Tests and screening strategies for the diagnosis of hepatitis C

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ABSTRACT

The threshold for testing for hepatitis C virus (HCV) should be low for persons with any risk factor for HCV infection. Current practice calls for first screening for antibodies to HCV and then testing for HCV RNA in those in whom antibodies are detected. Viral testing can distinguish between active and resolved HCV infection and also determine viral load, which can help predict response to antiviral therapy. Many highly sensitive assays are available for testing for HCV RNA. Once HCV infection is diagnosed, the HCV genotype should be determined to help predict treatment response and duration. Liver biopsy can aid in disease staging and help guide treatment decisions. Practical and efficient screening strategies for HCV are guided by risk factors for HCV infection.

Physicians need to understand effective strategies for establishing or excluding a diagnosis of hepatitis C, given the prevalence of hepatitis C virus (HCV) infection and its potentially serious complications. This article reviews current information to guide strategies for diagnosing or excluding hepatitis C and to support the correct interpretation of screening tests.

HEPATITIS C: COMMON, POTENTIALLY SERIOUS

Infection with HCV is the most common chronic blood-borne viral infection in North America. An estimated 3.9 million people in the United States have been exposed to HCV, and 2.7 million have measurable HCV RNA. An estimated 38,000 are newly infected annually. More than 5% of certain demographic groups are infected.[1]

Although the natural history of HCV infection is often benign, over time 20% of infected individuals develop serious sequelae, such as severe fibrosis, cirrhosis, end-stage liver disease, or hepatoma. Some develop extrahepatic manifestions, such as lichen planus, leukocytoplastic vasculitis, membranoproliferative glomerulonephritis, porphyria cutanea tarda, or B-cell lymphoma.

WHY TEST FOR HCV?

Testing and testing sequences for HCV infection depend upon the clinical question to be answered, which usually is one of the following:

- Does this patient have hepatitis C?
- Has this person (eg, a potential blood donor) ever been exposed to HCV?
- What effect has HCV infection had on the liver?
- What is the likelihood that treatment will be effective in this patient?
- Has treatment been effective?

CANDIDATES FOR HCV TESTING

Broadly speaking, there are four categories of candidates for HCV testing:

- Those who have risk factors for HCV infection
- Those with abnormal liver function test results
- Blood donors
- HCV-infected patients undergoing antiviral therapy.

Testing needs differ for each category. Testing the general population for HCV is not cost-effective. However, the threshold for testing should be low for persons with any risk factor. A history of relevant HCV exposure is important.

The principal mode of transmission is parenteral exposure to infected blood or blood products. Those

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with a history of illicit injection-drug use are at highest risk. Blood transfusions prior to 1992 carry a 5% to 7% risk of HCV infection for each unit of blood transfused. Exposure to blood from unclean needles used in tattooing or body piercing also risks HCV infection. Sexual contact with an infected person poses only a small risk.

Certain groups are at unusually high risk, such as prison inmates and people with low socioeconomic status. Many infected individuals deny all recognized risk factors. This suggests either forgetfulness or the possible presence of other, as-yet-unrecognized modes of transmission.

■ LAB TESTING: OPTIONS AND STRATEGIES

Laboratory testing forms the basis for establishing or rejecting the diagnosis of hepatitis C. Liver biopsy is needed in most patients if accurate staging is desired.

Laboratory tests have evolved in diversity, sensitivity, and specificity. Historically, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were used, even though they are nothing more than surrogate markers for HCV. Aminotransferase testing has been replaced by tests that measure either antibody to HCV (anti-HCV) or viral presence. The current practice of first screening for anti-HCV and then testing for the virus in those in whom antibodies are detected represents a compromise between cost and efficiency: antibody testing is inexpensive, while viral testing is costly. When viral testing becomes less expensive, it will be reasonable to use viral testing as the first screening test and primary diagnostic tool in most clinical situtations.

Aminotransferase levels: No longer useful

Levels of aminotransferases (ALT, AST) in the blood indicate the degree to which liver membrane injury has resulted in an increased release of hepatocellular enzyme into the bloodstream. Because ALT is more specific than AST for liver injury, ALT is used more often. In patients with risk factors for HCV infection (see above) and in whom there is no other explanation for increased enzyme levels, elevated aminotransferase levels are highly associated with HCV infection. Indeed, an elevated ALT level remains a reason to reject a potential blood donor.

Early studies of interferon alfa, which formed the basis for its original approval by the US Food and Drug Administration for treatment of hepatitis C, were done before the advent of testing for anti-HCV or viral RNA. In these studies, the presence of risk factors plus an elevated ALT level was taken as evidence that HCV was present.

At the same time, an elevated ALT level may be seen in a number of other disorders, limiting its specificity. Moreover, HCV infection may not elicit an ALT elevation.[2] As many as 30% of HCV-infected people have persistently normal ALT values. Some with normal ALT values may have advanced liver disease; ALT levels tend to fall as cirrhosis develops.[3] It remains true, however, that when the ALT level, all other standard liver function measures (AST, alkaline phosphatase, bilirubin, immunoglobulins, albumin), and the complete blood cell count are normal in an HCV-infected person, the likelihood of significant liver disease is very low.[4] This is especially true if the patient has no liver comorbidities, such as significant alcohol consumption, and is not obese.

Modeling studies have assessed the use of ALT values as the first screen for HCV, followed by more specific viral tests for those with elevated ALT levels.[5] As discussed in detail later, this was the most expensive of several strategies tested.

In summary, ALT and other markers of liver injury are no longer appropriate for selecting who should be tested for hepatitis C.

Antibody tests: A helpful first screen

A number of tests are available for detecting the presence of anti-HCV. The most commonly used are the enzyme immunoassay (EIA) and the enzyme-linked immunosorbent assay (ELISA), which is a type of EIA. These tests cost less than $50 and are rapid, easy to perform, and widely available. Early versions were plagued by frequent false-positive reactions, but the current "third-generation" assays are 99% specific and 99% sensitive in immunocompetent individuals.[6]

To confer specificity on a positive EIA result, a test has been introduced that contains four antigens from HCV, embedded on a strip, and uses a recombinant immunoblot assay (RIBA) technique. The presence of antibodies to two or more of the antigens embedded on the test strip represents a positive result. Detection of only one antibody represents an indeterminate result. Very rarely, antibodies to superoxide dismutase are present, so the test strip contains a fifth region to test for these antibodies. In such cases, the RIBA result is uninterpretable. A positive RIBA result is almost always a true positive—ie, a marker of current or past infection.

Many clinical laboratories automatically test for

anti-HCV by RIBA when an EIA result is positive, to confirm that the result is a true positive. Given the remarkable improvement in third-generation EIAs, RIBA testing is no longer necessary as a routine add-on to confirm all positive EIA results. A positive EIA should instead be followed by viral testing for HCV. RIBA testing remains useful when a patient tests positive for anti-HCV by EIA but has no viremia. In such a case, a negative RIBA result probably indicates a false-positive EIA antibody test, whereas a positive RIBA suggests resolved HCV infection.

Rarely, an HCV-infected person will not express antibody to HCV. This lack of antibody expression is described mostly in immunosuppressed patients, and most often in those on chronic hemodialysis. It is clear that HCV can persist in and be transmitted by these individuals even if they remain negative for anti-HCV by both EIA and RIBA. This possibility should be considered when screening for HCV in selected populations (eg, patients undergoing transplantation and patients on chronic dialysis).[7]

Viral testing: A watershed in HCV evaluation

The presence of antibodies to HCV cannot distinguish between current and resolved infection. Moreover, it does not have any bearing on the likelihood of successful antiviral treatment. The advent of serum-based tests of viral presence represents a watershed in the evaluation and management of hepatitis C.

Several assays are in use for the detection of HCV RNA, and infected patients may be tested in several laboratories, each using a different test procedure. Until recently, even the units of expression lacked standardization. Many studies of HCV therapy expressed the amount of virus present (viral load) in copies per mL. Several studies selected 2 million copies per mL as the threshold separating "low" from "high" viral load. However, because there was no comparability of quantified viral loads between assays, it was virtually impossible to interpret viral loads when different test systems were used.

Assays now are standardized and expressed in IU per mL of serum.[8] A viral load greater than 800,000 IU/mL is currently considered high, regardless of the assay used. **Table 1** lists the assays in common use in the United States and their lower limits of detection.

Target vs signal amplification. The test most commonly used to determine the presence or absence of HCV is based on the polymerase chain reaction (PCR). This test detects the presence of minute quantities of HCV by first amplifying the quantity of HCV RNA in the sample, a technique referred to as *target amplification*. Transcription-mediated amplification is another target amplification test.

Other tests, such as the branched DNA assay, operate by a different mechanism, referred to as *signal amplification*. The first step in branched DNA testing is to bind a signal to the virus, after which the signal is amplified.

Most target amplification tests such as PCR are more sensitive than currently available signal amplification tests, and so yield fewer false-negative results. Target amplification tests are more complicated and more costly than signal amplification tests, and also take longer to perform. Signal amplification tests are technically simple, highly automated, rapid, and easily reproduced. Their relative lack of sensitivity is their main drawback. Both types of tests are extremely specific. Apart from a

TABLE 1
Commonly used tests for detecting HCV RNA

Assay type and brands	Manufacturer	Detection limit* (IU/mL)
QUALITATIVE TESTS		
Polymerase chain reaction		
Amplicor HCV v2.0	Roche Molecular Systems	50
Transcription-mediated amplification		
Versant HCV RNA Qualitative Assay	Bayer Diagnostics	10
QUANTITATIVE TESTS		
Polymerase chain reaction		
LCx HCV RNA Quantitative Assay	Abbott Diagnostics	25
SuperQuant	National Genetics Institute	30
Amplicor HCV Monitor v2.0	Roche Molecular Systems	600
Cobas Amplicor HCV Monitor	Roche Molecular Systems	600
Branched DNA		
Versant HCV RNA 3.0 Quantitative Assay	Bayer Diagnostics	615

*Most untreated patients with HCV infection have 50,000 to 5,000,000 IU/mL, so differences in the lower limit of detection are usually not important. See text.

TABLE 2
Guide to the interpretation of hepatitis C testing

Antibody to HCV	HCV RNA	Usual interpretation	Other possible interpretation
Negative	Negative	No infection	—
Positive	Positive	HCV present	—
Positive	Negative	Resolved infection	1. False-positive (<1%) 2. Treated, HCV below detectable limits (verify with qualitative HCV RNA PCR)
Negative	Positive	Infection present (usually in immunocompromised patients or patients undergoing hemodialysis)	1. Early infection 2. False-positive or contaminated test system

contaminated system, false-positive results are rare.

The current generation of PCR tests are quite sensitive, detecting HCV viral loads as low as 25 IU/mL. Levels of circulating HCV in individuals with untreated infection usually range from 50,000 to 5,000,00 IU/mL.

Qualitative vs quantitative assays. The HCV RNA test kits designed to indicate viral load are not quite as sensitive as those that provide only a qualitative (present/absent) result. Because untreated individuals with HCV have viral levels so much higher than the threshold of detection, this small loss in sensitivity is not important, and quantitative HCV RNA testing should be ordered for these patients. **Table 1** illustrates the narrow gap between the most sensitive qualitative and quantitative tests.

Results of qualitative PCR tests for HCV RNA are expressed as either positive or negative; viral load is not provided. Because of the slight loss in sensitivity with quantitative assays, a negative result on a quantitative PCR or branched DNA assay may be falsely negative and, in a person with suspected HCV infection, should be confirmed with a qualitative PCR test for HCV RNA. This is especially true when assessing treatment response.

Why is viral load important? There is no correlation between viral load and histologic disease activity, but patients with high viral load have a lesser likelihood of responding to available antiviral therapy.[9]

In addition, viral load has implications for therapeutic "stopping rules." It is now clear that patients with HCV genotype 1 who do not achieve a 100-fold reduction in viral load after 12 weeks of antiviral therapy have less than a 5% chance of achieving such a response if therapy is continued for an entire year. As a result, antiviral therapy generally should be stopped after 12 weeks in such patients, since continuing treatment is usually not worth the associated cost and morbidity, given the low response rate. However, this criterion of a 100-fold reduction in viral load at 12 weeks does not apply to patients with HCV genotype 2 or 3, since such patients require only 6 months of antiviral therapy.[10]

■ HCV GENOTYPES

The genomic heterogeneity of HCV has impeded the development of effective vaccines. Every strain of HCV demonstrates genomic variability over time. Such changes are referred to as quasispeciation.

More fundamental and more stable genomic differences in HCV allow classification of HCV into genotypes. Until recently, six major genotypes were recognized. Some HCV isolates in Vietnam fall outside these major genotypes, and three additional genotypes are now recognized, bringing the number of principal genotypes to nine. Several genotypes are subclassified as a or b (eg, genotype 1a or 1b), but these distinctions are of little clinical usefulness. Line probe assays used to determine genotype may misclassify patients of Southeast Asian ancestry who have genotype 7, 8, or 9 as having genotype 1b.

The frequency of the different HCV genotypes varies significantly with geography. Genotypes 1, 2, and 3 account for the vast majority of cases of HCV infection in North America; among these, genotype 1 predominates, accounting for 70% to 75% of North American cases. Genotype 4 is most common in Egypt and the Arabian peninsula.

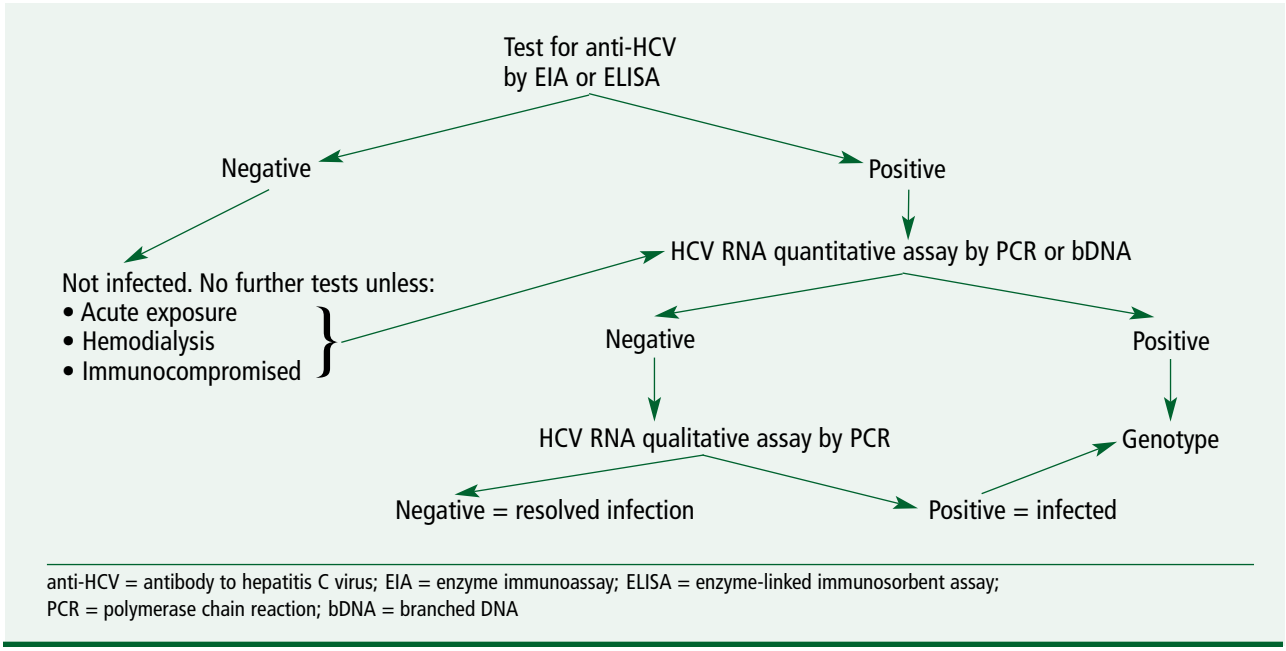

FIGURE 1. Algorithm for laboratory investigation of suspected HCV infection.

Genotype predicts treatment response, duration

Genotype does not have an important bearing on the virulence of HCV but instead relates most closely to anticipated treatment response and treatment duration.

HCV genotypes 2 and 3 are most likely to respond to antiviral therapy. Combination therapy with peginterferon alfa and ribavirin achieves a sustained virologic response in about 80% of previously untreated and noncirrhotic patients with HCV genotype 2 or 3, compared with about 50% of those with genotype 1. Moreover, patients with HCV genotype 2 or 3 require treatment for only 6 months to achieve maximal therapeutic benefit, whereas patients with genotype 1 require 12 months of therapy for maximal benefit. For these reasons, it is customary and appropriate to determine the HCV genotype in all infected patients who are being considered for antiviral therapy. Southeast Asian patients with genotype 7, 8, or 9 have a better response to antiviral therapy than do those with genotype 1.[11]

■ LIVER BIOPSY

The histologic features of chronic hepatitis C are well defined. Two components are considered: the degree of inflammation and hepatocyte necrosis (activity), and the hepatic response (fibrosis).

Activity is gauged by how many mononuclear inflammatory cells are present in and around the portal areas, and by the number of dead or dying hepatocytes. Activity changes do not imply progressive disease.

Fibrosis, more than inflammation, predicts progression to irreversible liver disease in patients with hepatitis C. HCV elicits a variable fibrotic response. Mild fibrotic reactions in the portal and periportal regions are the earliest changes that imply possible progression to cirrhosis. Intermediate fibrotic changes are present when the fibrosis extends from one portal area to another. This is termed "bridging fibrosis." In some, this reaction may evolve into frank cirrhosis. Other histologic changes, such as a mild or moderate amount of macrovesicular fat (steatosis), may also be seen in HCV-infected patients.

Standardized evaluation of liver histology in HCV infection is helpful, and several histologic grading scales have been developed and validated. Each considers the degree of liver pathology in terms of the amount of inflammation and the amount of fibrosis. **Table 3** profiles three common histology grading scales.[12–14] Among the three, the METAVIR system[14] is particularly simple and easy to learn. It has been extensively validated.[15]

TABLE 3
Histologic grading and staging in hepatitis C

Scale	Necro-inflammation	Fibrosis	Total score
Histology Activity Index (HAI)[12]	0–18	0–4	0–22
Ishak modified HAI[13]	0–18	0–6	0–24
METAVIR[14]	0–3	0–4	0–7

What's the role of liver biopsy in the evaluation of hepatitis C?

Liver biopsy is not necessary to establish the diagnosis of hepatitis C. All of the histologic findings seen in hepatitis C, individually and collectively, may be seen in other viral and nonviral liver diseases, so none is diagnostic of HCV infection. Serum-based tests are precise and unequivocal: an individual positive for HCV RNA is infected.

It is true, however, that histologic changes that are markedly different from those seen in hepatitis C (eg, Mallory hyaline, polymorphonuclear inflammation, granulomas, heavy pigment deposition from iron overload) may suggest a diagnosis in addition to hepatitis C. Still, absent other clinical or laboratory findings suggesting a second liver pathology, a liver biopsy will seldom alter the diagnosis. We have shown that liver biopsy in those with HCV infection diagnosed by serum-based tests never eliminates the diagnosis of HCV.[16] Moreover, additional liver diagnoses were made in only 2% of patients.[16]

Liver biopsy figures into the evaluation of hepatitis C by aiding with disease staging (ie, defining the amount of fibrosis and the presence or absence of cirrhosis) in ways that cannot be done without invasive testing. In a patient series at our institution, cirrhosis was found in 29% of cases of hepatitis C that came to biopsy.[16] Using a published cirrhotic discriminant score for clinical diagnosis of cirrhosis in these cases would have confidently established or excluded a diagnosis of cirrhosis in only 23% of these cirrhosis cases. In the remainder, liver biopsy was critical for proper staging. These findings have recently been confirmed.[17] Others have found that laboratory tests (eg, AST:ALT ratio; platelet counts; measures of hyaluronic acid, fibronectin, pseudocholinesterase, etc) are not sufficiently sensitive in predicting the histologic changes of severe fibrosis or cirrhosis.[18]

In many cases, knowledge of the presence (or absence) of cirrhosis is clinically relevant. All other features held constant, the presence of bridging fibrosis or cirrhosis reduces the expected response rate to antiviral therapy. Major shifts in expected outcome are far from trivial and often alter the clinical decision to treat.[19] Moreover, pretreatment liver biopsy can determine whether cirrhosis prevention is a reasonable treatment goal. Finding cirrhosis on biopsy also can influence management by prompting entry into surveillance programs for hepatoma and esophageal varices.

Liver biopsy remains an important tool in the thorough baseline evaluation of the HCV-infected patient. How frequently, or even if, sequential biopsies should be performed in the HCV-infected patient has not been established. There seems to be little need for routine biopsies after a course of antiviral therapy. In clinical practice, authorities differ with respect to rebiopsy at intervals to restage the liver in HCV infection. I do not recommend routine follow-up biopsies.

■ SCREENING STRATEGIES: HCV RISK FACTORS SHOULD BE THE GUIDE

The tests described above give rise to many possible screening strategies to find cases of HCV infection at an early stage. The costs and yield of several possible screening strategies were explored in an analysis constructed from a large database derived from the National Hepatitis Surveillance Program, conducted in 1992.[5] One strategy called for testing for HCV only in those individuals who had a greater than 7% likelihood of infection based on an empirically derived mathematical model. Other strategies tested for HCV only if significant risk factors were uncovered in a simple questionnaire. A final strategy tested for HCV only if the ALT level was elevated.

The analysis found that use of the predictive mathematical model was the most effective and efficient means of deciding who should have HCV testing.[5] Unfortunately, such a model is too arcane and unwieldy to be clinically applicable. However, one of the risk-based screening strategies was associated with very similar costs per 100 persons screened, costs per case detected, and marginal costs per case detected. Specifically, this strategy tested for HCV in those who reported risk factors:

- History of intravenous drug use
- Sex with an intravenous drug user
- Blood transfusion before 1992
- Hemodialysis
- Employment in health care.

(This list of risk factors should be expanded to include other modes of blood–blood transmission, including tattoos and body piercings, intranasal cocaine use, and having an HCV-infected mother.)

The least efficient strategy was to screen by measuring ALT and then testing for HCV in cases of an elevated ALT level. The lesson of this analysis is that testing for HCV infection should be offered to those with risk factors for infection, regardless of ALT level.

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