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Epidemiological Analysis of the Significance of Low-Positive Test Results for Antibody to Hepatitis B Surface and Core Antigens

STEPHEN C. HADLER,* BERT L. MURPHY, CHARLES A. SCHABLE, WILLIAM L. HEYWARD, DONALD P. FRANCIS, AND MARK A. KANE

Hepatitis Branch, Viral Disease Division and Arctic Investigations Laboratory, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Georgia 30333

Received 18 August 1983/Accepted 11 January 1984

To determine the significance of certain serological test results commonly encountered in hepatitis B virus testing, we reviewed serological test data from nine studies of hepatitis B conducted between 1980 and 1982. Three tests, for hepatitis B surface antigen and for antibodies to hepatitis B surface antigen and hepatitis B core antigen (anti-HBs and anti-HBc), were used to measure hepatitis B virus infection risk in various populations. Two results, low levels of anti-HBs alone and low levels of anti-HBc alone, occurred at constant frequencies (2.72 and 0.4%, respectively), regardless of the prevalence of HBV infection in the population. Positivity for low levels of anti-HBs alone persisted for 1 year in less than one-half of those studied; in addition, response to hepatitis B virus vaccine was augmented in only one-third of this group. Positivity for low levels of anti-HBc alone did not persist in any of 11 persons studied. These findings indicate that presently available tests for anti-HBs and anti-HBc at low levels are often nonspecific and should be interpreted with caution.

The availability of sensitive tests for hepatitis B virus (HBV) infection has led to widespread use of these tests for various purposes, including clinical diagnosis, screening of populations to determine HBV infection risk, and screening of persons selected to receive the new HBV vaccine. The interpretation of serological testing has generally been straightforward in prospective studies of natural HBV infection (5, 6). When the three tests, for hepatitis B surface antigen (HBsAg), antibody to HBsAg (anti-HBs), and antibody to hepatitis B core antigen (anti-HBc), are used simultaneously to monitor infection, two markers of infection (anti-HBc with either HBsAg or anti-HBs) are usually present at a given time. The major exceptions occur during early incubation, when HBsAg alone may be present, and during the window phase, when anti-HBc alone is present.

Certain serological results, however, have remained difficult to interpret. Positivity for anti-HBs without anti-HBc is frequently observed in prevalence studies, yet is rarely, if ever, observed in prospectively monitored patients unless HBV vaccine has been given (5, 6; P. Kerlin, M. Aschavai, A. Redeker, R. Peters, and M. Jones, Gastroenterology 77:A22, 1979). This pattern of test results has been hypothesized to be a primary immunization-like response to HBV exposure or to be anti-HBs persisting after anti-HBc has disappeared after natural infection (2, 5). Two studies, however, have shown no relation between the prevalence of anti-HBs alone and either age or risk of exposure to HBV, suggesting that neither hypothesis can explain this result (2, 9). Another study has shown that positive anti-HBs results in the low range (2.1 to 9.9 sample ratio units [SRU] by radioimmunoassay) may be nonspecific, particularly when the specimen is also negative for anti-HBc (8).

To determine the significance of certain serological test patterns frequently encountered in HBV infection screening programs, we reviewed results from several studies completed at our laboratory over the last 3 years. These data suggest that positive results in the low range for anti-HBs and anti-HBc are often nonspecific and are not indicative of past HBV infection.

MATERIALS AND METHODS

Data were compiled from serological testing done during nine different studies by the Division of Hepatitis and Viral Enteritis and the Arctic Investigations Laboratory of the Centers for Disease Control. Sera were collected between 1980 to 1982 from persons participating in programs for HBV vaccination (male homosexuals [3] and Alaskan Eskimos) or programs to measure the prevalence of previous HBV infection in different population groups (oral surgeons, dental hygienists, prison inmates, workers in small hospitals, heterosexuals attending a venereal disease clinic, and college students). Each group consisted of from 400 to 4,000 persons and was believed to be representative of the population studied. All individuals in every group except one were tested for HBsAg, anti-HBc, and anti-HBs; workers in the small hospitals were tested only for anti-HBs and anti-HBc.

In two studies, prospective follow-up was completed on certain persons to determine the persistence of various HBV markers. In the study of Alaskan Eskimos, persons positive only for anti-HBs or anti-HBc had follow-up testing 12, 18, or 12 and 18 months later for all three HBV markers. In the other study, male homosexuals who participated in a double-blind trial of HBV vaccine and who were initially positive only for anti-HBs were monitored for 1 year. These participants had been screened only for anti-HBc and HBsAg before study participation. Participants who originally received a placebo were retested to determine the persistence of low level of anti-HBs alone. Participants who received vaccine were monitored to determine whether they had a normal or an augmented response to the HBV vaccine. In each group, serological testing was done at 1, 2, 4, 6, 8, and 12 months after the initial test specimen was obtained.

All sera were tested by radioimmunoassay for HBsAg (AUSRIA II; Abbott Laboratories) anti-HBs (AUSAB; Abbott), and anti-HBc (CORAB; Abbott). Specimens in which anti-HBs was below 5 SRU (derived by dividing the sample

* Corresponding author.

counts per minute by the mean of negative controls, according to the manufacturer's instructions) on initial test were tested again and were only considered positive when the repeat test was also positive. All specimens having anti-HBs levels of between 2.1 and 9.9 SRU were defined as low anti-HBs positive (manufacturer's positive, ≥2.1 SRU). All specimens tested by CORAB which had blocking between 50 to 69% (calculated as described in the test kit) were considered low anti-HBc positive (manufacturer's positive, ≥50% blocking).

RESULTS

Hepatitis B prevalence testing. In the nine groups studied, 9,390 persons were tested for markers of HBV infection. Of these, 2,993 (31.9%) had at least one positive test for HBV infection, including 475 (5.1%) with HBsAg (429 also had anti-HBc) and 1,955 (20.8%) with both anti-HBs and anti-HBc. A total of 213 persons (2.3%) were positive only for anti-HBc, and 350 (3.7%) were positive only for anti-HBs. Among those with anti-HBc alone, 179 had high levels (>70% blocking) and 34 had low levels (50 to 69% blocking), whereas among those with anti-HBs alone, 139 had high levels (>10 SRU) and 211 had low levels (2.1 to 9.9 SRU).

To examine the significance of the four types of results (high levels of anti-HBc alone, high levels of anti-HBs alone, low levels of anti-HBs alone, and low levels of anti-HBc alone), we compared the frequencies of these results in groups having different risks of presumed HBV infection, as defined by positivity for two different markers of HBV infection. If these results were indicative of previous HBV infection, their frequency would be expected to increase with increasing prevalence of HBV infection of the group. If, on the other hand, these results were nonspecific, their frequency in persons without HBV infection should be constant, regardless of the HBV prevalence of the group. To determine which of these was the case, we divided the nine populations into 12 groups which varied in the prevalence of presumed HBV infection. Each study population was treated as a single group except the Alaskan Eskimos, who were divided into four groups of villages with various prevalences (8.2 to 55.2%) of presumed HBV infection. Groups were ordered by frequency of HBV infection, and the relative frequencies of the four types of results were compared among different groups (Table 1). The frequency of high anti-HBc correlated well with frequency of HBV infection in the group, suggesting this result to indeed be indicative of past HBV infection. In contrast, no correlation was found between the frequency of any other single marker and HBV prevalence. The frequencies of low levels of anti-HBs and low levels of anti-HBc tended to decrease with increasing prevalence of HBV infection in the groups, whereas the frequency of high levels of anti-HBs remained relatively constant.

Next, the frequency of the latter three results in persons without HBV infection in each group was examined (Fig. 1). Low levels of anti-HBs and low levels of anti-HBc were each found at a relatively constant frequency (3.1 and 0.5%, respectively), which was not related to the prevalence of HBV infection in the group. In contrast, high levels of anti-HBs occurred with a frequency increasing directly with increasing HBV prevalence ($r = 0.77$, $P < 0.01$). These findings indicate that neither low levels of anti-HBs alone nor low levels of anti-HBc alone are due to past HBV exposure and suggest that low levels of these antibodies reflect a constant nonspecific background positivity of these tests. The findings, however, do suggest that high levels of anti-HBs alone are related to past HBV exposure.

Reliability of low anti-HBc and low anti-HBs. The frequency with which low levels of anti-HBs and low levels of anti-HBc reliably indicated past HBV infection was also examined. For persons with low anti-HBs results, numbers with positive anti-HBc were compared with those without anti-HBc; for persons with low anti-HBc results, numbers with positive anti-HBs were compared with those without anti-HBs. This was done for the whole study population and for the four combined groups with various known HBV prevalences (Table 2).

A total of 410 persons were positive for low levels of anti-HBs; 199 of these persons also were positive for anti-HBc and 211 of them were negative for anti-HBc, indicating only 49% reliability of this test for indicating HBV infection. The prevalence of low levels of anti-HBs with positive anti-HBc increased directly with increasing prevalence of HBV infection, whereas the prevalence of low levels of anti-HBs alone was relatively constant. As a consequence of these trends,

TABLE 1. Frequency of positivity for anti-HBs alone and anti-HBc alone in various populations

Group (prevalence)	No. tested	HBV prevalence (%)[a]	% Positive for[b]:			
			High anti-HBc alone	Low anti-HBc alone	High anti-HBs alone	Low anti-HBs alone
Alaskan villagers (high)	919	55.2	3.3	0.11	1.5	1.1
Homosexual males	1,461	53.6	2.0	0.14	1.4	1.8
Prison inmates in N. Mex.	458	35.4	6.1	0.22	1.8	3.1
Alaskans (moderately high)	1,362	31.9	1.2	0.29	1.6	2.1
Prison inmates in Ark.	685	18.8	5.0	0.88	1.2	1.6
Oral surgeons	421	22.6	0.95	0	3.1	3.1
Alaskans (moderate)	624	13.8	1.4	0.69	1.9	2.9
Alaskans (low)	1,097	8.2	1.4	0.18	1.3	2.0
Heterosexuals	553	7.4	1.4	0.36	2.2	4.0
Dental hygienists	504	3.4	0.40	0.99	0.6	1.6
Workers in small hospitals	701	3.1	0.014[c]	0.57	0.7	2.7
College students	605	2.8	0.50	0.50	1.2	3.1

[a] These values are the percentages of subjects having two HBV markers.

[b] The average percentages positive for high levels of anti-HBc, low levels of anti-HBc, high levels of anti-HBs, and low levels of anti-HBs alone were 1.90, 0.36, 1.48, and 2.25, respectively.

[c] One subject with a positive result was not tested for HBsAg.

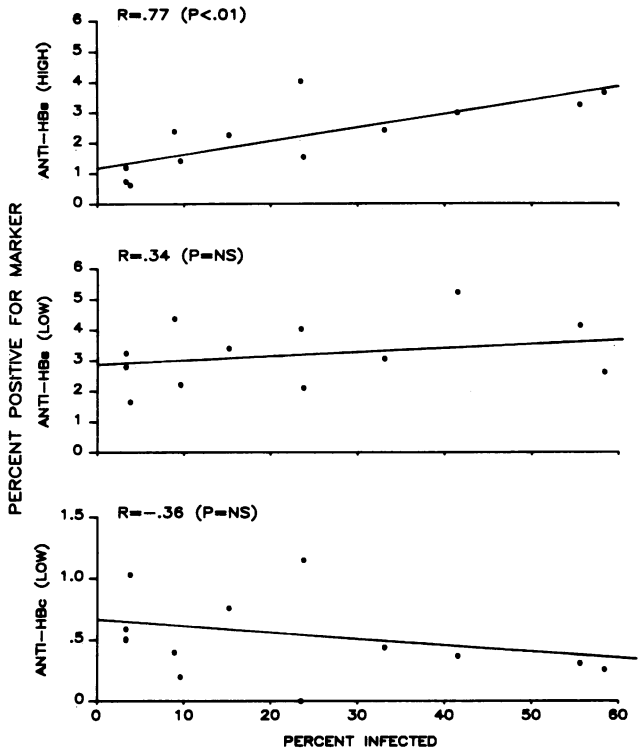

FIG. 1. Frequency of anti-HBs or anti-HBc alone among persons without known HBV infection versus prevalence of HBV infection in group. NS, Not significant.

the fraction of persons with low levels of anti-HBs who were positive for anti-HBc (true positives) decreased with decreasing HBV prevalence, from 71% in high HBV prevalence groups to 16% in low prevalence groups. Hence the reliability of this test result decreased with decreasing HBV prevalence of the group.

The prevalence of low levels of anti-HBc (110 positive, 1.2%) was only one-fourth that of low levels of anti-HBs but showed an identical pattern. The prevalence of low levels of anti-HBc with positive anti-HBs increased directly with increasing HBV prevalence, whereas the frequency of low levels of anti-HBc alone actually decreased. In high HBV prevalence groups, 94% of specimens with low levels of anti-HBc were positive for anti-HBs, whereas in low HBV prevalence groups, only 27% of such specimens were positive for anti-HBs. This also indicated a decreasing reliability of this test as HBV prevalence of the group decreased.

Longitudinal follow-up. To gain further insight into the significance of positive results for anti-HBs alone and for low levels of anti-HBc alone, we reviewed prospective follow-up testing obtained from the study of Alaskan Eskimos and the HBV vaccine trial in homosexual men (3). Follow-up at 12, 18, or 12 and 18 months was possible in 55 of 62 persons with high levels of anti-HBs from the former study. Eleven of these persons had been misclassified, owing to laboratory error: all had positive tests for anti-HBc on follow-up and the repeat test of the initial specimen was anti-HBc positive. Three persons with initial anti-HBs below 15 SRU became infected with HBV and developed high levels of both anti-HBs and anti-HBc. One person with initial high anti-HBs maintained anti-HBs at the same level but had repeatably weak positive anti-HBc 12 months later and reverted to anti-HBc negative at 18 months follow-up. Of the 40 others, antibody persisted at unchanged levels in 18, decreased in 17, and became negative in 5, all with initial levels of below 40 SRU. Thus, this group consisted of a proportion (20%) misclassified due to laboratory error and a majority with consistently positive but slowly decreasing anti-HBs, with those with the lowest initial levels (<15 SRU) often losing antibody and sometimes becoming infected with HBV.

Follow-up of 11 persons from the Alaska study with low levels of anti-HBc alone on initial testing revealed all to be negative for all HBV markers when tested 12 or 18 months later. Repeat testing of the initial specimens showed 10 of 11 to be negative, suggesting that the initial results were nonrepeatable false-positive results.

Follow-up from both studies of persons with low levels of anti-HBs showed this antibody to persist in only a fraction of those initially positive. (Table 3). Among 25 persons monitored in the study of homosexual men, 36% became negative for anti-HBs 1 month after the initial positive result, and 72 and 60% were negative at the 6- and 12-month follow-up tests, respectively. Among 62 such persons followed in Alaska, 5 (8%) became infected with HBV and developed either HBsAg or high levels of anti-HBs and anti-HBc; of the remainder, 52 and 49% were negative 12 and 18 months later, respectively.

Response to HBV vaccine in persons with low levels of anti-HBs. In 22 persons with low levels of anti-HBs alone who were given HBV vaccine, the antibody response to the vaccine was compared with that in persons who were

TABLE 2. Relative frequency of low levels of anti-HBs and low levels of anti-HBc in persons with and without other antibody markers

Prevalence group	n	HBV infection prevalence	Low anti-HBs positive[a]			Low anti-HBc positive[b]		
			No. with anti-HBc (%)	No. without anti-HBc (%)	Reliability[c]	No. with anti-HBc (%)	No. without anti-HBs (%)	Reliability[d]
High	2,380	54.2	92 (3.9)	37 (1.6)	71.3	50 (2.1)	3 (0.1)	94.3
Moderately high	1,820	32.8	62 (3.4)	42 (2.3)	59.6	10 (0.6)	5 (0.3)	66.7
Moderate	1,730	17.9	28 (1.6)	42 (2.4)	40.0	10 (0.6)	10 (0.6)	50.0
Low	3,460	5.4	17 (0.5)	90 (2.6)	15.9	6 (0.2)	16 (0.5)	27.3
Avg for all groups		25.4	(2.12)	(2.25)	48.5	(0.81)	(0.36)	69.1

[a] Positive for 2.1 to 9.9 SRU.
[b] Positive 50 to 69% inhibition.
[c] Percent of persons with low levels of anti-HBs who were anti-HBc positive.
[d] Percent of persons with low levels of anti-HBc who were anti-HBs positive.

TABLE 3. Persistence of low levels of anti-HBs over time in two studies

Mo after initial positive[a]	Alaskan Eskimos		Homosexual men	
	No. tested	No. (%) negative	No. tested	No. (%) negative
0	61	0 (0)	25	0 (0)
1			25	9 (36)
2			25	12 (48)
4			24	14 (56)
6			25	18 (72)
8			25	16 (64)
12	56[b]	29 (52)	21	13 (62)
18	53[b]	26 (49)		

[a] SRU values of 2.1 to 9.9 were considered positive.
[b] Number does not include five persons who developed HBsAg-positive or anti-HBc-positive infection.

seronegative when they received the vaccine (Fig. 2). There was no difference in the proportions of persons in each group who developed very high anti-HBs levels (>50 SRU) at any time during follow-up. However, a significantly higher proportion of the low-positive anti-HBs group developed intermediate anti-HBs levels (10 SRU < anti-HBs < 50 SRU) at all times during follow-up. This proportion ranged from 14% at 1 month after vaccination (versus <1% in seronegatives) to 48% at 2 months and 23% at 8 months after vaccination (versus only 2 to 5% among those without anti-HBs initially [$P < 0.01$]). Thus, in a proportion of individuals with low levels of anti-HBs alone, the antibody response to HBV vaccine suggested previous exposure to HBV, which allowed a somewhat more rapid and stronger antibody response than in persons without this marker.

DISCUSSION

This study attempted to put into perspective the interpretation of low-positive test results for anti-HBs and anti-HBc. Interest in this problem has been raised in several other studies and is due primarily to incomplete understanding of test results showing positive anti-HBs with negative anti-HBc in persons who have not received the HBV vaccine. Because this pattern has not been described in prospectively monitored natural HBV infection, it has been hypothesized to be a vaccine-like response to a low-dose HBV exposure. This remains controversial, however (2, 4, 7–9).

Investigation of suspect test results should be approached from both a laboratory and an epidemiological perspective. Laboratory analysis of test results may include repeat testing of the same specimen, specificity testing after absorption with specific reagents, and follow-up testing of specimens from the same individual over time. The epidemiological approach can include comparison of the frequency of the test results in populations at different risks of HBV infection, and, conversely, comparison of risk factors for HBV infection among persons with known HBV infection and those with positive results for a single antibody.

Both laboratory and epidemiological data from this and other studies provide a consistent explanation for each of the test results examined. Interestingly, each of these results appears to have a different specificity for previous HBV infection. Persons positive for high levels of anti-HBs alone generally can be considered to have previous HBV exposure based on the epidemiological data and the usual persistence of antibody over time. A proportion may be true infections (positive for both anti-HBs and anti-HBc), incorrectly classified, owing to errors in testing for anti-HBc. However, those persons with antibody levels at the low end of this spectrum may be expected to lose the antibody over time and possibly become infected with HBV. Unfortunately, the exact origin for this result, whether it is due to mild infection with failure to develop strong anti-HBc or to low-dose HBV exposure with vaccine-like response but without actual infection, still remains uncertain and requires further study.

In contrast, persons who test positive for low levels of anti-HBc alone should presently be considered not to have had prior HBV exposure. In our study, repeat testing of the same specimen was usually negative, as was follow-up testing for anti-HBc over time. This result, which was seen in constant proportions in all groups tested, appears to be due to nonspecificity of the test, which may be revealed by repeat testing of the same specimen.

Persons having serological tests positive for anti-HBs (low) alone fit between these extremes. In many such persons (30 to 50%), this antibody persists for 1 or more years, and in an equal proportion, there will be an augmented response to the HBV vaccine. Yet in the majority, the antibody does not persist and the response to the vaccine is identical to that of seronegative persons. These results are consistent with specificity testing that showed 60% nonspecificity of low levels of anti-HBs with negative anti-HBc (8). The high proportion of nonspecific results, combined with the occurrence of typical HBV infection in some persons with this test result, indicate that this result should be interpreted conservatively and as not indicative of previous HBV infection or exposure.

When all the above data are considered, the available

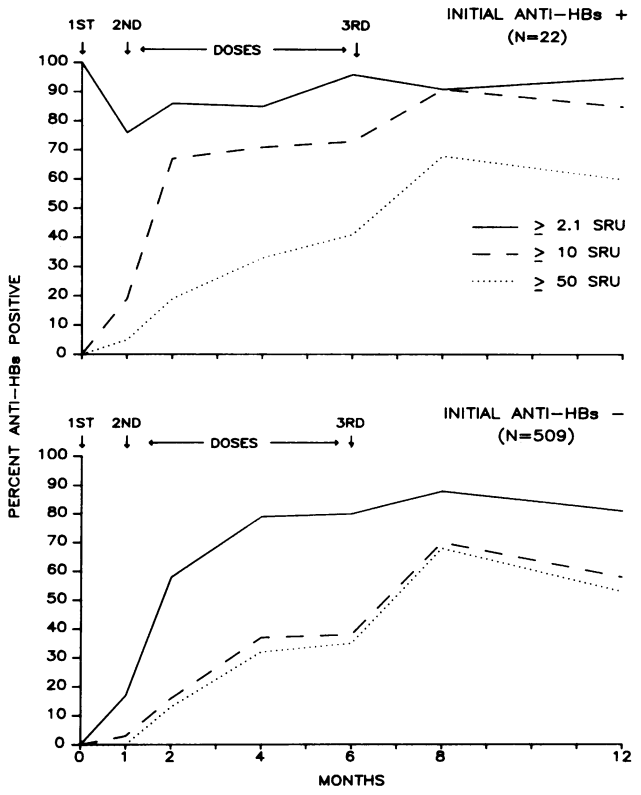

FIG. 2. Anti-HBs response to HBV vaccine in persons with or without low levels of anti-HBs at first dose.

radioimmunoassay tests for anti-HBs and anti-HBc appear to have poor reliability when positive at low levels. These data are not surprising, because the manufacturer's recommendations in the test kit for anti-HBs indicate that up to 80% of low anti-HBs results (between 2.1 to 5.0 SRU) may be negative on repeat testing of the same specimen. Our data suggest that, even when this test is repeatably positive in the 2.1 to 9.9 range, a high proportion of the results may be nonspecific. Although this study was completed by using highly sensitive tests from a single manufacturer over a 2-year period, it suggests that any low-positive tests for HBV markers should be viewed with skepticism until confirmed by repeat testing, specificity testing, or testing for other markers of HBV infection.

These findings have greatest importance when these serological tests are used for epidemiological studies of HBV infection or to determine susceptibility before HBV vaccination. In epidemiological studies, the importance of nonspecific test results varies with background HBV prevalence and is most significant in groups with low HBV infection risk. In low-risk groups, such as volunteer blood donors or health professionals just entering the field, up to 80% of persons with low-positive anti-HBs or anti-HBc test results will be negative for other markers and thus will be nonspecific positives. These persons with low-positive results may account for a high proportion of persons positive for any HBV marker, and cause both an overestimation of infection risk and misinterpretation of the risk factors for HBV infections in these groups.

In HBV vaccination programs, single low-positive test results should not lead to exclusion from vaccine eligibility. Vaccination has been previously recommended for persons with anti-HBs between 2.1 to 9.9 SRU (1), but has not been recommended for persons with low anti-HBc (alone). It is now reasonable to recommend HBV vaccination for all persons being positive for only one of either anti-HBs or anti-HBc at a low level. If only one test (anti-HBs or anti-HBc) is used for prevaccination screening and a low-positive result obtained, we would recommend retesting of that specimen for both antibodies and vaccination for those with nonrepeatable positives or with only a single positive result, but not for those persons for whom both tests are positive.

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