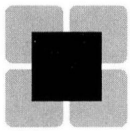

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Interpreting serologic tests for hepatitis C virus infection: balancing cost and clarity

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SUMMARY Although progress has been made toward developing a cheap and accurate method to diagnose hepatitis C virus (HCV) infection, current screening tests have an unacceptably high false-positive rate. Newer tests are more accurate, but also more costly. This paper outlines an approach for interpreting and using these different tests.

KEY POINTS The second-generation enzyme-linked immunosorbent assay (ELISA) for HCV antibodies, the current screening test for HCV infection, has a sensitivity of approximately 90% but a low specificity. Persons with risk factors for HCV infection, elevated aminotransferase levels, and a positive ELISA result most likely have HCV infection. Confirmatory testing with a recombinant immunoblot assay adds considerably to the cost of diagnosis and should only be used to confirm HCV infection in ELISA-positive patients at low risk or with conditions such as hyperglobulinemia that promote false-positive reactivity. Polymerase chain reaction (PCR) testing is the most sensitive and accurate method of diagnosing HCV infection, but its cost limits its use. PCR testing should be reserved for cases of diagnostic uncertainty, evaluation of possible acute hepatitis C, patients with normal serum aminotransferase levels and anti-HCV antibodies, and patients about to undergo interferon therapy.

INDEX TERMS: HEPATITIS C; ENZYME-LINKED IMMUNOSORBENT ASSAY; POLYMERASE CHAIN REACTION; SERODIAGNOSIS
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A 38-YEAR-OLD black man is being evaluated for renal transplantation. He has end-stage renal disease due to chronic membranous glomerulonephritis of unknown cause, a history of intravenous drug abuse, and has been in prison. An enzyme-linked immunosorbent assay (ELISA) shows that he has antibodies to hepatitis C virus (anti-HCV), but his serum aminotransferase levels are persistently normal (alanine aminotransferase [ALT] 39 U/L, aspartate aminotransferase [AST] 27 U/L). Does this patient have HCV infection?

To confirm his HCV status, a recombinant immunoblot assay is ordered. This reveals reactivity to the C33c and C22-3 viral antigens but not to C100-3 or 5-1-1, and is interpreted as positive. A polymerase chain reaction (PCR) test does not detect any HCV RNA. A liver biopsy reveals only minimal inflammatory changes. Thus, concluding that the patient has recovered from a previous HCV infection, the physician clears him for renal transplantation.

This case illustrates some of the ambiguities in interpreting the different tests for HCV.

TABLE
INTERPRETING SEROLOGIC TESTS FOR HEPATITIS C VIRUS INFECTION

Test result*				Interpretation
ELISA	RIBA	PCR	ALT	
Positive	Positive	Positive	Elevated	Chronic hepatitis C
Positive	Positive	Positive	Normal	"Healthy carrier"
Positive	Positive	Negative	Normal	Recovered from infection
Positive	Negative	Negative	Normal	False-positive ELISA

*ELISA, enzyme-linked immunosorbent assay for antibodies to hepatitis C; RIBA, recombinant immunoblot assay; PCR, polymerase chain reaction for hepatitis C RNA; ALT, alanine aminotransferase levels

SEROLOGIC TESTS FOR HEPATITIS C VIRUS INFECTION

HCV was first identified and its genome cloned in 1989. Almost immediately, researchers started to devise assays to detect antibodies to HCV in serum.[1] As these assays were developed, it became apparent that HCV is the predominant cause of non-A, non-B hepatitis. This single-stranded RNA virus accounts for about one third of cases of acute hepatitis in the United States, in the last decade infecting about 150 000 persons per year.[2] In more than 70% of cases, infection progresses to chronic hepatitis.[3] Improved serologic tests, used judiciously, can now detect HCV infection with a high degree of accuracy.

ENZYME-LINKED IMMUNOSORBENT ASSAY

How the test works

An ELISA for detecting anti-HCV is the primary screening test for HCV infection. The first-generation ELISA (ELISA 1.0, Ortho Diagnostic Systems, Emeryville, CA) used the C100-3 clone, derived from the less-conserved nonstructural region of the HCV genome. Although this early assay was better than any previous test, it was far from ideal. Its sensitivity was poor[4,5] because some patients lack antibodies to C100-3 and because a long time elapses between infection and seroconversion, averaging 22 weeks in one study.[6] The assay also lacked specificity[4,5]; false-positive results were very common in patients with connective-tissue diseases and other states associated with hypergammaglobulinemia.[7,8]

The second-generation ELISA was introduced in an effort to improve the usefulness of ELISA as a screening test. This test uses an extended version of the C100-3 protein (C200) and two additional recombinant viral antigens: the C33c protein from the nonstructural region of the genome and the C22 protein from the highly conserved core region. These changes substantially improved the sensitivity of the test to greater than 90% in patients with chronic hepatitis C.[9–12] In many cases, antibodies specific to the C22 and C33c proteins appear earlier than do the C100-3 antibodies detected by the earlier test, thus shortening the seronegative window.[3,10] Although the second-generation test has somewhat higher specificity than the earlier test had, concerns remain about its high rate of false-positive results, especially in low-risk persons (such as apparently healthy volunteer blood donors) and those with hypergammaglobulinemia.[9,10]

The third-generation ELISA (not yet licensed for use in United States) has been shown in European studies to have greater sensitivity and specificity.[13–15]

Interpreting positive ELISA results

Because of its low specificity, a positive HCV ELISA result must be interpreted with caution. To determine if a person is truly infected, supplemental assays are sometimes—but not always—needed.

High-risk patients with abnormal liver function. High-risk patients who have a reactive HCV ELISA and elevated serum aminotransferase concentrations most likely are infected, and therefore do not need further testing. In fact, in persons with known risk factors (ie, intravenous drug abuse, hemodialysis, or multiple blood transfusions) who have repeatedly reactive HCV ELISA results, the prevalence of infection is 70% to 100%.[2]

High-risk patients with normal liver function. The picture is less clear for ELISA-positive persons who have a risk factor for HCV infection but persistently normal serum aminotransferase levels. Possible explanations for these findings include ongoing infection without significant liver damage, resolved infection, or a false-positive ELISA result (*Table*). In such cases, further testing should be considered.

Low-risk patients with normal liver function. ELISA is much less specific in screening low-risk persons such as volunteer blood donors, in whom fewer than 50% found positive by ELISA are truly infected.[2,3] In such a patient, if a detailed history and physical examination fails to find risk factors for hepatitis C or evidence of liver disease, the ELISA result may have been falsely positive, but further testing is indicated.

Low-risk patients with abnormal liver function. Further testing is also indicated if a low-risk, ELISA-positive patient has elevated serum aminotransferase levels, although other chronic liver diseases should be considered, including chronic hepatitis B, hemochromatosis, Wilson's disease, and alpha-1-antitrypsin deficiency. Because false-positive HCV reactivity can occur in persons with hypergammaglobulinemia, autoimmune hepatitis, or primary biliary cirrhosis, these conditions should also be considered in the differential diagnosis.

RECOMBINANT IMMUNOBLOT ASSAY

How the test works

A recombinant immunoblot assay is used to confirm anti-HCV reactivity. The second-generation recombinant immunoblot assay (RIBA 2, Chiron Corporation, Emeryville, CA) uses the 5-1-1, C100-3, C22, and C33c HCV proteins as well as the human enzyme superoxide dismutase (SOD) applied as separate bands on nitrocellulose strips. These strips are incubated with patient serum, and adherent antibody is measured as a blue-black color change that can be compared to two control bands. A positive result is defined as reactivity to at least two of the four viral antigen bands, while reactivity to a single band or to the 5-1-1 and C100-3 bands only is interpreted as being indeterminate. The test is negative if no bands show reactivity.

Interpreting the recombinant immunoblot assay

Recombinant immunoblot assay's accuracy makes it a useful confirmatory test: its sensitivity approaches 98% and its specificity ranges between 95% and 100%.[9,13,14,16–18] False-positive results are uncommon in patients with hypergammaglobulinemia.[9,10]

Recombinant immunoblot assay testing adds $50 to $100 to the cost of diagnosis and should only be used to confirm HCV infection in ELISA-positive patients at low risk or with conditions such as hypergammaglobulinemia that promote false-positive reactivity.

Positive ELISA, negative recombinant immunoblot assay. An ELISA-positive person with a negative recombinant immunoblot assay result is unlikely to have HCV infection.

Positive ELISA, positive recombinant immunoblot assay. Conversely, a positive recombinant immunoblot assay result correlates highly with the capability of transmitting an infection and with abnormal liver histology.[13,14,16–18]

Indeterminate recombinant immunoblot assay. An indeterminate result warrants further evaluation. Depending on the sample size and the type of population, between 22% and 67% of persons with indeterminate recombinant immunoblot assay results have HCV RNA in their serum.[16,19] Despite this wide variation, many persons with indeterminate results are infected and need further testing by PCR.

DETECTING HCV RNA: USING POLYMERASE CHAIN REACTION

How the test works

HCV infection can also be diagnosed by the presence of viral RNA in serum or infected tissues. Because only small amounts of HCV RNA are present, PCR testing is usually required to detect it. This procedure requires that the HCV RNA first be reverse-transcribed into cDNA and then amplified multiple times to increase the minute quantities of DNA to detectable levels. During the procedure, care must taken to avoid cross-contamination, which can cause false-positive results.

PCR testing is still used primarily as a research tool, and methodologic problems and lack of standardization have raised concerns about its reliability.[20] Some researchers suggest that PCR may not detect HCV RNA in persons with intermittent viremia or with extremely low quantities of circulating HCV RNA,[9,21] though how often these occur has yet to be determined. PCR testing is also costly (approximately $200) and labor-intensive, inhibiting its widespread use. Despite these problems, PCR is considered the most sensitive and specific test for hepatitis C.[4,11,22] Further, HCV RNA is detectable in the serum within 1 to 2 weeks after infection, thus greatly shortening the seronegative window that limits the usefulness of other assays.[23] Rarely, patients may be PCR-positive but ELISA-negative.

Interpreting PCR results

Positive PCR results. Thus, PCR-positive patients almost surely have active HCV infection. Patients who have normal serum aminotransferase levels but who are PCR-positive have HCV infection, but very mild hepatitis. The long-term natural history of this condition is not known, and such patients should be observed at intervals for as long as HCV RNA remains detectable in the serum. If serum aminotransferase levels become elevated, interferon therapy could be considered, although it is currently not advised for patients with normal aminotransferase levels.

Negative PCR results. Persons in whom HCV RNA cannot be detected may have recovered from an infection or have levels so low as to be undetectable by even this ultrasensitive assay. Patients with normal serum aminotransferase levels and who are ELISA- and recombinant immunoblot assay-positive but PCR-negative (as was the case with our example of the man awaiting renal transplantation) most likely have recovered from an HCV infection, although the possibility that they may have an intermittent viremia or a circulating RNA level too low to be detected by PCR cannot be ruled out entirely. They should therefore be advised to have their serum aminotransferase levels checked periodically for up to 12 months; if these remain within the normal range, recovery from HCV infection can be presumed.

PCR testing should be reserved for cases of diagnostic uncertainty, evaluation of possible acute hepatitis C, patients with normal serum aminotransferase levels and anti-HCV antibodies, and patients about to undergo interferon therapy. The *Table* outlines how the results of different assays should be interpreted.

FUTURE DIRECTIONS

Much progress has been made toward developing a cheap and accurate method to diagnose hepatitis C. However, the current screening test, though inexpensive, still has an unacceptably high false-positive rate and, to a lesser extent, low sensitivity. The period between infection and seroconversion has been shortened with these newer assays,[24] and false-positive reactivity is not as common with hypergammaglobulinemia.[25] Efforts to make PCR testing less labor-intensive should improve its cost-effectiveness and make it more available for clinical use, and efforts to standardize PCR testing should make it more reliable.

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