Clinical Virology of Hepatitis C

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INTRODUCTION

There are two types of laboratory tests for the virological diagnosis and monitoring of hepatitis C virus (HCV) infection, namely serologic tests of anti-HCV antibodies (indirect tests), and tests that detect, quantify or characterize viral components such as HCV RNA and core antigen (direct tests). Both direct and indirect tests are useful for diagnosis, treatment choices, and therapeutic monitoring.

HCV MARKERS

The HCV genotype, HCV RNA, HCV core antigen, and anti-HCV antibodies are the four markers of HCV infection.

HCV genotyping

There are six HCV genotypes, which are subdivided into subclades or subtypes identified by lower-case letters (1a, 1b, 1c, etc) [1]. HCV types, subtypes and isolates are distinguished on the basis of average sequence divergences of about 30%, 20% and 10%, respectively [1]. The HCV genotype is an intrinsic characteristic of the infecting HCV strain that does not change over time.
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HCV RNA
The detection of HCV RNA in peripheral blood is a reliable marker of HCV replication. HCV RNA becomes detectable about one or two weeks after infection, then usually peaks before disappearing in cases that spontaneously resolve. During chronic infection, HCV RNA levels generally stabilize gradually, although HCV RNA can occasionally disappear for days or weeks before reappearing and reaching a plateau. HCV RNA levels are stable during chronic infection [2]. The HCV RNA level does not correlate to the severity of liver lesions, but it is generally low or undetectable in end-stage liver disease [3, 4].

HCV core antigen
Total HCV core antigen levels correlate with HCV RNA levels [5]. Prior to seroconversion, core antigen is generally detected 1 or 2 days later than HCV RNA [6, 7], and subsequent time course of core antigen levels then matches HCV RNA kinetics [5]. The HCV core antigen titer can therefore be used as an indirect marker of HCV replication.

Anti-HCV antibodies
Specific antibodies can be detected 7 to 8 weeks after infection with current tests [8-10]. From between 50% to 70% of patients have detectable anti-HCV antibodies at clinical onset [11]. Anti-HCV antibodies can persist throughout life in patients with spontaneously resolving infection, although in some cases they may fall slowly, or disappear after several years [12]. In chronically infected patients, antibodies persist for life. They may become undetectable during hemodialysis or profound immunodepression.

VIROLOGICAL TESTS

Antibody tests
Third-generation enzyme immunoassays (EIAs) detect mixed antibodies against HCV core, NS3, NS4 and NS5 antigens. The target antigens are coated on microtiter plates, microbeads or holders designed for “closed” automated devices. The specificity of current EIAs is greater than 99%. There is no gold standard, so sensitivity is
more difficult to determine. In routine use, more than 99% of immunocompetent patients with detectable HCV RNA are positive with current EIAs [13]. EIAs can be negative during hemodialysis and in profoundly immunodeficient patients despite ongoing HCV replication, but this is rare with the most recent tests [14]. Confirmation with immunoblot tests is no longer useful in the clinical setting, because of the excellent sensitivity and specificity of current EIAs [15].

**HCV serotyping**

The HCV genotype can be determined by competitive EIA testing for type-specific antibodies. One commercial test is available so far (Murex™ HCV Serotyping 1-6 Assay, Murex Diagnostics, Dartford, UK) and is interpretable in about 90% of immunocompetent patients with chronic hepatitis C [16]. Sensitivity is lower in hemodialysis and immunodepression [17, 18]. This test does not identify the subtype. Overall, the results are in agreement with molecular tests in about 95% of cases, although performance is better with genotype 1 than with other genotypes [16, 19, 20]. This test cannot distinguish true mixed infection from cross-reactivity.

**Molecular HCV genotyping methods**

The gold standard for genotyping is direct sequencing of the NS5B or E1 region. This is followed by sequence alignment with reference sequences and by phylogenetic analysis [1]. In practice, HCV is genotyped by direct sequence analysis, restriction fragment length polymorphism analysis, or reverse hybridization to genotype-specific oligonucleotide probes [21-23]. Two commercial kits are based on PCR amplification of the 5’ noncoding region. The Trugene™ HCV 5’NC genotyping kit (Bayer Corporation, Tarrytown, New Jersey) [24, 25] is based on direct sequencing of PCR amplicons and database interpretation. The INNO-LiPA HCV II line-probe test (Innogenetics, Gent, Belgium) [21, 22] is based on reverse hybridization of PCR amplicons, using a nitrocellulose strip coated with genotype-specific oligonucleotide probes, and colorimetric determination. The six HCV types, and also many subtypes, can be identified with the two tests, although subtyping errors occur in 10% to 25% of cases because of variability in the target 5’ noncoding region. However, clinical decision-making is solely based on the type, not the subtype.
HCV RNA detection

HCV RNA detection tests are far more sensitive than most quantitative tests, and involve target amplification by PCR or TMA [26, 27]. A commercial PCR-based test is available. It can be fully manual (Amplicor™ HCV v2.0, Roche Molecular Systems, Pleasanton, California) or comprise manual extraction and automated reverse transcription, amplification and reading in the Cobas Amplicor™ device (Cobas Amplicor™ HCV v2.0, Roche Molecular Systems, Pleasanton, California). The detection limit is 50IU of HCV RNA per mL. The manual TMA test (Versant™ HCV RNA Qualitative Assay, Bayer Corporation) has a slightly better detection limit of 10IU/mL. Specificity is 98%-99% with both tests.

HCV RNA quantification

Target amplification (PCR or TMA) and signal amplification (“branched DNA”) can be used to determine viral copy numbers. The World Health Organization (WHO) has established an international standard for HCV RNA units [28]. One IU represents the total amount of HCV RNA rather than the number of viral particles. Universal adoption of this reference standard [29] has facilitated recommendations and clinical guidelines.

The detection limits of current tests (30IU/mL to 615IU/mL) are shown in Figure 1. The upper end of the linear range ranges from <500,000IU/mL to 20,000,000IU/mL with the real-time PCR assay (Cobas TaqMan 48 HCV, Roche Molecular Systems, Rotkreuz, Switzerland). Samples exceeding the upper end of the linear range must be diluted 1:10 or 1:100. The specificity of these tests is 98%-99%, regardless of the genotype [30-36]. Variations of less than 0.5 log (less than three-fold) may be due to intrinsic or between-patient variability and should not be taken into account in the clinical setting [37].
Figure 1: Linear ranges of quantitative HCV RNA tests. HCV RNA levels are shown in IU/mL. About 90% of patients' HCV RNA levels remain in the gray area without antiviral treatment.

HCV core antigen

An EIA test is available that can be used to detect and quantify total HCV core antigen (Ortho-Clinical Diagnostics, Raritan, New Jersey). The HCV core antigen titer (in pg/mL) correlates closely with the HCV RNA level, and can thus be used to monitor viral replication [5]. One pg of total HCV core antigen per mL is equivalent to about 8000IU of HCV RNA in most patients [5]. This test does not currently detect HCV core antigen in samples with HCV RNA levels under 20,000IU/mL, which restricts its clinical use [5].
PRACTICAL USE OF VIROLOGIC TESTS

Diagnosis

Acute hepatitis C

Patients with acute hepatitis of unknown origin must be tested with an anti-HCV EIA and with a sensitive technique (detection limit of 50IU/mL or less) for HCV RNA [38]. HCV RNA positivity in an anti-HCV-negative patient with acute hepatitis strongly indicates acute hepatitis C, and this is subsequently confirmed by seroconversion. Acute hepatitis C is unlikely when both markers are absent, and when anti-HCV is positive and HCV RNA negative; most of these patients have encountered (and cleared) HCV some time previously and therefore have another etiology. Such patients should nonetheless be tested for HCV RNA a few weeks later, as HCV RNA can disappear transiently before chronic replication becomes detectable. The presence of both anti-HCV antibodies and HCV RNA indicates either acute hepatitis C or an acute exacerbation of chronic hepatitis C. It is also difficult to diagnose acute hepatitis due to other causes when the patient also has chronic hepatitis C.

Chronic hepatitis C

A diagnosis of chronic hepatitis C is almost certain when a patient with chronic liver disease has both anti-HCV and HCV RNA (detected with a sensitive technique) [15]. Anti-HCV negativity with HCV RNA positivity is exceptional in immunocompetent patients, but more frequent (albeit still rare with current EIAs) [14] in patients who are on hemodialysis or profoundly immunodepressed.

HCV RNA detection with a sensitive technique confirms chronic HCV infection in patients found to be anti-HCV-positive during blood donation or screening of at-risk populations. Patients who still have antibodies after spontaneously resolving HCV infection in the past are difficult to distinguish from patients with false-positive reactivity when HCV RNA is undetectable on at least two occasions 6 months apart. A high EIA optical density ratio favors a true-positive result, but low optical density ratios are inconclusive, as anti-HCV antibody titers can fall gradually after the virus has been spontaneously cleared. However, all these patients can be reassured that they are not infected.
Diagnosis after occupational exposure

HCV RNA becomes detectable one to two weeks after infection, and diagnosis of acute infection is based on HCV RNA detection with a sensitive technique, starting at least one week after exposure.

Mother-child transmission

Babies born to HCV-infected mothers should be tested for HCV RNA with a sensitive method rather than for anti-HCV, because passively transferred antibodies can persist for up to a year after birth [39-42]. HCV RNA can be detected in the infected infant as little as a few days after delivery, and may then persist or clear spontaneously. There is no consensus on the timing of diagnostic HCV RNA testing, but about 6 to 12 months after birth seems optimal. High titers of anti-HCV antibodies persisting after the first year of life suggest chronic infection, which is confirmed by HCV RNA detection [39-42].

Prognostic value of virologic tests during the course of HCV disease

The HCV RNA level and HCV genotype do not predict the severity of liver damage or fibrosis, or the risk of extra-hepatic disease.

Antiviral treatment of chronic hepatitis C: decision-making and monitoring

The treatment decision

Pegylated interferon-alpha ribavirin combination therapy is only warranted for patients in whom HCV RNA is detected by a sensitive technique. The indication and duration of treatment depends on the genotype. All patients with genotype 2 or 3 infection should be offered this treatment, as they have a good chance of a sustained virologic response (70 to 80%) and treatment only lasts 24 weeks with a low dose of ribavirin [43-45]. The response rate in patients infected by genotype 1 is only 40 to 45%, and treatment lasts 48 weeks with a higher dose of ribavirin [43-45]. The likely risk-benefit ratio must thus be considered case by case. Patients with necroinflammatory activity and/or fibrosis on liver biopsy should be treated, contrary to patients with “mild” hepatitis. Pending further studies, patients infected by genotype 4, 5 or 6 must be treated like patients infected by genotype 1. The baseline HCV RNA level currently has no bearing on the
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decision-making process. Baseline HCV RNA assay is not necessary in genotype 2 or 3 infection, but can help when assessing the treatment response at week 12 in patients infected by genotype 1 [45, 46].

Follow-up and treatment monitoring

A sensitive HCV RNA test is used to judge the virologic response at the end of treatment; persistence of HCV RNA is highly predictive of relapse after treatment withdrawal. Non-detection of HCV RNA at the end of treatment defines a virologic response, but such patients should be retested for HCV RNA 24 weeks later, again using a sensitive method.

HCV RNA testing before and after 12 weeks of treatment is used to monitor pegylated interferon-alpha and ribavirin treatment of genotype 1 chronic hepatitis C [45, 46]. Treatment is continued for a total of 48 weeks when, at week 12, a 2-log (100-fold) fall in HCV RNA level occurs or when HCV RNA is undetectable in patients whose baseline HCV RNA level was less than 100 times the detection limit. The likelihood of a sustained virologic response is virtually nil in other patients, and treatment can thus be stopped, or else be continued in an attempt to slow liver disease progression [45, 46]. Total HCV core antigen assay can be used for the same purpose, provided the antigen titer is more than 200pg/mL (detection cut-off 1 to 2pg/mL) [5].

Figure 2 shows a decision algorithm for the use of virologic tests in the treatment of chronic hepatitis C.
Treatment of acute hepatitis C

Encouraging results were recently obtained with standard interferon alpha monotherapy of acute hepatitis C [47], but the optimal schedule is unknown, and the role of virologic tests in the decision to treat is uncertain [48]. Regardless of the type, dose, and duration of interferon treatment, a sensitive HCV RNA technique must be used to assess the virologic response at the end of treatment. When HCV RNA is negative at the end of treatment, the nature of the response (sustained or transient) should be assessed 24 weeks later; and sustained HCV RNA negativity indicates that treatment has been successful.
Monitoring of untreated patients
Repeat virologic testing has no prognostic value in untreated patients, in whom follow-up is based on regular liver biopsy.

CONCLUSION
The advent of virologic tests has vastly improved the management of HCV infection. These tests can be used to diagnose infection, to guide treatment decisions, and to monitor therapeutic response. They still have to be fully standardized and automated, and more clinically relevant cut-off values are required. The availability of more sensitive and more accurate HCV RNA tests will improve treatment monitoring and help to elucidate the mechanisms of antiviral resistance. Together with the development of new antiviral drugs, these advances should markedly improve the management of HCV infection.
REFERENCES


34. **Hepatitis C**


