Do We Have to Rethink Serologic Markers Used to Diagnose Hepatitis B Infection?

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ABSTRACT

The recent addition of testing volunteer blood donors for antibody to hepatitis B core antigen (anti-HBc) has raised questions not apparent when such testing is applied to hepatitis B (HB) infected patients. The review of published studies, and our own, demonstrates that it is difficult to evaluate false positive results. Some patients appear to lose antibody to hepatitis B surface antigen (anti-HBs) but not antibody to hepatitis B core antigen (anti-HBc), thus raising the possibility of disease transmission. It is anticipated that additional investigation of individuals positive for anti-HBc only by response to hepatitis B vaccine permits clarification of their immune status and absence of infectivity.

The virus responsible for hepatitis B (HBV) infection is probably the most extensively studied pathogenic human virus. Laboratory test procedures have been developed for the antigenic viral components and their respective antibodies. Some of these are the hepatitis surface antigen (HBsAg) or envelope lipoprotein, and the antibody to the surface antigen (anti-HBs). The hepatitis B core antigen can only be demonstrated in liver tissue,7 but the antibody to the hepatitis core antigen (anti-HBc) is present in serum. Patients with HBV infection have been followed with these test procedures to document progress and/or resolution of their infection.

After HBV infection, laboratory testing first demonstrates the presence of HBsAg in the patient’s serum.4 This is followed by anti-HBc and, finally, anti-HBs. The presence of both anti-HBc and anti-HBs is considered as an immune, non-infectious state. Persistence of HBsAg denotes infectivity even if anti-HBc and anti-HBs can be demonstrated. If only anti-HBc is present without anti-HBs during a short window period following the presence of HBsAg, the patient is considered infectious.

In 1972, blood banks began testing donors for HBsAg to prevent HBV transmission by transfusion. This is a departure from the use of the test for the diagnosis, as it had been used up to that time. Anti-HBc testing was added in 1987. It is believed to be a surrogate test for non-A, non-B hepatitis for which there is at present no test available.

Hepatitis B infection is associated with two distinct clinical patterns. One is characterized by jaundice, malaise, and
anorexia, which is probably seen in the smallest group of patients following HBV infection. The largest group of HBV infected patients has no symptoms to alert the physician or patient that there is an infection with the HBV. This clinically silent infection is the greatest obstacle to the prevention of HB since it assists in spreading the infection to close contacts of the infected individual. Complete recovery is the usual outcome of both types of infection. If chronic hepatitis ensues, after either clinical course, cirrhosis and sometimes carcinoma of the liver are the serious late complications.

Transmission of the HBV occurs to the fetus during pregnancy, by close personal contact, sexual activity, blood transfusion, or parenteral transmission of body fluids through needle sticks.

In areas of low HBV prevalence, the introduction in 1972 of HBsAg testing was difficult to evaluate as to the impact on the few cases of post-transfusion hepatitis. Constant testing of blood donors has kept the HBV infection surveillance under observation, raising unresolved questions. The Central Kentucky Blood Center (CKBC) recently documented a curious decline in the prevalence of donors positive for HBsAg by 13 percent with a male prevalence. At the same time, a decline in reported HBV infection is reported by the Centers for Disease Control by 12 percent. This pattern suggests that introduction of additional questioning, not testing, influences safety measures for the blood supply.

Following the introduction of anti-HBc testing on volunteer donors, it came as a surprise that 2,028 blood donors or 2.09 percent of CKBC blood donors and no apheresis donors had a reactive test for anti-HBc. All these donors were negative for HBsAg. They also had a normal alanine aminotransferase (ALT) level, the enzyme which is usually elevated in the presence of liver cell damage. Similar results were obtained by the American Red Cross study recently published which showed an anti-HBc reactive rate of 2.54 percent among their many donor centers.

Because the prevalence at CKBC was unexpected, a pilot study of 200 anti-HBc positive donor samples was undertaken, resulting in 197 valid testing procedures. The donors selected had frequently donated at CKBC and had never been implicated in HBV transmission. The samples were tested by another licensed blood collecting facility for anti-HBc by radioimmunoassay (RIA), AB-COREK,* and enzyme-linked immunosorbent assays (EIA) CORZYME.* In addition, all samples were tested for anti-HBs by EIA AUSAB.* The additional testing for anti-HBs was chosen to search for serologic markers confirming exposure to the HBV. It was found that 71.07 percent of the donors were reactive by all three test procedures. This finding suggests that all these donors had recovered from their silent HBV infection and must be considered immune. The discrepancies noted on 57 samples are shown in table I.

It would be hazardous to designate all anti-HBc positive donors with a negative anti-HBs test as falsely positive. Some of these donors could be infectious and later convert to the antibody to the surface antigen. The five percent with nega-

* Abbott Laboratories.

| Anti HBs | Anti HBc EIA | RIA | neg | 13 22.8% Anti HBs | Anti HBc EIA | RIA | neg | 3 5.26% |
|---------|--------------|-----|-----|-----|-------------------|--------------|-----|-----|-------|
| Anti HBc EIA | RIA | pos | | Anti HBc EIA | RIA | neg | |

Precisely 26 (45.61 percent) were in the gray zone for either enzyme-linked immunosorbent assays (EIA) or radioimmunoassay (RIA) testing and negative for anti HBs.
tive anti-HBc by both procedures are most likely in the falsely reactive category. It is most difficult to judge the test results if only one of the test procedures is positive for anti-HBc. It can not be concluded that the EIA procedure is more sensitive and specific than the RIA procedure nor that the RIA procedure is less sensitive and specific.

Because all abnormal test results are reported to the donor, a flood of inquiries usually ensues. The heightened public awareness on the impact of hepatitis infection following recent intense media campaigns is most likely responsible. However, it was not simple to answer the many questions concerning immunity, lack of infectivity, or mode of infection, considering published data and personal experience from HBV testing to date.

Interpreting the presence of both anti-HBs and anti-HBc as immune is justified, but anti-HBs testing is not part of donor examination. Such testing would have to be carried out through the donor’s personal physician. As the next step in search of a possible answer, all positive anti-HBc donor records were reviewed. Thirty donors were found with documented donation records from two days to 16 weeks prior to the positive HBc test result. All previous donations had been found negative for HBsAg and anti-HBc results to an HBV infection occurring between the two tested donations. The HBsAg usually persists for about two months and should easily have been detected at the first documented donation. Since most donors have a longer interval between donations, history of the last donation, in general, can not be used to clarify whether or not the patient has acquired an infection since the last donation.

Because the pilot study demonstrated 71.07 percent of donors having a positive anti-HBc and anti-HBs test confirming prior HBV infection resulting in immunity, it would be expected that 587 donors (table II), a considerable number, could possibly be contagious or represent donors with false positive anti-HBc reactions. There is little help available to clarify this dilemma. A small number of patients have been studied by Zito, et al., concerning loss of the anti-HBs with retention of anti-HBc. Conceivably, this pattern is not associated with reapparance of infectivity as seen in the window period following acute infection. Their study demonstrates that following well documented hepatitis B infection, 19 percent of patients have lost anti-HBs reactivity while retaining anti-HBc activity.

Therefore, if it can be demonstrated that the anti-HBc reactivity is falsely positive and does not reflect exposure to HBV infection, no further action needs to be taken. However, if the reactivity of anti-HBc indicates that the subject can transmit HBV to close contacts, additional action is required, such as vaccination. Only two studies have tried to identify patients further with isolated anti-HBc by documenting the immune response following the hepatitis B vaccination. The study groups are too small, but an anamnestic response was seen in some of the subjects following vaccination, as well as a primary response. This approach to the dilemma deserves continued investigation.

Because both vaccination studies and serial serologic procedures are costly, additional expenditures for healthy blood donors for the evaluation of abnormal test results may have to be justified.

**TABLE II**

<table>
<thead>
<tr>
<th>1987 - 1988 Fiscal Year Central Kentucky Blood Center</th>
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<tr>
<td>Blood collections 97,115</td>
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<tr>
<td>Apheresis products 4,900</td>
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<td>Problem donors 587</td>
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*Derived by applying the sample study with 71.07 percent of the donors positive for anti-HBs and anti-HBc.
If it is demonstrated which healthy blood donors would spread HBV infection, additional measures must be taken to protect their close contacts.

Summary

Testing healthy individuals without history of exposure to hepatitis may yield unexpected results. For those demonstrating the immune pattern of positive anti-HBs and anti-HBc tests, no further action is required. A positive anti-HBc and negative anti-HBs test result may denote infectivity or be the result of a false positive anti-HBc. The expensive step of vaccinating close contacts may be indicated since, unfortunately, falsely positive anti-HBc reactions do occur but can not be resolved with the present testing available. It appears worthwhile to study the anti-HBc-only individual’s response to hepatitis B vaccination if a clear-cut immune response pattern will evolve in those individuals who have lost the antibody to HBsAg.

References


