Interpretation of Laboratory Tests for Diagnosing Viral Hepatitis

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Viral hepatitis is a major cause of morbidity and mortality worldwide. The Centers for Disease Control and Prevention estimated that there were nearly 200,000 new cases of viral hepatitis in the United States in 2001. Although many cases are subclinical or present with only flu-like symptoms, viral hepatitis has a significant impact on society. There are 5 hepatitides that have their principal site of injury in the liver: hepatitis A, B, C, D, and E. All the major hepatitides can cause acute viral hepatitis, but only hepatitis B (with or without co-infection with hepatitis D) and hepatitis C cause chronic liver disease. Chronic infection can lead to cirrhosis and even hepatocellular carcinoma.

Over the past several decades, significant advances have been made in our understanding of hepatitides, which has led to the development of various serologic and molecular tests that have made it possible to accurately diagnose the specific type of viral hepatitis (Table 1). A firm understanding of these tests and serologic markers will lead to the accurate diagnosis and appropriate management. This review will focus on the serologic and molecular tests available, rational ordering of the tests, and their interpretation in the diagnosis of viral hepatitis.

HEPATITIS A
Epidemiology

Hepatitis A virus (HAV), an RNA-containing virus that is transmitted exclusively through the fecal-oral route, is considered to be the most common cause of acute viral hepatitis. Worldwide, it is estimated to infect 1.4 million people annually, most prominently in South America, Africa, and Asia. The Centers for Disease Control and Prevention estimated 90,000 symptomatic cases of HAV infection and 180,000 asymptomatic infections in the United States in 1997. Accurate diagnosis is not only important for the care of the infected individual but also for close contacts who may be candidates for immunoglobulin prophylaxis.

Laboratory Testing for HAV

After infection, the incubation period for HAV can range from 15 to 49 days, averaging 30 days. The gold standard for the diagnosis of acute HAV is the detection of IgM anti-HAV in the serum by radioimmunoassay or enzyme-linked immunoassay (EIA). No confirmation test is necessary if there is a positive IgM anti-HAV result in conjunction with elevated transaminases and clinical symptoms consistent with acute hepatitis. IgM anti-HAV is invariably detected at the presentation of acute HAV. IgG anti-HAV also may be detectable during an acute HAV infection. Other methods of documenting HAV infection exist, such as detecting HAV RNA by reverse transcriptase polymerase chain reaction (RT-PCR) or direct HAV antigen detection in stool, but these are expensive, impractical, and have no routine clinical application.

IgM anti-HAV is detectable in the serum for 4 to 6 months after infection, although an infection that is relapsing can have a positive IgM anti-HAV for up to 12 months before resolution. HAV infection does not cause chronic liver disease. The presence of IgG anti-HAV is indicative of immunity. Anti-HAV IgG becomes detectable after vaccination or natural infection. HAV vaccination provides immunity for an estimated 10 to 20 years, whereas natural infection provides lifelong immunity.

HEPATITIS B
Epidemiology and Viral Structure

Hepatitis B virus (HBV) is the ninth leading cause of death worldwide, with estimates of more than 350 million carriers worldwide. In the United States, approximately 1 to 1.25 million people are chronically infected.

HBV is a complex double-shelled DNA virus. The
outer membrane contains hepatitis B surface antigen (HBsAg), while the inner core of the virus contains hepatitis B core antigen (HBcAg) and hepatitis B e antigen (HBeAg). Tests for these specific particles and their corresponding antibodies aid in the diagnostic evaluation of an HBV-infected patient.

HBV Virology and Viral Markers

The incubation period for HBV can vary from approximately 2 to 6 months. HBsAg is detectable by enzyme-linked immunosorbent assay (ELISA) approximately 6 weeks after infection and usually is present when an infected person is symptomatic from HBV. With successful clearance of the virus, HBsAg should begin to disappear and become undetectable by about 4 to 6 months. The disappearance of HBsAg is followed by the appearance of antibody to HBsAg (anti-HBs), a neutralizing antibody that confers immunity. Occasionally, the appearance of anti-HBs may trail the disappearance of HBsAg by several weeks to months. During this window period, both HBsAg and anti-HBs may be below the level of detection; if this occurs, the sole markers of recent HBV infection during this time are antibodies against the HBV core antigen, IgM and IgG anti-HBc. Like HBsAg, IgM anti-HBc is detectable at the onset of clinical symptoms. Although both anti-HBc IgM and IgG rise together, levels of IgM anti-HBc usually will decrease while IgG remains increased. The presence of IgM anti-HBc is suggestive of a recent infection (< 6 months). However, IgM anti-HBc may become detectable during an exacerbation of chronic hepatitis as well. Thus, differentiating an acute HBV infection from a reactivation of chronic HBV using the presence of IgM anti-HBc may be difficult.6 After resolution of an HBV infection, both anti-HBc and anti-HBs will be present. Vaccination leads to the production of only anti-HBs. The titers of anti-HBs may fall below the level of detection over time.

Although uncommon, it is possible to see both HBsAg and anti-HBs present simultaneously. The presence of both may suggest either resolving acute HBV infection or the presence of non-neutralizing antibodies, indicating a chronic carrier state.9 There are several explanations for an isolated anti-HBc IgG, which are discussed in Table 2 along with the interpretation of other serologic outcomes for the diagnosis of HBV infection.

Laboratory Testing for Chronic HBV

Fewer than 10% of all HBV-infected patients develop chronic HBV. Patients who tend to be at greater risk for chronic disease are the very young (up to 90% in newborns)10,11 and the immunocompromised, including hemodialysis patients.12,13 Chronic HBV is suggested by the presence of HBsAg in the serum for at least 6 months. After it has been determined that HBV is chronic, the level of viral activity or replication is assessed by testing for HBeAg and HBV DNA in the serum using ELISA. Although HBeAg and HBV DNA become apparent immediately after the incubation period along with HBsAg, these tests are not routinely ordered at that time unless there is evidence of chronic HBV infection. The presence of HBeAg in the serum correlates with HBV DNA synthesis and thus is a marker of viral replication and infectivity.14

The two types of chronic HBV infection are the active and inactive forms. Active chronic HBV is characterized by HBeAg positivity, HBV DNA exceeding 10^5 copies/mL, elevated transaminases, and liver biopsy findings showing chronic hepatitis. Inactive chronic HBV is characterized by seroconversion from HBeAg to antibody for HBeAg (anti-HBe), HBV DNA below 10^5 copies/mL, normal transaminases, and the absence of significant hepatitis on liver biopsy.11 Both active and inactive HBV infection will continue to exhibit detectable HBsAg.

HEPATITIS C

Epidemiology and Natural History

Currently, approximately 3 to 4 million individuals in the United States are infected with hepatitis C virus (HCV), an RNA virus.15 There are 6 different genotypes of HCV. Genotype 1 accounts for 70% to 75% of HCV infections in the United States and is associated with a lower rate of response to treatment.16

Symptoms of acute HCV infection usually are mild and nonspecific. The incubation period for HCV is 14 to 160 days. HCV typically is diagnosed in the chronic phase. Approximately 80% of infected individuals develop chronic hepatitis. Patients with chronic hepatitis are
at risk of cirrhosis and subsequently hepatocellular carcinoma. HCV is currently the leading cause for liver transplantation.

Laboratory Testing for HCV

Both serologic and molecular tests are available for the diagnosis and management of HCV.17 Screening for HCV is performed using EIA.18,19 Both its sensitivity and specificity for the diagnosis of HCV are greater than 99%.20,21 Positive EIA results can be confirmed with a recombinant immunoblot assay (RIBA). Note that anti-HCV is not neutralizing and thus does not confer immunity.

Molecular tests utilizing RT-PCR to detect HCV RNA are extremely sensitive tools for the diagnosis and management of HCV. HCV RNA can be detected in the serum within 1 to 3 weeks of exposure and at the onset of symptoms.16 Both quantitative and qualitative assays exist. Qualitative testing can detect as few as 100 HCV RNA copies/mL, and the results are read as either positive or negative. Quantitative assays provide an estimate of viral load. However, the HCV viral load neither correlates with severity of disease nor helps in predicting the prognosis. Nevertheless, low levels of viremia may correlate with a better probability of response to antiviral therapy.22 Besides estimating the probability of achieving a therapeutic response to antiviral therapy, RT-PCR testing also may be useful in diagnosing seronegative patients who are immunosuppressed when there is a high suspicion of disease23 and assessing treatment efficacy during therapy.24

Historically, confirmation testing was done using RIBA after a positive EIA. More recently, RT-PCR testing has become the confirmatory test of choice. An algorithm of tests used in the diagnosis of HCV is shown in the Figure.

HEPATITIS D

Natural History

Hepatitis D virus (HDV), also called the delta virus, is a defective RNA virus that is unable to cause disease
on its own without the presence of a concurrent HBV infection. The areas with the highest endemic rates are the Middle East, Mediterranean countries, and certain parts of South America.\textsuperscript{25–27} HDV is uncommon in Western Europe, North America, and East Asia. HDV can occur as a co-infection during an acute HBV infection or as a superinfection in chronic HBV carriers. HDV infections also have been noted in liver transplant patients with a prior history of HBV before overt reinfection of the graft with HBV.\textsuperscript{28} In co-infection, severe and fulminant hepatitis can develop. In superinfection, there may be acute worsening of liver disease, but the greatest risk is the development of chronic liver disease with rapid progression to cirrhosis.\textsuperscript{29,30}

**Laboratory Testing**

The delta antigen (HDV Ag) becomes present in the body during the late incubation period of acute infection, which lasts 21 to 45 days. The presence of HDV Ag is followed by anti-HDV IgM and IgG. IgM anti-HDV lasts about 10 to 20 days when the infection is acute and self-limited but may persist longer if the infection becomes chronic. Anti-HDV, like anti-HCV, is not neutralizing and does not confer immunity. RT-PCR used to detect serum HDV RNA is the most reliable test, with a near 100% sensitivity.\textsuperscript{31} IgM anti-HDV does not help distinguish between co-infection and superinfection. This distinction can be made by serologic HBV testing, specifically, the presence or absence of IgM anti-HBc. Patients with co-infection will be positive for IgM anti-HDV and IgM anti-HBc, whereas patients with superinfection will be positive for IgM anti-HDV and negative for IgM anti-HBc. Another clue pointing toward co-infection would be a biphasic aminotransferase elevation in the face of positive IgM anti-HBc, with one peak resulting from the HBV-induced liver injury and the other peak from the HDV individual liver injury.

**HEPATITIS E**

Hepatitis E virus (HEV), an RNA virus transmitted through the fecal-oral route, is an uncommon cause of acute hepatitis in the United States. Like HAV, HEV does not cause chronic liver disease. Most cases occur in underdeveloped countries, with the highest incidence in Africa, Asia, the Middle East, and Latin America. It should be considered in a traveler returning from an area where HEV is endemic.\textsuperscript{32} The incubation period is 40 days on average and ranges from 15 to 60 days. Both anti-HEV IgM and IgG are detectable at the onset of illness, but the sensitivities with current available tests are only 53% and 87%, respectively, with specificities of 99% and 92%, respectively.\textsuperscript{33} Levels of IgM anti-HEV may decline rapidly, whereas IgG anti-HEV may remain elevated although declining in titer. Detection of HEV RNA by RT-PCR is not widely available. HEV infection should be suspected in patients with a travel history to areas endemic for HEV along with symptoms and laboratory findings consistent with acute hepatitis, who are also negative for HAV, HBV, HCV, cytomegalovirus, and Epstein-Barr virus on serologic testing.

**CONCLUSION**

Viral hepatitis remains a major cause of morbidity and mortality worldwide. It is virtually impossible to clinically differentiate among the various causes of viral hepatitis; accurate diagnosis can only be achieved with serologic and molecular testing; these techniques have revolutionized our ability to diagnose the offending pathogen. It is crucial to have a firm understanding of the serologic markers that characterize the viral hepatitides at various stages of the disease process. Knowledge of the strengths and limitations of these tests allows for rational ordering and interpretation. Ongoing research of the major hepatotropic viruses, along with further development of better diagnostic techniques and treatments of viral hepatitis, will continue to aid in the care of patients.

**REFERENCES**