Hepatitis Serologic Markers and Nucleic Acid Testing

Hepatitis A virus (HAV) – Hepatitis A virus is an RNA virus of the picornavirus family. HAV is spread by the fecal-oral route, and causes hepatic injury after an incubation period of only a few weeks. HAV RNA is present in stool and plasma for most of the period before onset of clinical symptoms, but disappears soon after onset of clinical illness. Immunoglobulin M (IgM) antibodies to HAV (anti-HAV IgM) are typically present at onset of symptoms, and remain detectable for an average of 3 to 6 months after infection (range <30-420 days, with 13.5% positive longer than 4 months). (128) Total anti-HAV persists for long periods after infection, perhaps for life (129); seroprevalence increases with increasing age, ranging from 11% in children < 5 years to 74% in those > 50 years. (130) HAV vaccine induces detectable anti-HAV within 2 to 4 weeks of the initial dose of vaccine (131) and antibody remains detectable at 5 years in 99% of individuals completing vaccination. (132) There are no commercially available antigen or nucleic acid detection tests for HAV. Immune electron microscopy, and immunoassay methods have been used to detect HAV antigen in stool filtrates and other specimens in research settings, and HAV RNA assays have been employed to document sources of epidemics and in research studies. .

Recommendations

IgM anti-HAV should be used to diagnose acute HAV infection (IB)

Total antibody should be used for determining immune status for HAV (IB)

Hepatitis B virus (HBV) – Hepatitis B is a DNA virus of the hepadnavirus group. These viruses replicate by forming an RNA intermediate, which is copied using the enzyme reverse transcriptase to regenerate DNA strands. HBV is transmitted by exchange of body fluids; major methods of transmission include serum, sex, and transmission from mother to infant (usually occurring after birth). While HBV infection is typically acute with complete recovery in adolescents and immunocompetent adults, chronic infection can also occur. Approximately 1-3% of healthy adults, 5-10% of immunocompromised adults, and 90% of neonates exposed to HBV develop chronic infection.

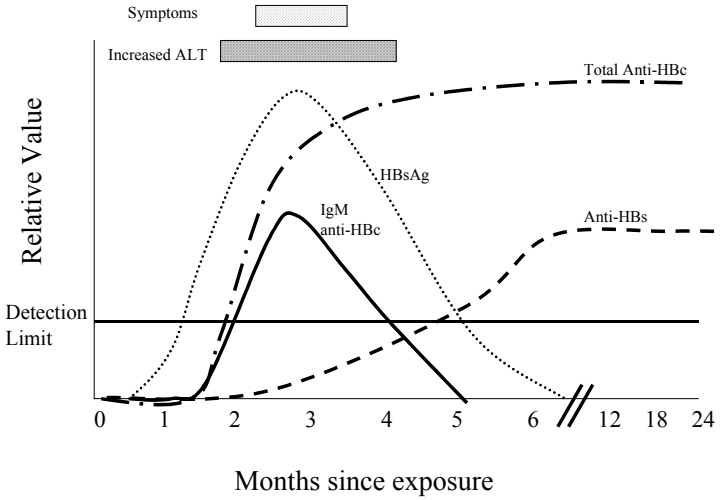

Figure 6. Time Course of Serologic Markers in Acute Hepatitis B Infection with Resolution. Following infection, the first marker of infection to appear is the surface antigen (HBsAg), from 1 to 3 months after exposure. Approximately 1-2 months later, the first antibody response is IgM antibody to the hepatitis B core antigen (IgM anti-HBc), generally around the time of increase in AST and ALT activities in plasma. At the time of onset of jaundice, most patients have both HBsAg and IgM anti-HBc. With clearance of virus, anti-HBs will become detectable. In a small percentage of patients, there may be a transient period where neither HBsAg nor anti-HBs can be measured; the only commonly measured marker present at this time will be IgM anti-HBc, a pattern termed the "core window". Although not illustrated in this diagram, usually such patients will be positive for anti-HBe, if a second test is needed to confirm the anti-HBc result. With recovery from HBV infection, anti-HBc and anti-HBs persist for life in most individuals.

HBV produces several protein antigens that can induce an antibody response. The most abundant, HBV surface antigen (HBsAg) is produced in excess along with viral particles, but can also be present when HBV DNA is integrated into cellular DNA and no longer produces infectious virions. HBV core antigen and e antigens (HBcAg and HBeAg) are produced by an overlapping genetic region in the virus and are found in infectious particles. A typical serological and clinical course of acute HBV infection is shown in Figure 6. (133) IgM antibody to HBcAg (anti-HBc) is usually considered the gold standard for diagnosis of acute hepatitis B. (134) It may also be present at fluctuating, low titers in patients with chronic hepatitis B, particularly when patients also have positive plasma HbeAg, HBV DNA, or episodes of rising ALT indicating reactivation of disease. (135) Total anti-HBc typically persists for life. (136) HBsAg is characteristically present and anti-HBs absent at presentation in patients with acute HBV infection, but both are occasionally absent (134), leaving IgM anti-HBc the only marker of infection ("core window"). Isolated positive anti-HBc also may represent low level viremia, loss of anti-HBs many years after recovery, or a false positive result (136, 137, 138) Two factors are associated with likelihood of false positive results: low level of anti-HBc reactivity and absence of anti-HBs using sensitive immunoassays. In several studies, virtually none of those with low levels of anti-HBc and negative anti-HBs showed an anamnestic response to a single injection of HBsAg vaccine, whereas 35-40% of those with weakly positive anti-HBs and 50-80% of those with high level of anti-HBc responded. (137, 139, 140) Convalescence from infection is indicated by loss of HBsAg and development of anti-HBs. Concomitant HBsAg and anti-HBs may be seen in a small number of patients with chronic HBV infection. This phenomenon appears to be particularly common in patients on maintenance hemodialysis (7%) compared to other HBsAg positive patients (2%). (141) The presence of anti-HBs

in these settings does not appear to have clinical importance. Patterns of serological markers in various forms and phases of HBV infection are shown in Table 9. (142) Examples of discordant or unusual hepatitis profiles are given in Table 10. Tests with discordant results should be repeated and testing for additional serological markers may be indicated to establish the correct diagnosis. (143)

Table 9. Serological diagnosis of hepatitis B virus infections (modified from reference 142)

Marker	Incubation	Acute Infection	Past Infection	Chronic Infection	Vaccination
HbsAg	+[a]	+	-	+	-
HbeAg	+	+	-	+/-	-
HBV DNA	+	+	-	+/-	-
Anti-HBc					
IgM	-	+	-	+/-[c]	-
Total	-	+	+	+	-
Anti-Hbe	-	-	+/-	+/-[d]	-
Anti-HBs	-	-	+	-	+

[a]+, detectable; -, not detectable; +/- may be detectable.
[b]Non-PCR methods
[c]May be positive in 10-15% patients with reactivation of infection
[d]Patients with chronic HBV infection usually have detectable HBeAg or anti-HBe.

Table 10. Discordant or unusual hepatitis B serologic profiles requiring further evaluation.

- HBsAg positive/Anti-HBc negative
- HBsAg, anti-HBs, and anti-HBc positive
- Anti-HBc positive only
- Anti-HBs positive only in a non-immunized patient
- HBsAg negative/HBeAg positive
- Positive for HBeAg and anti-HBe
- Total anti-HBc negative/IgM anti-HBc positive

Recommendations

Tests for HBsAg, anti-HBs, and anti-HBc should be performed for diagnosis of current or past HBV infection. In suspected acute HBV infection, tests for IgM anti-HBc should be utilized (IB).

HBeAg and anti-HBe are not required for diagnosis of acute hepatitis B or for routine evaluation of HBV status (IIIB, E).

In patients with discordant results, tests should be repeated; persistently discordant results should be evaluated by a hepatologist or gastroenterologist (IIIB).

In patients with chronic presence of HBsAg, HBeAg and anti-HBe are useful tests for determining the status of infection. HBV DNA can be present in hepatocytes in two forms: as replicating virus, leading to production of infectious particles, or integrated into the host DNA, a non-replicative form. HBeAg is only produced as part of replicating virus, and thus can be used to indirectly determine the state of HBV DNA production in

the hepatocyte. In the HBeAg positive patient, loss of HBeAg and seroconversion to anti-HBe positivity is typically associated with loss of circulating HBV DNA by methods other than polymerase chain reaction (PCR), normalization of aminotransferases and histologic improvement, implying a low replication state and significant clinical improvement. (144) HBV DNA measurements are more useful in following chronic hepatitis B patients receiving antiviral therapy. Loss of detectable HBV DNA by a solution phase hybridization assay is an earlier indicator of response to antiviral therapy than loss of HBeAg (143). Several assays for detection of serum HBV DNA are commercially available; sensitivity limits are given in Table 11. There is currently no standardization of HBV DNA assays between laboratories.

Table 11 – Lower Detection Limits of HBV DNA Assays

Method	Detection Limit (copies/mL)[a]
Hybrid Capture	3.0×10^6
Branched DNA	0.7×10^6
Liquid Hybridization	4.0×10^4
Polymerase Chain Reaction	10^2 - 10^3

[a]Results can also be expressed as pg/mL of HBV DNA by dividing by $2.85 * 10^5$

Circulating HBV DNA can be found by sensitive PCR methods in a high percentage of patients with negative HBsAg and positive anti-HBs, anti-HBe, and anti-HBc months or years after clinical recovery from acute hepatitis (145) or chronic hepatitis. (146) The significance is not clear, as most viral DNA is found in immune complexes, (145) and may not represent the entire genome. A recent study of 7 potential liver donors who were HBsAg negative and anti-HBs and anti-HBc positive found replicating forms of the virus within the hepatocyte in 6 of the 7 individuals. (147) Similarly, in patients with chronic hepatitis C infection, HBV viral DNA is commonly found (using sensitive PCR methods) in both liver and serum, particularly in patients with anti-HBc as an isolated HBV marker. (137, 138) These studies suggest that many patients who were formerly thought to have recovered from HBV actually have low, but controlled, levels of viral replication persisting for many years after clinical recovery. It is not clear what level of HBV DNA viremia should be used to consider a patient "cured" of HBV infection for clinical purposes.

Recommendations

HBeAg and anti-HBe are useful in monitoring patients with chronic HBsAg positivity (IB)

Quantitative HBV DNA should be used for monitoring response to antiviral therapy (IIB).

An international standard for HBV DNA test should be established and manufacturers should calibrate kits against it. (IIIB).

Tests for HBV DNA should be quantitative and the clinically useful dynamic range for HBV DNA tests should be defined (IIIB).

Hepatitis C virus (HCV) - Hepatitis C virus (HCV) is an RNA virus of the flaviviridae family. To date, HCV has not been cultured; it was recognized by detecting viral sequences through recombinant technology, and the entire genome has now been sequenced. Currently, no commercial assays are available to detect HCV antigens, although a highly sensitive assay for HCV core protein has been developed. (148)

Most HCV tests measure antibodies to HCV. Screening tests for HCV infection detect antibodies to HCV proteins, usually apparent by an average 80 days (range 33-129 days) after infection using second generation anti-HCV enzyme immunoassays (EIA-2). (149) Immunocompromised patients and those on dialysis may rarely lack detectable antibodies by EIA-2 despite other evidence of active viral infection. (150) A third generation EIA (EIA-3) for anti-HCV has been approved by the FDA for screening blood products; it contains reconfigured core and NS3 antigens and an additional antigen (NS5) not found in EIA-2. EIA-3 provides a slight increase in sensitivity but lower specificity than EIA-2, and shortens the time to detection of antibody to an average of 7-8 weeks after infection. (151) In patients who have cleared HCV from the circulation, titers of anti-HCV gradually fall (152, c218), and eventually become negative in 6-10% of infected individuals. (153, 154) In evaluating possible perinatal transmission of HCV, maternal antibody clears by 12 months in 90% of non-infected infants and by 18 months in 100%. (155) Approximately 90% of infected infants have detectable plasma HCV RNA by 3 months of age. (156)

Supplemental tests for anti-HCV help resolve suspected false-positive EIA test results. Recombinant immunoblot assays (RIBA) contain the same HCV antigens as do the EIA tests, along with superoxide dismutase (SOD) to detect non-specific antibodies to yeast proteins (recombinant HCV antigens are typically derived using yeast as the vector). A positive RIBA is defined as reactivity against two or more HCV antigens from different regions of the genome, without reactivity to SOD. Reactivity to a single HCV antigen or multiband reactivity with reactivity to SOD are considered indeterminate. In populations at high risk for HCV infection less than 1% of EIA-2 positive specimens will be false-positives. Additionally, in recently infected individuals, RIBA is positive in only 85% of cases. (157) Therefore, RIBA testing in high-risk populations is not necessary for the diagnosis of hepatitis C. (158)

Active HCV infection is defined by presence of HCV RNA in plasma. HCV RNA can be detected within 1-2 weeks after acute infection, weeks before ALT becomes abnormal and prior to appearance of anti-H[illegible] in typical HCV infection is illustrated in Figure 7. Although not FDA-approved, reverse transcription (RT) PCR assays for HCV RNA are use[illegible]inical practice; t[illegible]e most sensitive can detect > 100 HCV RNA copies/mL. HCV RNA assays are not standardized, and quantitative assay results may vary significantly between different laboratories using different assays. (158, 159) HCV RNA is very susceptible to degradation by the high activities of RNase present in blood; therefore, serum specimens for HCV RNA should be centrifuged as soon as possible after clot formation. EDTA or sodium citrate plasma are preferred specimens for HCV RNA tests. Heparinized plasma is inhibitory for many nucleic acid amplification assays, and serum specimens provide suboptimal stability unless serum is frozen soon after specimen collection. If

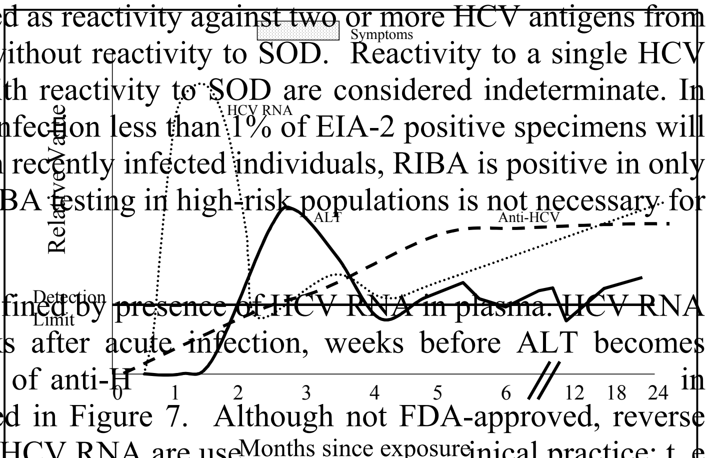

Figure 7 – Time Course of Serologic Markers in Acute Hepatitis C Infection. Following infection, the first marker to appear is HCV RNA, usually detectable by 1-2 weeks following exposure to the virus. HCV RNA concentration gradually increases, but begin to fall with development of antibody response; they may be transiently negative in about 15% of cases. Anti-HCV appears at an average of 8-10 weeks after exposure; the time is shorter with third generation than with second generation anti-HCV assays. After the acute episode, which is clinically silent in most individuals, 75-85% will develop chronic infection with HCV. During the transition from acute to chronic infection, both ALT and HCV RNA may be intermittently positive; they are more likely to be persistently positive many years after infection, although 15-25% of chronically infected individuals may have persistently normal ALT.

centrifugation is performed immediately, less than 10% of HCV RNA is lost even if the plasma or serum is not separated from the formed elements for up to 6 hours. (160) If a serum separator tube is used, specimens are stable after centrifugation for up to 24 hours. (160) Short term (< 7 days) storage of serum or plasma at 4°C is acceptable. Once frozen, samples are stable through at least three freeze-thaw cycles. (160) Quantitative HCV RNA assays are often less sensitive than qualitative RNA assays using the same technology, but this is not universal. The current version of the branched DNA assay is the least sensitive, with a lower limit of detection of 200,000 copies/ml; however, branched DNA assays have better linearity and reproducibility than do PCR assays. In patients with chronic HCV who are untreated, it is unusual to encounter specimens with undetectable HCV RNA by branched DNA but positive by PCR. (160a) Results from different methods cannot be directly compared because different standards are used. A World Health Organization international standard for HCV RNA for nucleic acid amplification assays is now available (161), and is being introduced to use by kit manufacturers.

Recommendations

EIA screening tests for HCV antibody are adequate for diagnosis of past or current HCV infection in a patient population with a high prevalence of disease; supplemental testing is not needed in such patients. If confirmation of active infection is required, HCV RNA should be used. (IIB, E).

Supplemental anti-HCV tests (RIBA) should be used in populations with low prevalence of disease, or to confirm prior infection by HCV in a patient who is HCV RNA negative (IIIB, E).

Improved inter-method agreement and precision are needed for HCV RNA tests; methods should use a standard such as that developed by the World Health Organization (IIB)

Specimens for HCV RNA should either be collected as EDTA or citrated plasma, or be promptly centrifuged to prevent falsely low results (IIB).

There are six major genotypes and >90 subtypes of HCV that vary in their world-wide distribution. In addition, HCV has a high rate of spontaneous mutation, producing discrete “quasispecies” that vary from one individual to the next. (162) Genotypes 1a and 1b account for about 2/3 of infections in the United States; genotype 1 represents 90-95% of infections in African-Americans compared to about 60% in white patients. (163). Genomic amplification and sequencing, followed by sequence comparison and phylogenetic tree construction is the reference method for genotype determination (164). A variety of genotype screening assays have been described, including PCR using genotype-specific primers (165), restriction fragment length polymorphism of amplified sequences (166), and a commercially available line probe assay. (167) These methods compare favorably with the reference method for determining HCV genotype. (168)

Hepatitis D virus - HDV is a defective RNA virus that replicates only in the presence of HBsAg. Testing for evidence of HDV infection should be considered in HBsAg-positive patients with symptoms of acute or chronic hepatitis, particularly in those with fulminant hepatitis or where there is a high risk for HDV infection. The only HDV serological test widely available commercially detects total anti-HDV. In patients in whom virus is cleared, antibody typically disappears between 1 and 5 years. (169) In most clinical situations, HBsAg, IgM anti-HBc, and total anti-HDV are adequate to diagnose HDV infection. Patients with acute HDV co-infection are usually positive for IgM anti-HBc, while patients with HDV superinfection are usually negative for IgM anti-HBc.

Hepatitis E virus - HEV is an enterically transmitted RNA virus that causes sporadic and epidemic acute hepatitis in developing countries; it does not cause chronic hepatitis. In the United States, HEV infection has been seen rarely as a cause of hepatitis, predominantly among those who have traveled to endemic areas, although at least one case has occurred without history of travel. (170). Immunoassays for anti-HEV have been developed for diagnostic use (171). An evaluation of multiple anti-HEV methods showed significant variation in titers reported, and discordance between methods, although tests detecting antibodies to ORF2 were most accurate. (172) Antibody reactive with HEV antigens was found in 15-25% of homosexual men, intravenous drug users, and blood donors in Baltimore, suggesting lack of specificity of assays. (173)