Testing for Viral Hepatitis

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Advances made within the past decade in the delineation of the structural components of the hepatitis B virus (HBV), including the hepatitis B surface antigen (HBsAg) and the hepatitis B core antigen (HBc), have resulted in the recent commercial availability of new methods of serologic testing. These tests, which include the core antigen antibody (anti-HBc) and the surface antibody (anti-HBs), facilitate the diagnosis and management of this illness by primary care physicians. In addition, a serologic test (anti-HAV) is available to diagnose or exclude hepatitis A, and research efforts are progressing in an attempt to identify a specific marker for non-A non-B hepatitis.

Viral hepatitis was first recognized as a distinct clinical entity in the United States and Europe in the 19th century. At that time, the literature began to note scattered outbreaks of a syndrome that was identified by several names such as catarrhal, infectious, and epidemic jaundice. In studies using human volunteers conducted during World War II, two distinct types of viral hepatitis (subsequently designated A and B) were noted. The existence of two etiologic agents was suggested by the lack of cross immunity between the two forms of viral hepatitis.

In 1964 Blumberg reported the existence of the hepatitis associated (or Australia) antigen now called hepatitis B surface antigen (HBsAg), in the sera of patients with hepatitis B.[1] In 1973 Feinstone reported the association of a specific virus-like particle with hepatitis A infection.[2] In the same year a second antigen, the hepatitis B core antigen (HBc), was reported.[3] In 1975 non-A non-B hepatitis (also called hepatitis C) was described.[4]

Structural Components

Hepatitis B surface antigen (HBsAg) refers to the small spherical and long filamentous forms, with an average diameter of 22 nm, found in the sera of patients with hepatitis B. It is also found on the surface of the larger Dane particle. The HBsAg does not appear to contain nucleic acid and thus probably represents an incomplete or defective virus or virus coat particle. Although the presence of HBsAg in serologic testing represents infectivity to the clinician, these viral coat particles are probably not infectious. HBsAg has also been demonstrated in oropharyngeal secretions, seminal fluid, urine, and stool. HBsAg, in its various forms, is seen in Figure 1.

Hepatitis B core antigen (HBc), antigenically different from HBsAg, has been noted in very small amounts in the blood of most patients with acute hepatitis B and in some carriers.[5] But, because it is present in such minute amounts, testing for this antigen is not a reasonable alternative. HBc is represented on electron microscopy as the 28 nm diameter electron dense core of the Dane particle. It is released by detergent treatment of the Dane particle. Of more clinical significance is the antibody to hepatitis B core antigen (anti-HBc) which will be discussed shortly.

The Dane particle is considered to be the leading candidate for the complete hepatitis B virion (a virion is a virus as a structural entity) among the

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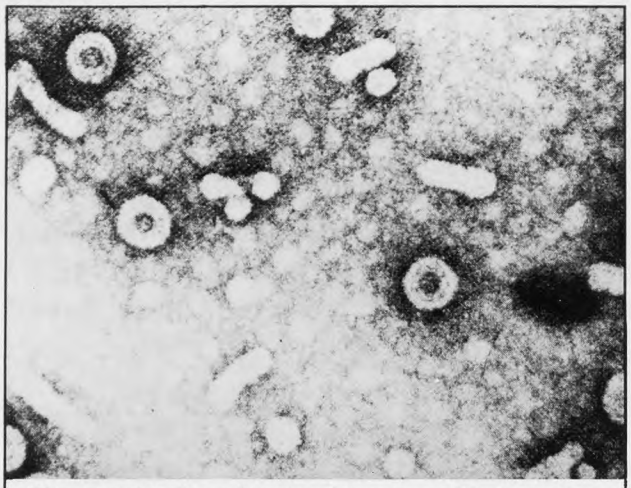

Figure 1. Various forms of the hepatitis B surface antigen (HBsAg). Photograph used with the permission of Abbott Diagnostics

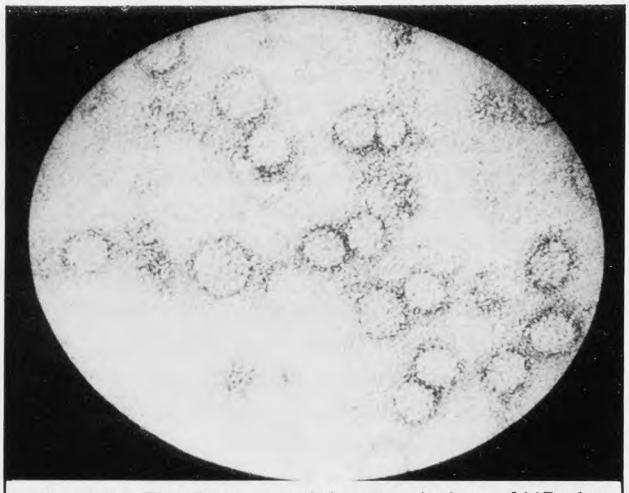

Figure 2. The Dane particle, consisting of HBsAg on its surface and HBcAg in its core. Photograph used with the permission of Abbott Diagnostics

recognized particles in infectious plasma. The Dane particle, with a 42 nm diameter, consists of HBsAg on the surface and HBcAg in its core. The core also contains a DNA polymerase and a small (smaller than any other animal virus) double stranded, circular DNA. It has been speculated that overproduction of the surface component of the Dane particle is responsible for HBsAg present in the sera of patients with hepatitis B virus (HBV) infection. It should be noted that the Dane particle has not yet been conclusively shown to be directly infectious. The Dane particle is seen in Figure 2. The e antigen (HBeAg), first detected in 1971, is a nonparticulate component of the viral core. It is a soluble protein detectable frequently in serum containing HBsAg. The e antigen, together with its antibody (anti-e), may be a potential marker of infectivity.[6]

Hepatitis A infection is associated with a 27 nm diameter virus-like particle as reported by Feinstone in 1973.[2] The antibody to this antigen is, at present, the only practical method for diagnosing hepatitis A virus (HAV) infection.

Clinical Aspects

Hepatitis B, formerly known as serum or long incubation hepatitis, has a mortality rate that varies from one to ten percent in various series. The onset is usually insidious with an incubation period that ranges from 6 to 26 weeks. Nonspecific symptoms such as anorexia, fatigue, and abdominal discomfort follow. These nonspecific symptoms may mimic those of other viral infections of the respiratory or gastrointestinal tract. There may be also a striking distaste for cigarettes. One to two weeks later, the usual symptoms and signs of

hepatitis are noted, ie: jaundice, light colored stools, dark foamy urine, and an enlarged tender liver. Joint pains or acute migratory arthritis along with urticarial or erythematous maculopapular rashes can be present in HBV infection. Serum transaminases (glutamic-oxaloacetic and glutamic-pyruvic) are maximally elevated early in the icteric phase and are usually in the range of 100 to 1000 IU. During the icteric phase itself, the levels start to fall toward normal.

There is approximately a ten percent carrier rate and a ten percent incidence of chronic hepatitis following HBV infection. The carrier state is defined as a persistently positive HBsAg serologic test with no symptomatic, biochemical, or histologic evidence of disease. The ten percent incidence of chronic hepatitis is broken down into approximately a seven percent incidence of chronic persistent hepatitis and a three percent incidence of chronic active (formerly called aggressive) hepatitis.[7] The latter is further differentiated into HBsAg negative (also called lupoid hepatitis) and HBsAg positive, which differ in many epidemiologic and clinical aspects. Chronic hepatitis is defined as a chronic inflammatory reaction of the liver, continuing without improvement for at least six months. The diagnosis is based on clinical and biochemical criteria plus the definitive microscopic changes seen in needle liver biopsy. Chronic hepatitis following HBV infection is more frequent early in life or when the immunologic reaction to infection is modified by disease (ie, in patients with chronic renal failure or following a renal transplant).

Hepatitis B was formerly known as serum hepatitis because it was believed it could be transmitted only by the parenteral route. However, as noted by the presence of HBsAg in various body secretions, it can be transmitted by a variety of non-parenteral routes.

Hepatitis A, formerly known as infectious or short incubation hepatitis, has a mortality rate less than that of hepatitis B, approximately 0.5 percent. The onset is acute following an incubation period of two to six weeks. As compared to hepatitis B, the disease is usually milder with lower bilirubin levels and shorter duration. Symptomatology is as described for hepatitis B except that joint pain and rashes are uncommon. There is no carrier and probably no chronic state associated with hepatitis A infection. Thus, transmission of disease depends solely on exposure to the virus from an acutely infected subject. Although the fecal-oral route is the prime route of transmission, it can be transmitted by parenteral routes. Unlike hepatitis B, it does not spread via salivary droplets. It occurs most frequently in children and young adults. The endemic and epidemic occurrence is determined to a large extent by environmental and socioeconomic factors.[8] Thus, poor sanitation, overcrowding, and exposure of a non-immune population will contribute to an epidemic situation with a high frequency of infection in the young. A comparison of the clinical aspects of the two types of viral hepatitis is summarized in Table 1.

It should be noted that the true incidence of viral hepatitis (both A and B) is actually much higher than what is reported (approximately 15,000, 30,000, and 8,000 cases of HBV, HAV, and non-A non-B, respectively, per year)[9] because many cases are mild or subclinical, a number of cases present as nonspecific infections, and physicians often fail to report the disease even when it is detected. A third form of viral hepatitis, designated non-A non-B, or hepatitis C, is diagnosed by excluding the other two types of hepatitis. The incubation period has not been conclusively identified but is believed to be in between that of hepatitis A and B. It can be transmitted by both parenteral and non-parenteral routes. In one study it has been reported to account for 90 percent of the present cases of post-transfusion hepatitis.[10]

Serologic Testing

Since the discovery of hepatitis associated antigen (HAA) in 1964, testing for this antigen, now referred to as hepatitis surface antigen (HBsAg), has passed through several "generations" of testing with each successive generation of tests being more sensitive. At the present time the Bureau of Biologics, a division of the Food and Drug Administration, requires that only third generation tests be used for detection of HBsAg in blood banking. However, second generation tests are still used by some laboratories to screen for

Table 1. Clinical Aspects of Viral Hepatitis

	Type A	Type B
Mortality	~.5%	1-10%
Incubation Period	2-6 weeks	6-26 weeks
Onset	Acute	Insidious
Sequelae Carrier	0	10%
Chronic	0 (?)	10%
Mode of Transmission		
Oral (fecal)	Usual	Infrequent (not fecal)
Parenteral	Infrequent	Usual (intimate contact)
Age Group	Mainly children and young adults	All Ages

carriers in patients or employees. Approximate sensitivities of second generation tests, which include counterimmunoelectrophoresis and reverse passive hemagglutination, are in the range of 1.0 μg/ml of HBsAg.[11] Commercially available third generation tests for HBsAg are of three main types. The radioimmunoassay and enzyme immunoassay methods are of the direct noncompetitive type employing the "sandwich" principle (ie, antibody-antigen-antibody). The third type of third generation test employs latex agglutination. Approximate sensitivities of third generation tests, in general, are in the order of 2.5 ng/ml of HBsAg.[12]

Besides screening of blood donors and testing in patients suspected of having viral hepatitis, other indications for HBsAg testing include helping to determine possible progression from acute to chronic hepatitis, testing recipients prior to transfusion, and screening of certain groups who are at high risk both for accidental exposure to HBV and transmission of disease. This includes operating room personnel, patients, and staff in renal dialysis units, laboratory technicians, personnel of blood bank and blood donor stations, and primate animal handlers.

The time sequence of HBsAg positivity in acute HBV in relation to the clinical course is noted in Figure 3. As can be seen, it is found most frequently during the late incubation and early acute illness phases lasting an average of three to six weeks. However, in some patients, it may be present for only a few days. Thus, if a patient has a negative test for HBsAg but is strongly suspect of having hepatitis B, not only should it be repeated in one to two weeks but other serologic testing should be considered. In addition, even if the patient improves clinically, HBsAg should be tested for approximately two to three months after the acute illness to detect a possible carrier or chronic hepatitis state. It should also be noted that there is a high incidence of HBsAg positivity in patients with Down's syndrome, leukemia, leprosy, and hepatomas. Although the exact mechanism is not known, it is believed to be due to a lowered immunity and an increased incidence of infections with hepatitis B virus. There are several distinct immunologic subtypes of the hepatitis B surface antigen. Although tests for these subtypes are not commercially available, they may be of importance, not in predicting severity or chronicity of disease, but in tracing epidemics and comparing several types of HBV infections in various geographical locations.[13]

The fact that even the third generation tests noted above may not be sensitive enough was shown by one study where, using a known infectious plasma pool, 1 ml of a 10^{-7} dilution was infectious in volunteers; yet this pool was negative on the radioimmunoassay third generation test.[14] This may account for the fact that, in several studies of healthy volunteer blood donors negative for HBsAg by third generation testing, one to three percent were positive for core antigen antibody (anti-HBc).[15] It should be noted that because the core antigen is present in such minute

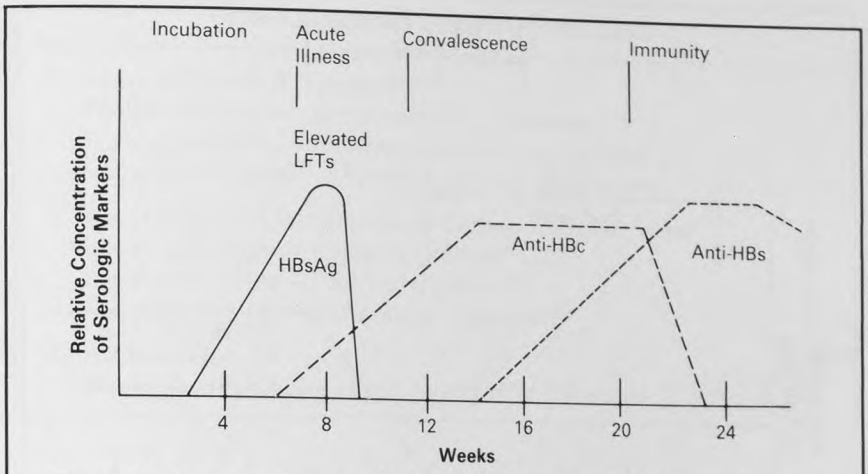

Figure 3. Typical sequence of HBV markers in acute HBV infection with resolution. With the permission of Abbott Diagnostics

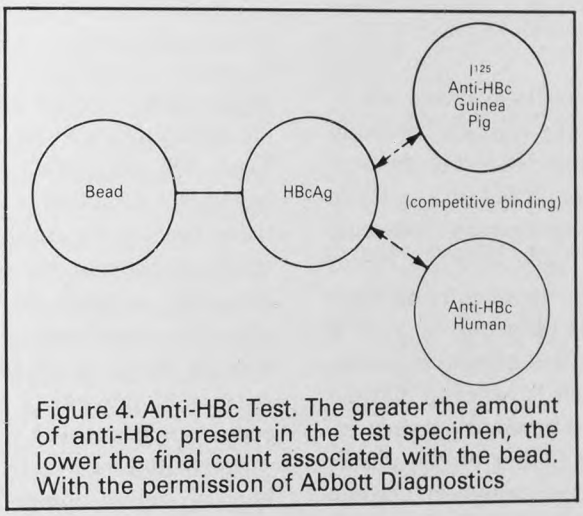

Figure 4. Anti-HBc Test. The greater the amount of anti-HBc present in the test specimen, the lower the final count associated with the bead. With the permission of Abbott Diagnostics

amounts only the antibody to this antigen can be used in serologic testing for hepatitis B. The test for anti-HBc is commercially available and the mechanism is based on competitive binding as depicted in Figure 4. The usual time sequence of anti-HBc in relation to the clinical course is depicted in Figure 3. As can be seen, it is usually detected two to three months after exposure, usually at, or close to, the time when clinical symptoms and abnormal liver enzymes are present. Thus, it may be a useful adjunct in the situation described above when, despite a negative HBsAg, the clinician strongly suspects hepatitis B. Although not presently used for this purpose, the possible role of anti-HBc in screening blood donors is suggested by the fact that, in addition to being present in one to three percent of HBsAg negative volunteer blood donors, it has been shown that, when the dilutional titer is equal to or greater than 1:100, hepatitis B is transmitted.[16] In addition, in carriers of hepatitis B (ie, HBsAg positive), anti-HBc is positive almost 100 percent of

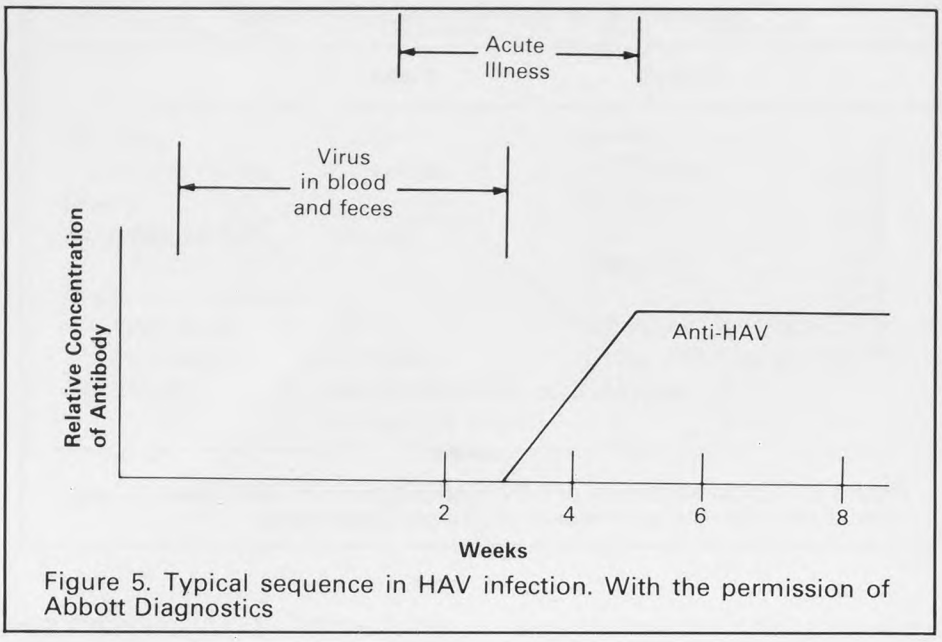

Figure 5. Typical sequence in HAV infection. With the permission of Abbott Diagnostics

the time. The third commercially available test of HBV to be discussed is the surface antibody (anti-HBs). The mechanism of this test is identical to that of anti-HBc. As noted in Figure 3, a rising titer of anti-HBs within the two months following an acute hepatitis episode is indicative of a recent HBV infection. Thus, paired acute and convalescent phase sera for anti-HBs titers may serve as a second possible test in the HBsAg negative patient with suspected hepatitis B. It is believed that the rise in anti-HBs titer confers immunity against reinfection by HBV and that failure of its appearance is indicative of either a carrier or chronic state. The detection of anti-HBs in an asymptomatic patient indicates that the patient previously had HBV infection and is probably immune to it.

The e antigen and its associated antibody were initially reported to be predictive of chronicity and resolution, respectively, but subsequent studies have suggested that this applies only to large groups of patients and not to individuals.[17] Testing for these HBV markers is supposed to become commercially available within the next year, although its use in individual patients needs to be definitely established by further studies.

With regard to serologic testing for hepatitis A, only one test, the antibody to the hepatitis A virus (anti-HAV), is presently available. This is because at the time the patients are first seen most no longer have viremia and have stopped excreting the hepatitis A virus in their feces (Figure 5). Thus, the only practical method for diagnosing hepatitis A infection is with the demonstration of a rising titer of the antibody. So it is important to start sampling at the earliest clinical suspicion. However, a single positive reading, particularly after the acute clinical state, does not establish that the recent acute episode was due to hepatitis A since 25 percent or more of the United States population is anti-HAV positive.[8] The diagnosis of various disease state manifestations of viral hepatitis using commercially available serologic tests is summarized in Table 2.

Future Directions

Recently, investigators in France have claimed to identify a specific serologic marker for non-A non-B hepatitis. If this test eventually becomes commercially available, it may prove to play a very important role in screening donor blood. In addition, it may help to identify those cases of viral hepatitis that were negative by current serologic tests for hepatitis A and B. The future

Table 2. Diagnosis Using Serologic Testing

1. Acute HBV with Resolution
   Positive HBsAg---->Negative HBsAg 3-4 weeks
   Postive anti-HBc---->Negative anti-HBc 4-6 months
   Negative anti-HBs---->Positive anti-HBs 4-5 months
2. Acute HBV with Progression to Carrier or Chronic state
   Positive HBsAg---->Remains positive
   Positive anti-HBc---->Remains positive
   Negative anti-HBs---->Remains negative
3. Acute HAV
   Negative anti-HAV---->Positive anti-HAV 2-3 weeks

commercial availability of testing for the e antigen-antibody system may prove to be of benefit to the clinician if subsequent studies can establish its relevance in individual hepatitis patients. In addition, it remains to be established whether HBsAg negative donor blood should be screened for anti-HBc. This may possibly be determined by noting what percentage of the above subgroup of donors have anti-HBc titers in excess of what has been shown to be infectious in recipients.

The status of anti-HBs in both donor and recipient blood, in terms of a protective effect, needs to be established. Although anti-HBs comprises the hyperimmune globulin used in accidental exposure to the HBV, one study showed that recipients of donor blood with anti-HBs titers equal to or greater than 1:128 developed post-transfusion hepatitis more often (67 percent vs 40 percent) than did those recipients receiving blood with absent or only low antibody titers.[18] Also, since there have been no studies reported in the literature comparing the relative sensitivity and specificity of the three types of third generation HBsAg tests now commercially available, this would appear to be another important avenue of investigation.

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