas R, Matas L and Ausina V provided overall intellectual input into the review’s topic and edited the manuscript; all authors approved the final version to be published.

Supported by A Miguel Servet contract No. MS09/00044 funded by FIS-ISICII (Spanish Government) to Martró E; grant P110/01734 within the “Plan Nacional de I+D+I” and co-financed by “ISCIII-Subdirección General de Evaluación y el Fondo Europeo de Desarrollo Regional” (FEDER) to González V, Saludes V, Martró E

Correspondence to: Elisa Martró, PhD, Researcher in the National Health System, Microbiology Service, Fundació Institut d’Investigació en Ciències de la Salut Germans Trias i Pujol, Hospital Universitari Germans Trias i Pujol (Universitat Autònoma de Barcelona), Ctra. del Canyet s/n, 08916 Badalona, Spain. emartro.igtp.germanstrias@gencat.cat
Telephone: +34-934-978894 Fax: +34-934-978895
Received: September 27, 2013 Revised: December 7, 2013
Accepted: March 6, 2014
Published online: April 7, 2014

Abstract

Hepatitis C virus (HCV) infection represents a major public health issue. Hepatitis C can be cured by therapy, but many infected individuals are unaware of their status. Effective HCV screening, fast diagnosis and characterization, and hepatic fibrosis staging are highly relevant for controlling transmission, treating infected patients and, consequently, avoiding end-stage liver disease. Exposure to HCV can be determined with high sensitivity and specificity with currently available third generation serology assays. Additionally, the use of point-of-care tests can increase HCV screening opportunities. However, active HCV infection must be confirmed by direct diagnosis methods. Additionally, HCV genotyping is required prior to starting any treatment. Increasingly, high-volume clinical laboratories use different types of automated platforms, which have simplified sample processing, reduced hands-on-time, minimized contamination risks and human error and ensured full traceability of results. Significant advances have also been made in the field of fibrosis stage assessment with the development of non-invasive methods, such as imaging techniques and serum-based tests. However, no single test is currently available that is able to completely replace liver biopsy. This review focuses on approved commercial tools used to diagnose HCV infection and the recommended hepatic fibrosis staging tests.

© 2014 Baishideng Publishing Group Co., Limited. All rights reserved.

Key words: Hepatitis C virus; Diagnosis; Real-time polymerase chain reaction; Serology; Hepatitis C virus-RNA quantification; Hepatitis C virus genotyping; Hepatic fibrosis staging

Core tip: About 150 million people are chronically infected with hepatitis C virus (HCV) worldwide, making them at risk for cirrhosis, hepatocellular carcinoma and end-stage liver disease. Recent advances in hepatitis C therapy may bring the opportunity of eradicating this infection. However, ongoing HCV transmission, under-
diagnosis of HCV-infected persons, and difficulties in accessing treatment remain great challenges that require public health responses. In this review, we focus on diagnostic methods used to control HCV infection, including laboratory and point-of-care tests. We also discuss available non-invasive methods to assess liver fibrosis, as the severity of liver disease has important implications in the prognosis and treatment of hepatitis C.

INTRODUCTION

HCV is an enveloped, single-strand RNA virus that belongs to the Flaviviridae family. Its genome of approximately 9.6 kb contains a single open reading frame that encodes for three structural (core, E1 and E2) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A and NS5B) and is flanked by untranslated regions (UTR)\(^\text{[7]}\). The HCV genome exhibits significant genetic variability, which has led to its classification into seven genotypes and multiple subtypes within each genotype\(^\text{[2,3]}\).

While genotypes 1, 2 and 3 are distributed worldwide, the prevalence of HCV genotypes and subtypes varies geographically according to transmission route and ethnicity\(^\text{[4]}\).

Hepatitis C virus (HCV) has a seroprevalence of 2.8% (over 185 million people) worldwide, making it the major causative agent of chronic liver disease, cirrhosis and hepatocellular carcinoma\(^\text{[5,6]}\). Although acute HCV infection can be spontaneously cleared, it leads to a chronic infection in the majority of persons. Given the asymptomatic nature of a high proportion of acute and chronic HCV infections, unrecognized infection is a global public health problem that should be promptly addressed using appropriate screening strategies and diagnostic assays. Serological and molecular markers of HCV infection are key to correctly diagnose past exposure versus active infection, and acute versus chronic infection, as well as to assess treatment indication.

Pegylated-interferon alpha (PegIFN-α) and ribavirin (RBV) combination therapy is the current standard of care for treating chronic hepatitis C by non-1 genotypes. A triple therapy that also contains an HCV-specific protease inhibitor has recently been approved to treat chronic infection by HCV genotype 1 in many countries around the world\(^\text{[7]}\). Over the past decade, HCV genotyping assays have been improved and ultrasensitive quantitative molecular assays have been developed. These technical improvements are mainly due to changes in the treatment algorithms and the use of response-guided therapy, which is based on how rapidly HCV responds to treatment (“on-treatment virologic response”). Acutely infected patients have higher response rates to antiviral treatment than those with an established chronic infection. Thus, effective screening and fast diagnosis of HCV are highly relevant steps in preventing disease progression and virus spread, since they allow infected persons to be identified and treated. We discuss the assays approved for in vitro diagnostics in the following sections.

TOOLS FOR THE DIAGNOSIS AND MANAGEMENT OF HCV INFECTION

Two major types of assays have been developed to diagnose and manage HCV infection: those that detect HCV-specific antibodies that are used to indirectly diagnose infection, and those that detect viral components (e.g., the core antigen or the viral genome) that are used to directly diagnose hepatitis C and manage HCV infected patients.

Indirect diagnosis

Serological assays detect HCV-specific total antibodies (IgM and IgG) and are used to screen and diagnose HCV exposure. However, these assays do not discriminate between active and resolved infections.

Screening assays: Since 1989, when HCV was discovered and its immunodominant epitopes were identified, HCV infection has been mainly diagnosed by detecting HCV antibodies from serum samples using enzyme immunoassays (EIA). Over time, serologic assays have evolved, and current third-generation assays now include multiple recombinant HCV antigens from the core, NS3, NS4 and NS5 regions. This has resulted in the reduction of the window period and in an overall improved detection of patients exposed to HCV (with excellent sensitivity and specificity)\(^\text{[8]}\).

Currently, immunoassays can be fully automated using high-throughput, random access instruments that are widely used in clinical laboratories. Characteristics of the most commonly used assays are summarized in Table 1. Most of these are chemiluminescence immunoassays, which have improved the specificity and positive predictive value (PPV) of conventional EIAs\(^\text{[9]}\). Briefly, HCV antigens are immobilized on different types of solid phases (microwell, magnetic and paramagnetic particles). The presence of HCV-specific antibodies in the clinical specimen is then detected with a conjugate antibody (anti-human IgG labeled with acridinium or horseradish peroxidase) that catalyzes the oxidation of a luminol, producing light. The light signal is measured by the system and then normalized relative to the cut-off value [signal/cut-off (S/CO)] or as relative light units\(^\text{[9,10]}\).

Anti-HCV assays have several disadvantages, including: (1) the prolonged duration of the window period between the time of infection and the detection of HCV antibodies (approximately 45-68 d)\(^\text{[11]}\); (2) the low PPV in low-risk populations (as false-positive results may result from the presence of multiple circulating immunoglobulins that can interact non-specifically with HCV
antigens); and (3) the possibility of false-negative results in immune-compromised persons or in those who are undergoing haemodialysis due to an inadequate antibody response. Furthermore, all available assays have a gray zone from which results are not interpretable. In cases with uninterpretable results, the sample should be centrifuged to completely remove all cells, cellular debris and fibrin, and the assay should be repeated in duplicate to verify its status. If the results of the duplicated repetition are below the assay cut-off for both replicates, the sample should be considered negative. If either duplicate retest result is above or equal to the cut-off, the sample should be tested by supplementary assays to confirm the result.

**Confirmatory assays:** Recombinant immunoblot assays (RIBA) can be used to confirm the presence of HCV-specific antibodies for individuals who have tested positive by EIA, especially when screening populations with a low prevalence of HCV infection. This assay is highly specific, as the presence of antibodies against each of several HCV proteins is assessed as individual bands on a membrane strip.

The CHIRON® RIBA® HCV 3.0 SIA assay, which was previously cleared by the United States Food and Drug Administration (FDA), has been recently discontinued. The INNO-LIA™ HCV Score (Fujirebio) assay is CE-marked and can be automated on the Auto-LIA 48 instrument. This assay includes recombinant proteins and synthetic peptides from the E2 hypervariable region, the helicase (NS3), and the NS4A, NS4B and NS5A regions.

However, a main problem of RIBA is the occurrence of indeterminate results, especially in those specimens with grey-zone results in the screening assays. Currently, this assay has been substituted as a confirmatory test by widely-used molecular techniques, which can additionally distinguish between active and resolved infections.

**Rapid, point-of-care screening tests:** Both serologic and molecular assays to detect HCV infection have to be carried out in a laboratory, which forces patients to return for their results. Improving this by obtaining the results during the patient’s visit has lead to the development of simple, rapid and non-instrumented point-of-care tests (POCTs). POCTs also make it possible to test for HCV outside of clinical settings in hard-to-reach, high-risk populations, such as injecting drug users (IDU), which are unlikely to be screened following conventional test regimens. POCT technologies have been successfully used to detect human immunodeficiency virus (HIV) infections and may be useful in addressing the problem of under-diagnosis of HCV infection.

Several POCTs have been developed to detect HCV-specific antibodies with a relatively high sensitivity and specificity. However, results from such tests should be interpreted with caution, as population-selection biases and the use of different reference standards used to ascertain true disease status could influence test performances. The only test currently approved by the FDA is the OraQuick HCV Rapid assay (OraSure). This test detects HCV antibodies in fingerstick and venipuncture whole blood, serum, plasma, or oral fluid specimens by an indirect lateral flow immunoassay. Core, NS3 and NS4 antigens are immobilized on a nitrocellulose membrane, and the results are directly visualized using colloidal gold labeled with protein A, which generates a reddish-purple line within 20 min in the presence of HCV-specific antibodies. This test showed a sensitivity of 97.8%-99.3%, and a specificity of 99.3%-99.6%, in serum specimens from a population of IDU, depending on the reference standard used. A sensitivity of 83.3% was observed from oral fluids. In another study, the sensitivity varied between 98.1% (oral fluid) and 99.9% (plasma or serum), with a specificity between 99.6% (oral fluid) and 99.9% (blood, plasma or serum), in a population of individuals who were symptomatic for hepatitis or asymptomatic for hepatitis but with risk factors for HCV infection (mostly IDU and their sex partners, incarcerated individuals and IDU and their sex partners).

### Table 1 Main commercial immunoassays to detect anti-hepatitis C virus antibodies approved for in vitro diagnostics

| Analyzer and manufacturer | Assay principle | Solid phase | HCV antigens | Reaction sample volume (μL) | Time of reaction (min) | IVD registration |
|---------------------------|----------------|------------|--------------|----------------------------|------------------------|-----------------|
| Architect i2000SR, Abbott Laboratories | CMIA | Paramagnetic particles | HC43 (Core and NS3), c100-3 (NS4A) | 20 | 20 | FDA, CE |
| AsSYM, Abbott Laboratories | MEIA | Paramagnetic particles | HC43 (Core), c200 (NS3), c100-3 (NS4A) | 33 | 30 | FDA, CE |
| LiaisonXL, DiaSorin | CLIA | Paramagnetic particles | Core, NS3, NS4 | 25 | 46 | CE |
| VITROS ECL, VITROS 3600, Ortho-Clinical Diagnostics | CLIA | Microwell | c22-3 (Core), c200 (NS3 and NS4), NS5 | 20 | 55 | FDA, CE |
| Elecsys, Roche Diagnostics | ECLIA | Paramagnetic particles | Core, NS3, NS4 | 40 | 18 | FDA, CE |
| ADVIA Centaur, Siemens | CLIA | Magnetic particles | c22-3 (Core), NS3, c200, NS5 | 10 | 58 | FDA, CE |

IVD: Certified as in vitro diagnostic test or device; CE: Conformité Européenne (European Union); FDA: Food and Drug Administration (United States of America); ECLIA: Electrochemiluminescence immunoassay; CMIA: Chemiluminescent microparticle immunoassay; CLIA: Chemiluminescence immunoassay; HCV: Hepatitis C virus; MEIA: Microparticle capture enzyme immunoassay.
Table 2  Hepatitis C virus-RNA qualitative assays approved for in vitro diagnostics

| Assay and manufacturer | Method | Reaction sample volume (μL) | Lower limit of detection (IU/mL) | Instrumentation for automated processing | IVD registration |
|------------------------|--------|-----------------------------|---------------------------------|----------------------------------------|-----------------|
| COBAS® AMPLICOR HCV Test v2.0, Roche Molecular Systems | RT-PCR | 500 | 50 (plasma) 60 (serum) | COBAS® AMPLICOR® Analyzer (amplification and detection) | CE, FDA, Japan, Canada |
| COBAS® AmpliPrep/COBAS® AMPLICOR HCV Test v2.0, Roche Molecular Systems | RT-PCR | 250 | 50 (plasma) 60 (serum) | COBAS® AmpliPrep (extraction), COBAS® AMPLICOR® Analyzer (amplification and detection) | CE, FDA, Japan, Canada |
| COBAS® AmpliPrep/COBAS® TaqMan® HCV Qualitative Test v2.0, Roche Molecular Systems | Real-time RT-PCR | 650 | 15 | Fully automated; cobas p 630 Instrument (primary tube handling), COBAS® AmpliPrep (extraction and MM setup), COBAS® TaqMan® Analyzer or the COBAS® TaqMan® 48 Analyzer (amplification and detection) | CE, FDA, Japan, Canada |
| APTIMA HCV RNA Qualitative Assay¹, Hologic - Gen-Probe | TMA | 500 | 5.3 | Not automated. PANTHER System’s functionality currently in development | FDA |
| VERSANT® HCV RNA Qualitative Assay, Siemens | TMA | 50 | 5.3 | TMA modules (TCS, luminometer HC+, etc.) | CE, FDA |

¹The performance of this assay has not been demonstrated for monitoring hepatitis C virus (HCV) infected patients. IVD: Certified as in vitro diagnostic test or device; CE: Conformité Européenne (European Union); FDA: Food and Drug Administration (United States of America); RT-PCR: Reverse transcription-polymerase chain reaction; MM: Master mix.

individuals with other sexually-transmitted diseases, including HIV)²⁸. Additional studies would be required to assess: (1) the performance of this test in populations with a lower HCV prevalence; (2) the effect of the HCV genotype on the test results; and (3) the influence of HIV co-infection on the test accuracy. Further studies are also required to determine the cost-effectiveness of POCT testing in resource-limited countries with high HCV prevalence.

Recently, the Home Access® Hepatitis C test system (Home Access Health Corporation) has been cleared by the FDA. The kit can be purchased online, by fax, or by mail and is based on collecting a blood sample on a blood specimen card using a fingerstick at home, shipping the sample anonymously to an accredited laboratory and calling back for test results. Pre- and post-test counseling and referrals are also provided.

The use of POCTs may lead to low proportions of false-negative results (i.e., in immunosuppressed individuals) and false-positive results that may require additional confirmatory tests. Even so, by reaching more at-risk populations, POCTs could allow more cases to be identified and treated than laboratory-based tests.²⁷

**Direct diagnosis**

The detection of viral components is needed to diagnose an active HCV infection.

**HCV Core antigen detection and quantification:**

The HCV Core antigen can be detected in the serum of HCV-infected patients, and its levels are significantly related to those of HCV-RNA.²⁸ HCV Core assays cost less than molecular assays and can be easily performed in an immunoassay format. Therefore, they could be used as an alternative to HCV-RNA assays for three different situations: (1) to distinguish active from resolved HCV infections;²⁹,³⁰,³¹ (2) to identify HCV infection in the antibody window period;³² (3) to identify HCV infection in seronegative individuals at high risk for HCV infection, such as in hemodialysis patients.³³ Several studies have suggested that quantification of the Core antigen could be used to monitor the response to IFN-α plus RBV therapy in chronically infected patients.³⁴ Currently, Core antigen detection can be fully automated in the Architect HCV Core antigen test (Abbott Laboratories).³⁵ Although this assay is not sensitive enough to replace HCR-RNA testing for treatment monitoring according to the current clinical practice guidelines, it could be used as a supplemental test in resource-constrained settings.³⁶

Several combination assays that detect both HCV antibodies and Core antigen have been developed. Currently, the MONOLISA™ HCV Ag-Ab ULTRA (Bio-Rad) is a CE-marked microtiter-based assay that, despite not being as sensitive as the HCV antigen-specific assays, improves the detection of HCV infection within the window period of antibody assays.³⁷

**Molecular HCV assays:**

Molecular assays to detect the HCV genome are used for several purposes in the clinical setting. First, the presence of circulating HCV-RNA reflects viral replication, such that sensitive molecular assays (with a lower limit of detection < 50 IU/mL) are used to diagnose active HCV infection in patients with a positive antibody test. Second, molecular testing is required for an early diagnosis of acute HCV infection, as the HCV-RNA can be detected before specific antibodies become detectable (within 1-3 wk after exposure). Finally, the diagnosis of a chronic HCV infection is confirmed by the presence of both HCV antibodies (with the exception of severely immunosuppressed patients) and HCV-RNA over 6 mo.²⁷,³⁸

Commercially available assays that have been approved for in vitro diagnostics are listed in Tables 2 and 3.
The highly conserved HCV 5'UTR region is the target of choice for HCV genome detection across different genotypes. For this, several laboratory processes are necessary, including nucleic acid extraction from clinical specimens (serum or plasma), nucleic acid or signal amplification, and detection. These steps may be fully or partly automated with commercial platforms. However, these assays are time-consuming and require sophisticated technical equipment, trained personnel, dedicated laboratory areas and expensive reagents. Historically, quantitative assays were more sensitive than qualitative tests. Nowadays, most quantitative assays are highly sensitive and could replace qualitative tests.

Real-time reverse-transcription PCR (RT-PCR) is the method of reference for the quantification of HCV-RNA levels in clinical practice according to European and American guidelines[7,30], given its high sensitivity and wide dynamic range of quantification. Reverse transcription of the HCV-RNA as well as PCR amplification and real-time detection is performed in a closed system, thus avoiding carryover contamination with amplified products. The COBAS TaqMan assays (Roche Molecular Systems) are the most widely used worldwide. Although the first version of the assay performed worse with HCV genotype 4 than with the other genotypes, this has been improved in version 2.0[31,34]. Abbott Molecular[35], Siemens and Qiagen[36,37] also offer real-time RT-PCR assays.

Alternatively, Siemens offers an assay based on the branched-DNA (bDNA) signal-amplification technology[38,39]. No nucleic acid extraction is needed, and the process can be fully automated in the VERSANT™ 440 Molecular System. Although both the 5'UTR and core regions are targeted, the lower limit of detection of this assay limits its applicability.

Quantitative tests are also used to monitor antiviral therapy. In order to minimize side-effects, emergence of resistance and costs, HCV-RNA must be periodically quantified to strictly follow treatment stopping rules. With the advent of new treatment regimens that include a protease inhibitor and the response-guided treatment algorithms, only assays with a lower limit of quantification of ≤ 25 IU/mL and a lower limit of detection of approximately 10-15 IU/mL, should be used[40]. Additionally, the presence of detectable but not quantifiable HCV-RNA below those levels is clinically relevant, as it reflects true viremia[41].

**HCV genotyping assays:** Since the HCV genotype is

| Table 3 | Hepatitis C virus-RNA quantitative assays approved for \textit{in vitro} diagnostics |
|---------|--------------------------------------------------------------------------------|
| **Assay and manufacturer** | **Method** | **Reaction sample volume (µL)** | **Lower limit of detection (IU/mL)** | **Linear range of quantification (IU/mL)** | **Instrumentation for automated processing** | **IVD registration** |
| Abbott RealTime HCV, Abbott Molecular artus HCV RG RT-PCR Kit, Qiagen | Real-time RT-PCR | 500 | 12 | 12 - (1 × 10^6) | m2000lp (extraction and assay setup), m2000rt (amplification and detection) | CE, FDA |
| | Real-time RT-PCR | 500 | 34 | 65 - (1 × 10^6) | Manual extraction (QIAamp DSP Virus Kit and assay setup, Rotor-Gene Q (amplification and detection) | CE |
| | Real-time RT-PCR | 1000 | 21 | 35 - (1.77 × 10^5) | QIASymphony RQq QIA Symmorny SP (extraction), QIASymphony AS (assay setup), Rotor-Gene Q (amplification and detection) | CE |
| COBAS® AmpliPrep/COBAS® TaqMan® HCV Test, Roche Molecular Systems | Real-time RT-PCR | 850 | 15 | 43 - (6.9 × 10^5) | COBAS® AmpliPrep (extraction), COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer (amplification and detection) | CE, FDA, Canada, Japan |
| COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test v2.0, Roche Molecular Systems | Real-time RT-PCR | 650 | 15 | 15 - (1 × 10^6) | Fully automated: cobas p 680 (primary tube handling), COBAS® AmpliPrep (extraction), COBAS® TaqMan® Analyzer or COBAS® TaqMan® 48 Analyzer (amplification and detection) | CE, FDA |
| COBAS® TaqMan® HCV Test v2.0 for use with the High Pure System, Roche Molecular Systems | Real-time RT-PCR | 500 | 10 | 25 - (3.9 × 10^6) (CE) | Manual extraction (High Pure System Viral Nucleic Acid Kit), COBAS® TaqMan® Analyzer (amplification and detection) | CE, FDA |
| COBAS® TaqMan® HCV Quantitative Test v2.0, Roche Molecular Systems | Real-time RT-PCR | 500 | 15 | 15 - (1.0 × 10^8) | VERSANT® kPCR Molecular System Sample Preparation (SP) Module and Amplification/Detection (AD) Module | CE |
| VERSANT® HCV RNA 1.0 Assay (kPCR), Siemens | Real-time RT-PCR | 500 | 15 | 615^1 | No nucleic acid extraction needed. System 340 bDNA Analyzer (S340) (US) or VERSANT™ 440 Molecular System (CE) | CE, FDA |
| VERSANT® HCV RNA 3.0 Assay, Siemens | Real-time RT-PCR | 50 | 615^1 | 615 - (7.7 × 10^5) | No nucleic acid extraction needed. System 340 bDNA Analyzer (S340) (US) or VERSANT™ 440 Molecular System (CE) | CE, FDA |

^1The intended use of this assay is limited to the measurement of hepatitis C virus (HCV) viral loads at baseline and after 12 wk of therapy. IVD: Certified as \textit{in vitro} diagnostic test or device; CE: Conformité Européenne (European Union); FDA: Food and Drug Administration (United States of America); kPCR: Real-time kinetic polymerase chain reaction; bDNA: Branched DNA; RT-PCR: Reverse transcription-polymerase chain reaction.

WJG | www.wjgnet.com 3435 April 7, 2014 | Volume 20 | Issue 13 |
predictive of the response to IFN-α-based therapy, genotyping is mandatory to tailor dose and duration of treatment. Furthermore, it is necessary for deciding on triple therapy eligibility with currently approved protease inhibitors, which are effective against HCV genotype 1.

Commercialized HCV genotyping assays may result in <5% of indeterminate results due to the high genetic variability of HCV. Clinicians are forced to treat patients with an indeterminate result as if they were infected with genotypes 1 or 4 (resulting in longer treatment duration and higher RBV doses than for genotypes 2 and 3) and cannot decide on triple therapy eligibility. Therefore, patients with an indeterminate genotype result should be retested either using an alternative commercial assay or the reference method.

**TOOLS FOR HEPATIC FIBROSIS STAGING**

Chronic hepatitis C is characterized by a continuous process of liver inflammation that progresses to liver fibrosis. Liver fibrosis leads to cirrhosis over the decades in 10%-40% of the cases. The most important end stages of liver cirrhosis are esophageal varices, ascites, hepatic encephalopathy, hepatocellular carcinoma and, finally, liver failure. Importantly, cirrhosis can progress to hepatocellular carcinoma with an incidence of 1%-5% per year, and death associated to complications of cirrhosis has an incidence of 4% per year.

Liver fibrosis results from the accumulation of extracellular matrix mainly composed of collagens, proteoglycans, fibronectin and hyaluronic acid (HA). Hepatic stellate cells are the main producers of this matrix after its transdifferentiation from a quiescent to a myofibroblast phenotype in the setting of chronic liver injury. Unfortunately, a specific therapy against liver fibrogenesis does not currently exist because, among other reasons, the exact mechanisms involved in this process are not completely understood yet. This is especially problematic for patients who do not respond to current antiviral therapies.

The management of chronic hepatitis C strongly depends on hepatic fibrosis staging, since this is important
for treatment decision making and disease prognosis. The presence of an advanced (METAVIR score F3-F4) or moderate (METAVIR score F2) stage of liver fibrosis is an indicator for antiviral therapy [30,61], as these patients are at high risk for disease progression. Additionally, liver fibrosis progression is mostly reverted in successfully-treated patients [62,63]. Thus, fibrosis staging methods ideally should be able to differentiate between non-significant (METAVIR scores F0-F1) and significant (score ≥ F2) fibrosis. Furthermore, hepatic fibrosis staging helps in the early detection of cirrhosis, which allows clinicians to monitor the appearance of related decompensation events and hepatocellular carcinoma.

**Liver biopsy**

Liver biopsy remains the best accepted standard method for fibrosis staging [64]. Typically, liver fibrosis is classified semi-quantitatively in several stages by different scoring methods [65]. METAVIR is the best evaluated system in patients with chronic hepatitis C; fibrosis is scored as F0 (no fibrosis), F1 (portal fibrosis without septa), F2 (portal fibrosis with few septa), F3 (numerous septa without cirrhosis) or F4 (cirrhosis) [66]. However, liver biopsy presents several limitations: this procedure is expensive, time consuming and invasive with possible complications, and its interpretation is associated with sampling error and intra- and inter-observer variability. As a result, liver biopsy often does not accurately assess the fibrosis stage and is not useful for monitoring the disease progression [64].

Due to the limitations of liver biopsy, an extensive number of alternative non-invasive tests have been developed, including both imaging and serum-based tests. However, only some of these have been validated and accepted for chronic hepatitis C management [30,66], as reviewed below. These alternative tests are useful for establishing the two ends of the fibrosis spectrum (of minimal fibrosis and cirrhosis) but are less helpful in assessing the mid-ranges of fibrosis.

**Transient elastography**

Transient elastography (FibroScan®, Echosens) is so far the most common, accurate and validated alternative method [67]. It is an ultrasound-based imaging technology that measures liver stiffness, which directly correlates with the degree of fibrosis [68]. FibroScan® is painless, fast (5-10 min) and easy to analyze and has low intra- and inter-observer variability [69]. Consequently, it can be performed periodically, allowing monitoring of either fibrosis progression during its natural course [70] or regression while on treatment [70]. In addition, it predicts 5-year clinical outcomes in chronic hepatitis C [71]. FibroScan® reports the liver stiffness measurements (LSM) as the median expressed in kilopascals (kPa), the interquartile range (IQR) and the percentage of valid measurements. According to the manufacturer’s instructions, results are not reliable when the number of valid measurements is < 10, the IQR of the median LSM value is ≥ 30% and/or the percentage of successful measurements is ≤ 60%. This situation takes place in about 16% of all cases, mainly due to factors such as obesity and inadequate operator experience [72]. More recently, the introduction of the XL probe has increased the reliability of liver stiffness results as compared to the M probe when used in obese patients [73]. Several meta-analyses have evaluated the diagnostic accuracy of transient elastography in the staging of liver fibrosis. Those meta-analyses that evaluated accuracy by means of the AUROC concluded that FibroScan® is able to diagnose the presence of cirrhosis with excellent accuracy (AUROC ≥ 0.90) and the presence of significant fibrosis with moderate accuracy (AUROC from 0.80 to < 0.90) [74-76]. Similar conclusions were obtained from meta-analyses that evaluated FibroScan® accuracy by calculating sensitivity and specificity values: sensitivity and specificity were 70%-72% and 82%-84%, respectively, for the diagnosis of significant fibrosis, and 83%-87% and 89%-95% for cirrhosis [76-78]. Thus, transient elastography is now recommended in the clinical practice for assessing liver fibrosis in patients with chronic hepatitis C, and especially for detecting cirrhosis when other clinical manifestations are not present [30,66]. However, different stiffness cut-off values have been proposed to discriminate among stages of liver fibrosis, and a general consensus is still needed. In Spain, the use of FibroScan® instead of liver biopsy is accepted in patients with chronic hepatitis C in order to identify significant fibrosis (cut-off ≥ 7.6 kPa) and cirrhosis (cut-off ≥ 14.6 kPa) [79,80].

**Serum-based tests and scores**

Certain cytokines involved in fibrosis and factors associated with extracellular matrix deposition or degradation may be used as individual biomarkers of liver fibrosis. Additionally, numerous serum-based tests combining direct and/or indirect markers of fibrosis have been proposed as alternatives to liver biopsy. Direct biomarkers reflect the deposition of extracellular matrix in the liver by fibrogenesis/fibrolysis and include HA, pro-collagen III amino-terminal peptide, tissue inhibitor of matrix metalloproteinase-1, alpha 2-macroglobulin, haptoglobin and matrix metalloproteinase-1. Indirect biomarkers include routine laboratory data on the levels of alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase, apolipoprotein A1, cholesterol, bilirubin and urea as well as the platelet count and the prothrombin index. Nonetheless, only some serum-based tests have been widely validated in chronic hepatitis C and are recommended in the clinical practice for detecting significant fibrosis [30,66] (Table 5). However, these tests are not helpful for tracking fibrosis progression. ELF™, Fibrospect II™, Hepascore™, Fibrometer™ and FibroTest™ are patented and commercially available tests.

Although abundant studies have been performed to assess the different tests, we have only included contrasted results of meta-analysis studies in this review. Meta-analyses aimed to assess the diagnostic accuracy of serum-based tests to identify significant fibrosis/cirrhosis in comparison with liver biopsy have shown moderate
Table 5 Serum-based tests recommended in the clinical practice for the detection of significant fibrosis

| Serum-based tests                  | Parameters considered                                      | Ref.   |
|-----------------------------------|------------------------------------------------------------|--------|
| Tests combining direct markers of liver fibrosis | HA, PiNP, TIMP-1, age                                     | [88]   |
| Enhanced liver                    | HA, PiNP, TIMP-1, age                                     | [88]   |
| Fibrotest (ELF™)                  | MMP-1, PiNP                                              | [89]   |
| MP3™                              | MMP-1, PiNP                                              | [89]   |
| Fibrospect II™                    | HA, TIMP-1, alpha-2-macroglobulin                        | [90]   |
| Tests including indirect markers of liver fibrosis | AST to platelet ratio                                    | [91]   |
| AST to ALT ratio                  | AST, ALT                                                 | [92]   |
| AST index (APRI)                  | AST, platelet count                                       | [91]   |
| AST/ALT ratio                     | AST, ALT                                                 | [92]   |
| Forms index                       | GGT, platelet count, cholesterol, age                    | [93]   |
| Tests including combinations of direct and indirect markers of fibrosis | Hepascore™ Bilirubin, GGT, HA, alpha-2-macroglobulin, urea, age | [94]   |
| Fibrometer™                       | HA, AST, platelet count, prothrombin index, alpha-2-macroglobulin, urea, age | [95]   |
| FibroTest™                        | alpha-2-macroglobulin, haptoglobin, apolipoprotein A1, GGT, total bilirubin | [96]   |

HA: Hyaluronic acid; PiNP: Pro-collagen III amino-terminal peptide; TIMP-1: Tissue inhibitor of matrix metalloproteinase-1; MMP-1: Matrix metalloproteinase-1; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: Gamma-glutamyltranspeptidase.

accuracies in patients with chronic hepatitis C. The most widely validated tests are APRI and FibroTest™. A comprehensive meta-analysis on the APRI index performed by Lin et al. [81] revealed an AUROC of 0.77 for the diagnosis of significant fibrosis and of 0.83 for cirrhosis. The authors concluded that the APRI index is a good alternative for its use in clinical practice for confirming the presence of severe fibrosis/cirrhosis when other tests or symptoms are non-conclusive. The main strengths of the APRI index are its low cost and wide availability, which make this simple test an excellent first-line screening in developing countries. Poynard et al. [82] analyzed the diagnostic accuracy of FibroTest™ for significant fibrosis and showed a mean standardized AUROC of 0.85 in patients with chronic hepatitis C. However, the disadvantage of FibroTest™, similar to the other patented tests, is that it is relatively expensive and is not readily available in all centers. In addition, co-morbidities such as hemolysis, inflammation or Gilbert’s syndrome can lead to potential mistakes by changing the levels of some of the serum markers included in the test. In a later meta-analysis performed by Leroy et al. [83], FibroMeter™ had a higher AUROC (0.84) than FibroTest™ (0.80), APRI (0.79) or Hepascore™ (0.78) for significant fibrosis. In a recent meta-analysis assessing tests that combine direct and/or indirect markers, a moderate accuracy for the identification of significant fibrosis was described for ELF™ (median AUROC, 0.81), FibroMeter™ (0.82) and Fibrospect™ (0.86), and for the identification of cirrhosis in the case of APRI (0.84), FibroTest™ (0.86), Forns Index (0.87), ELF™ (0.88) and Hepascore™ (0.89). FibroMeter™ showed an excellent accuracy for diagnosing cirrhosis (0.91) [84]. Thus, in patients with chronic hepatitis C, both transient elastography and serum-based markers have a similar moderate accuracy for diagnosing significant fibrosis. Nevertheless, transient elastography is the most accurate non-invasive method for cirrhosis diagnosis.

To increase the accuracy of diagnosing significant fibrosis and to decrease the number of liver biopsies needed, several algorithms based on a combination of serum-based tests or a combination of transient elastography and a serum-based test have been recommended for use in clinical practice [85]. The sequential algorithm for fibrosis evaluation (SAFE) first uses the APRI index and then the FibroTest™. When a final diagnosis cannot be reached, liver biopsy is needed [86]. The Castéra algorithm uses transient elastography and FibroTest™ and relies on liver biopsy to resolve discrepancies [87]. Both SAFE and Castéra algorithms have shown an excellent accuracy in diagnosing significant fibrosis, but a higher number of liver biopsies could be avoided using the second algorithm (of 72% with Castéra or 48% with SAFE).

CONCLUSION

Despite great advances in HCV treatment, the lack of recognition of infection will hamper the control of hepatitis C. The World Health Organization announced in 2012 a framework for global action to prevent and control viral hepatitis infections [88]. Early diagnosis provides the best opportunity for effective medical support and prevention of further spread of the infection. Expanded HCV testing is required, and diagnostic tests with high sensitivity and specificity are available. Additionally, POCT tests could improve the number of diagnosed individuals and their access to care.

Despite significant advances in the field of fibrosis stage assessment, a single non-invasive method that is able to completely replace the liver biopsy does not exist at the current time. Liver biopsy should be performed when non-invasive methods or algorithms cannot be used or when inconclusive results are obtained, but it is not needed when clinical manifestations of cirrhosis are present.

ACKNOWLEDGMENTS

We would like to thank Veronica A Raker for editing.

REFERENCES

1 Lindenbach BD, Rice CM. Unravelling hepatitis C virus replication from genome to function. Nature 2005; 436: 933-938 [PMID: 16107832 DOI: 10.1038/nature]
2 Simmonds P, Bukh J, Combet C, Deléage G, Enomoto N, Feinestone S, Halfon P, Inchauspé G, Kuiken C, Maertens G, Mizokami M, Murphy DG, Okamoto H, Pawlotsky JM, Penin F, Sablon E, Shin-I T, Stuyver LJ, Thiel HJ, Viazov S, Weiner AJ, Widell A. Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. Hepatology 2005; 42: 962-973 [PMID: 16149085 DOI: 10.1002/hep.20819]
3 Kuiken C, Simmonds P. Nomenclature and numbering of
Swades V et al. HCV diagnosis and hepatic fibrosis staging

The European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. J Hepatol 2011; 55: 245-264 [PMID: 21371579 DOI: 10.1016/j.jhep.2011.02.023]

Orentl H, Deltenre P, Franquè S, Laleman W, Moreno C, Bourgeois S, Colle I, Delwaide J, De Maeght S, Mulkay JP, Stärkel P, Reynaert H. Update of the Belgian Association for the Study of the Liver guidelines for the treatment of chronic hepatitis C genotype 1 with protease inhibitors. Acta Gastroenterol Belg 2012; 75: 249-259 [PMID: 22870791]

Chevaliez S. Virological tools to diagnose and monitor hepatitis C virus infection. Clin Microbiol Infect 2011; 17: 116-121 [PMID: 21054664]

Chevaliez S, Bouvier-alias M, Rodriguez C, Soulier A, Poveda JD, Pawlotsky JM. The Cobas AmpliPrep/Cobas Taqman HCV test, version 2.0, real-time PCR assay accurately quantifies hepatitis C virus genotype 4 RNA. J Clin Microbiol 2013; 51: 1078-1082 [PMID: 23325825 DOI: 10.1128/JCM.02044-12]

Zittr S, Heilek G, Truchon K, Susser S, Vermehren J, Sizmann D, Cobb R, Sarrazin C. Second-generation Cobas AmpliPrep/Cobas TaqMan HCV quantitative test for viral load monitoring: a novel dual-probe assay design. J Clin Microbiol 2013; 51: 571-577 [PMID: 23241371 DOI: 10.1128/JCM.01784-12]

Vermehren J, Yu ML, Monto A, Yao JD, Anderson C, Berizuis R, Schneider G, Sarrazin C. Multi-center evaluation of the Abbott RealTime HCV assay for monitoring patients undergoing antiviral therapy for chronic hepatitis C. J Clin Virol 2011; 52: 133-137 [PMID: 21803650 DOI: 10.1016/j.jcv.2011.07.007]

Drexler JF, Reber U, Wuttkopf A, Eiss-Hübbinger AM, Drosten C. Performance of the novel Qiagen artus QS-RQG viral load assays compared to that of the Abbott RealTime system with genetically diversified HIV and hepatitis C virus plasma specimens. J Clin Microbiol 2012; 50: 2114-2117 [PMID: 22405357 DOI: 10.1128/JCM.00854-11]

Papa P, Fabeni L, Perno CF, Ciotti M. Performance evaluation of the new Roche Ampliprep/cobas Taqman HCV test, version 2.0, for detection and quantification of hepatitis C virus RNA. J Clin Microbiol 2013; 51: 238-242 [PMID: 23152551 DOI: 10.1128/JCM.01729-12]

Harrington PR, Zeng W, Naeger LK. Clinical relevance of detectable but not quantifiable hepatitis C virus RNA during boceprevir or telaprevir treatment. Hepatology 2012; 55: 1048-1057 [PMID: 22995516 DOI: 10.1002/hep.24791]

Lontok E, Mani N, Harrington PR, Miller V. Closing in on the target: sustained virologic response in hepatitis C virus genotype 1 infection response-guided therapy. Clin Infect Dis 2012; 54: 1466-1470 [PMID: 23362287 DOI: 10.1093/cid/cit025]

Ghany MG, Strader DB, Thomas DL, Siffel LB. Diagnosis, management, and treatment of hepatitis C. An update. Hepatology 2009; 49: 1335-1374 [PMID: 19330875 DOI: 10.1002/hep.22759]

Swiss Association for the Study of the Liver. Treatment of chronic hepatitis C genotype 1 with triple therapy comprising telaprevir or boceprevir. Swiss Med Wkly 2012; 142: w13516 [PMID: 22367957 DOI: 10.4414/smw.2012.13516]

Jacobson IM, Pawlotsky JM, Afshali NH, Dusheiko GM, Foros N, Jensen DM, Poordad F, Schulz J. A practical guide for the use of boceprevir and telaprevir for the treatment of hepatitis C. J Viral Hepat 2012; 19 Suppl 2: 1-26 [PMID: 22404758 DOI: 10.1111/j.1365-2893.2012.01950.x]

Murphy DG, Willems B, Deschênes M, Hilzenrat N, Mousseau R, Sbabah S. Use of sequence analysis of the NS5B region for routine genotyping of hepatitis C virus with reference to C/E1 and 5’ untranslated region sequences. J Clin Microbiol 2007; 45: 1102-1112 [PMID: 17287528 DOI: 10.1128/jcm.02366-06]

Kieffer TL, Sarrazin C, Miller JS, Welker MW, Forestier N, Reesink HW, Kwong AD, Zeuzem S. Telaprevir and pegylated interferon-alpha-2a inhibit wild-type and resistant genotype 1 hepatitis C virus replication in patients. Hepatology 2007; 46: 631-639 [PMID: 17680654 DOI: 10.1002/hep.21781]

McCown MF, Rajayaguru S, Kular S, Cammack N, Nâjera I. GT-1a or GT-1b subtype-specific resistance profiles for hepatitis C virus inhibitors telaprevir and HCV-796. Antimicrob Agents Chemother 2009; 53: 2129-2131 [PMID: 19273674 DOI: 10.1128/aac.01598-08]

Cento V, Landonio S, De Luca F, Di Maio VC, Micheli V, Mirabelli C, Niero F, Magni C, Rizzardini G, Perno CF, Ceccherini-Silberstein F. A boceprevir failure in a patient infected with HCV genotype 1g: importance and limitations of virus genotyping prior to HCV protease-inhibitor-based therapy. Antivir Ther 2013; 18: 645-648 [PMID: 23411358 DOI: 10.3851/imp2529]

Verbeek J, Stanley M, Shieh J, Celis L, Huyck E, Wollants E, Morimoto J, Farrow A, Sablon E, Jankowski-Hennig M, Schaper C, Johnson P, Van Ranst M, Van Brussel M. Evaluation of Versant hepatitis C virus genotype assay (LiPA) 2.0. J Clin Microbiol 2008; 46: 1901-1906 [PMID: 18400913 DOI: 10.1128/JCM.02390-07]

Bouchard F, Cantaloube JF, Chevaliez S, Portal C, Azer A, Lefrère JF, Pawlotsky JM, De Micco P, Laperche S. Improvement of hepatitis C virus (HCV) genotype determination with the new version of the INNO-LiPA HCV assay. J Clin Microbiol 2007; 45: 1140-1145 [PMID: 17251399 DOI: 10.1128/JCM.01982-06]

Ciotti M, Marcuccilli F, Guentu T, Babakir-Mina M, Chiodo F, Favarrato M, Perno CF. Multicenter evaluation of the Abbott RealTime HCV Genotype II assay. J Virol Methods 2010; 167: 205-207 [PMID: 20362009 DOI: 10.1016/j.jviromet.2010.03.017]

Shinol RC, Gale HB, Kan VL. Performance of the Abbott RealTime HCV Genotype II RUO assay. J Clin Microbiol 2012; 50: 3099-3101 [PMID: 22760043 DOI: 10.1128/JCM.01249-12]

Chevaliez S, Bouvier-alias M, Brillat R, Pawlotsky JM. Hepatitis C virus (HCV) genotype 1 subtype identification in new HCV drug development and future clinical trials. PLoS One 2009; 4: e8129 [PMID: 19997618 DOI: 10.1371/journal.pone.008129]

Lam TH, Cheng RS, Lai ST, Tsang TY, Cheng VC, Ho SL, Yam WC. Evaluation of in-house and commercial genotyping assays for molecular typing of hepatitis C virus in Hong Kong. Br J Biomed Sci 2010; 67: 82-85 [PMID: 20696764]

Larrat S, Poveda JD, Coudret C, Fussliker K, Magnat N, Signorini-Schmuck A, Thibault V, Morand P. Sequencing assays for failed genotyping with the versus hepatitis C virus genotype assay (LiPA), version 2.0. J Clin Microbiol 2013; 51: 2815-2821 [PMID: 23616453 DOI: 10.1128/JCM.00586-13]

Gonzalez V, Gomes-Fernandes M, Basçunéna E, Casanovas S, Saludes V, Jordana-Lluch E, Matas L, Ausina V, Martró E. Accuracy of a commercially available assay for HCV genotyping and subtyping in the clinical practice. J Clin Virol 2013; 58: 249-253 [PMID: 23731847 DOI: 10.1016/j.jcv.2013.05.005]
Schupp D, Afdhal NH. Liver cirrhosis. Lancet 2008; 371: 838-851 [PMID: 18328931 DOI: 10.1016/s0140-6736(08)68385-9]

Friedman SL. Hepatic stellate cells: protein, multifunctional, and enigmatic cells of the liver. Physiol Rev 2008; 88: 125-172 [PMID: 18198985 DOI: 10.1152/physrev.00133.2007]

Mormone E, George J, Nieto N. Molecular pathogenesis of hepatic fibrosis and current therapeutic approaches. Chem Biol Interact 2011; 193: 225-231 [PMID: 21803030 DOI: 10.1016/j.chembiol.2011.07.001]

Schupp D, Pinzani M. Anti-fibrotic therapy: lost in translation? J Hepatol 2012; 56 Suppl 1: S66-S74 [DOI: 10.1016/j.jhep.2011.06.008]

Liang TJ, Ghany MG. Current and future therapies for hepatitis C virus infection. N Engl J Med 2013; 368: 1907-1917 [PMID: 23675659 DOI: 10.1056/nejma1213651]

Poynard T, McHutchison J, Manns M, Trepo C, Lindsay K, Goodman Z, Ling MH, Albrecht J. Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. Gastroenterology 2002; 122: 1303-1313 [PMID: 11949517 DOI: 10.1053/gast.2002.30329]

Hézode C, Castéra L, Roudot-Thoraval F, Bouvier-Alias M, Rosa I, Roulot D, Leroy V, Mallat A, Pawlotsky JM. Liver stiffness diminishes with antiviral response in chronic hepatitis C. Aliment Pharmacol Ther 2011; 34: 656-663 [PMID: 21752038 DOI: 10.1111/j.1365-2036.2011.04765.x]

Grünhage F, Lammert F. Assessment of Hepatic Fibrosis and Steatosis. In: Mauss S, Berg T, Rockstroh J, Sarrazin C, Wedemeyer H, editors. Hepatology 2013 - A Clinical Textbook, Spanish Flying Publisher, 2013: 336-349

Bedossa P, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAIVIR Cooperative Study Group. Hepatology 1996; 24: 289-293 [PMID: 8690394 DOI: 10.1002/hep.5102402011]

Omata M, Kanda T, Yu ML, Yokosuka O, Lim SG, Jafari W, Tateishi R, Shindo Y, Amagasa T, Amamoto K, Komamura K, Kida K. SAFE biopsy: a validated method for large-scale staging of liver fibrosis in chronic hepatitis C. Aliment Pharmacol Ther 2008; 27: 705-714 [PMID: 18093383 DOI: 10.1111/j.1365-2036.2008.03688.x]

Sebastiani G, Hafon P, Afton AE, Celeste E, Saludes V. HCV diagnosis and hepatic fibrosis staging. Ann Intern Med 2013; 159: 372 [PMID: 24026329 DOI: 10.7326/0003-4819-159-5-201309030-00021]

Chou R, Wasson N. Blood tests to diagnose fibrosis or cirrhosis in patients with chronic hepatitis C virus infection. Ann Intern Med 2013; 159: 1368-1376 [PMID: 18655779 DOI: 10.1016/j.ann internmed.2008.06.020]

Chour A, Wegelin K, Czaja MJ, Bedossa P, Afton AE, Celeste E, Saludes V. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. Hepatology 2013; 58: 376-376 [PMID: 24216198 DOI: 10.1002/hep.24105]

Poynard T, Morra H, Hafon P, Castéra L, Ratziu V, Ibetta-Bismut F, Naveau S, Thabut D, Lecrubier F, Ziol M, Bourliere M, Cacoub P, Messous D, Munteanu M, de Ledinghen V. Meta-analyses of FibroTest diagnostic value in chronic liver disease. BMC Gastroenterology 2007; 7: 40 [PMID: 17937811 DOI: 10.1186/1471-230X-7-40]

Leroy V, Hafon P, Baccy J, Boursier J, Rousselet MC, Bourliere M, de Muret A, Sturm N, Hunault G, Penaranda G, Bréchot MC, Trocme C, Cales P. Diagnostic accuracy, reproducibility and robustness of fibrosis blood tests in chronic hepatitis C: a meta-analysis with individual data. Clin Biochem 2008; 41: 1368-1376 [PMID: 18655779 DOI: 10.1016/j.clinbiochem.2008.06.020]

Mormone E, Castéra L, Perretti M, Romiti R, de Nolfo G, Berti M, Paliata A, Cavallino G, Petrella A. Feasibility and diagnostic performance of the FibroScan XL probe for liver stiffness measurement in overweight and obese patients. Hepatology 2012; 55: 199-208 [PMID: 22198438 DOI: 10.1002/hep.24624]

Friedrich-Rust M, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology 2008; 134: 960-974 [PMID: 18395077 DOI: 10.1053/j.gastro.2008.01.034]

Shaheen AA, Wan AF, Myers RP. FibroTest and FibroScan for the prediction of hepatitis C-related fibrosis: a systematic review of diagnostic test accuracy. Am J Gastroenterol 2007; 102: 2589-2600 [PMID: 17850410 DOI: 10.1111/j.1572-0241.2007.01466.x]

Tsochatzis E, Gurusamy KS, Ntaoula S, Cholongitas E, Davidson BR, Burroughs AK. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. J Hepatol 2011; 54: 650-659 [PMID: 21146892 DOI: 10.1016/j.jhep.2010.07.033]

Dubois CM, Santilli A, Duarte-Rojo A, Wong D, Beaton M, Levstik M, Crotty PA, Elshabh M. Feasibility and diagnostic performance of the FibroScan XL probe for liver stiffness measurement in overweight and obese patients. Hepatology 2012; 55: 199-208 [PMID: 22198438 DOI: 10.1002/hep.24624]

Duarte-Rojo A, Wong D, Beaton M, Levstik M, Crotty PA, Elshabh M. Feasibility and diagnostic performance of the FibroScan XL probe for liver stiffness measurement in overweight and obese patients. Hepatology 2012; 55: 199-208 [PMID: 22198438 DOI: 10.1002/hep.24624]
86 Castéra L, Vergnol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Lédoringhen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; 128: 343-350 [PMID: 15685546 DOI: 10.1053/j.gastro.2004.11.018]

World Health Organization. Prevention & Control of Viral Hepatitis Infection: Framework for Global Action. 2012. Available from: URL: http://who.int/csr/disease/hepatitis/GHP_Framework_En.pdf

88 Rosenberg WM, Voelker M, Thiel R, Becka M, Burt A, Schuppan D, Hubscher S, Roskams T, Pinzani M, Arthur MJ. Serum markers detect the presence of liver fibrosis: a cohort study. *Gastroenterology* 2004; 127: 1704-1713 [PMID: 15578508 DOI: 10.1053/j.gastro.2004.08.052]

89 Leroy V, Monier F, Bottari S, Trocmé C, Sturm N, Hilleret MN, Morel F, Zarski JP. Circulating matrix metalloproteinases 1, 2, 9 and their inhibitors TIMP-1 and TIMP-2 as serum markers of liver fibrosis in patients with chronic hepatitis C: comparison with PIIINP and hyaluronic acid. *Am J Gastroenterol* 2004; 99: 271-279 [PMID: 15046217 DOI: 10.1111/j.1572-0241.2004.04055.x]

90 Patel K, Gordon SC, Jacobson I, Hézode C, Oh E, Smith KM, Pawlowski JM, McHutchison JG. Evaluation of a panel of non-invasive serum markers to differentiate mild from moderate-to-advanced liver fibrosis in chronic hepatitis C patients. *J Hepatol* 2004; 41: 935-942 [PMID: 15582126 DOI: 10.1016/j.jhep.2004.08.008]

91 Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, Lok AS. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; 38: 518-526 [PMID: 12883497 DOI: 10.1053/jhep.2003.50346]

92 Williams AL, Hooftnagle JH. Ratio of serum aspartate to alanine aminotransferase in chronic hepatitis. Relationship to cirrhosis. *Gastroenterology* 1988; 95: 734-739 [PMID: 3135226]

93 Foronx X, Ampurianès S, Llovet JM, Aponte J, Quintó L, Martínez-Bauer E, Bruguera M, Sánchez-Tapias JM, Rodes J. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. *Hepatology* 2002; 36: 986-992 [PMID: 12297848 DOI: 10.1053/jhep.2002.36128]

94 Adams LA, Bulsara M, Rossi E, DeBoer B, Speers D, George J, Kench J, Farrell G, McCaughan GW, Jeffrey GP. Hepascore: an accurate validated predictor of liver fibrosis in chronic hepatitis C infection. *Clin Chem* 2005; 51: 1867-1873 [PMID: 16055434 DOI: 10.1373/clinchem.2005.048389]

95 Calès P, Oberti F, Michalak S, Hubert-Fouchard I, Rousselet MC, Konaté A, Gallois Y, Ternisien C, Chevailler A, Luel F. A novel panel of blood markers to assess the degree of liver fibrosis. *Hepatology* 2005; 42: 1373-1381 [PMID: 16317693 DOI: 10.1002/hep.20935]

96 Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Bennhamou Y, Poynard J. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; 357: 1069-1075 [PMID: 11297957 DOI: 10.1016/S0140-6736(00)04258-6]
