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Antibody to Hepatitis B Core Antigen as a Paradoxical Marker for Non-A, Non-B Hepatitis Agents in Donated Blood

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The relationship between the presence of antibody to hepatitis B core antigen (anti-HBc) in donor blood and the development of hepatitis in recipients of that blood was studied in 6293 blood donors and 481 recipients who were followed for 6 to 9 months after transfusion. Of 193 recipients of at least 1 unit of blood positive for anti-HBc, 23 (11.9%) developed non-A, non-B hepatitis compared with 12 (4.2%) of 288 recipients of only anti-HBc-negative blood ($p < 0.001$). Donor anti-HBc status was not significantly associated with the development of hepatitis B in the recipient and was negatively associated with the development of cytomegalovirus hepatitis. The relationship of donor anti-HBc status and the development of non-A, non-B hepatitis in the recipient was independent of transfusion volume and elevated donor transaminase level. Although 88% of anti-HBc-positive blood units were not associated with recipient non-A, non-B hepatitis, calculation of maximal corrected efficacy predicted that exclusion of anti-HBc-positive donors might have prevented 43% of the cases of non-A, non-B hepatitis with a donor loss of 4%. Because of the serious chronic consequences of non-A, non-B hepatitis, surrogate tests for non-A, non-B virus carriers must be seriously considered.

THE INCIDENCE OF transfusion-associated type B hepatitis has been reduced markedly by the exclusion of commercial blood donors and the introduction of routine donor screening for the hepatitis B surface antigen (HBsAg) (1-3), but some cases of type B hepatitis continue to occur. Antibody to hepatitis B core antigen (anti-HBc) has been long recognized to be a sensitive indicator of hepatitis B virus infection (4-7), and it has been suggested that testing donors for anti-HBc might enhance detection of those harboring low levels of this virus (4, 5, 8-10). Although an association between donor anti-HBc status and the development of hepatitis B virus infection in a recipient is not unexpected, a surprising finding has been the report by the Transfusion-Transmitted Viruses Study Group (11) of a significant association between donor anti-HBc status and recipient non-A,

non-B hepatitis. The reported incidence of transfusion-associated hepatitis in the United States ranges from 5.4% to 27.1% (12), and greater than 90% of cases are estimated to be related to the agent(s) designated non-A, non-B (13). No confirmed, specific test suitable for screening blood donors for the non-A, non-B agent(s) has yet been established. The Transfusion-Transmitted Viruses Study has suggested that because of the similar modes of transmission of hepatitis B and the non-A, non-B virus, screening for anti-HBc might serve indirectly to identify donors with epidemiologic risk factors that would enhance transmission of both agents. The present study, which was conducted simultaneously with, but independently of, the Transfusion-Transmitted Viruses Study, further investigates the association of anti-HBc in donor blood and the development of transfusion-associated hepatitis.

Materials and Methods

The details of the prospective study design have been reported previously (13). Briefly, 729 consecutive adult patients undergoing open-heart surgery at the Clinical Center of the National Institutes of Health from November 1973 through December 1980 were entered into the study and prospectively followed for 6 to 9 months. Patients with HBsAg or transaminase elevations before transfusion were excluded. Blood samples were obtained weekly or biweekly during the first 3 months after transfusion, monthly for the next 3 months, and a final sample was obtained at 9 months. In addition to the study population, a control population of 203 patients undergoing cardiac catheterization without transfusions was similarly followed to determine the frequency of hepatitis and hepatitis B seroconversion in hospitalized patients undergoing invasive cardiac procedures without transfusions.

SEROLOGIC TESTS

All recipient specimens were assayed for alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin levels and for HBsAg (Ausria II; Abbott Laboratories, North Chicago, Illinois). Recipient ALT and AST levels were determined by a three-point kinetic assay with a sequential computer-controlled biochemical analyzer (SMAC). Hepatitis was diagnosed when between 2 and 26 weeks after transfusion, the recipient ALT level exceeded 2.5 times the upper limit of normal (110 IU/L) and when a repeat sample 1 or more weeks later was at least 2 times the upper limit of normal (88 IU/L).

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Table 1. Frequency of Hepatitis Events in Groups of Transfused Patients Included in and Excluded from Study

Group*	Hepatitis Events in Transfused Patients				
	Hepatitis B	Hepatitis B Virus Seroconversion	Cytomegalovirus Hepatitis	Non-A, Non-B Hepatitis	Total
Included in study (n = 481)	3 (0.6)†	9 (1.9)†	7 (1.5)†	35 (7.2)†	54 (11.2)†
Excluded from study (n = 248)	1 (0.4)	0 (0)	3 (1.2)	10 (4.0)	14 (5.6)
Total (n = 729)	4 (0.5)	9 (1.2)	10 (1.4)	45 (6.2)	68 (9.3)

* n refers to the number of recipients. Patients were included or excluded according to the availability of samples from all donor blood units they had received.

† Distribution of specific hepatitis events within the included group as compared with distribution within the excluded group: chi square = 2.18; degrees of freedom = 3; $p > 0.50$ (see text for method).

‡ Included group as compared with excluded group in total events: chi square = 6.03; degrees of freedom = 1; $p < 0.02$ (see text for method).

Other nonviral causes of ALT elevation, such as drug toxicity, anesthesia, alcoholism, anoxia, shock, congestive heart failure, and sepsis, were reasonably excluded. Hepatitis was considered chronic when the serum bilirubin level exceeded 2.5 mg/dL.

The samples from before and 3, 6, and 9 months after transfusion were tested for antibody to HBsAg (anti-HBs) by radioimmunoassay (AUSAB; Abbott Laboratories). The pretransfusion and 3- and 6-month samples were also tested for anti-HBc by radioimmunoassay (CORAB, Abbott Laboratories) and, when indicated by elevated ALT levels, for antibodies to cytomegalovirus by indirect hemagglutination, Epstein-Barr virus by immunofluorescence, and hepatitis A virus by radioimmunoassay (HAVAB, Abbott Laboratories). With the exception of 13 paid apheresis donors whose platelets were transfused to 10 patients, all blood donors were volunteers; only 2 of the 10 patients receiving platelets from paid donors were among the 481 patients included in the study analysis (see below). All donor blood products were negative for HBsAg by radioimmunoassay (AUSRIA II, Abbott Laboratories).

CLASSIFICATION OF HEPATITIS

In patients who developed ALT elevations consistent with transfusion-related viral hepatitis, the following criteria were used to define the cause of the hepatitis event. *Type B hepatitis* was diagnosed if the recipient developed HBsAg, or seroconverted for anti-HBs or anti-HBc (or both) simultaneously with the onset of ALT elevation. When anti-HBc or anti-HBs appeared in the 3-month sample, earlier samples were tested to distinguish passive transfer of antibody from active formation of the detected antibody. Hepatitis B virus seroconversion alone represented the de-novo appearance of anti-HBs or anti-HBc (or both) in the absence of ALT elevation. *Cytomegalovirus hepatitis* was diagnosed when the hepatitis event was associated with anticytomegalovirus antibody seroconversion and when there was no evidence for hepatitis B or hepatitis A virus infection. A rise in preexisting cytomegalovirus antibody titer was not considered evidence for an etiologic role for this agent. The diagnosis of *type A hepatitis* or *Epstein-Barr virus hepatitis* depended on the de-novo appearance of antibodies to these viruses in patients with elevated transaminase levels who were seronegative before transfusion. *Non-A, non-B hepatitis* was diagnosed when enzyme elevations suggested transfusion-associated hepatitis, when nonviral causes of an ALT elevation could be reasonably excluded, and when there was no serologic evidence of infection with hepatitis B virus, hepatitis A virus, cytomegalovirus, or Epstein-Barr virus. However, two patients with cytomegalovirus antibody seroconversion were considered to be also infected with the non-A, non-B virus, because of the development of chronic hepatitis and the presence of a fluctuating ALT pattern typical of non-A, non-B hepatitis.

Records of all patients and controls who had ALT levels that met the diagnostic criteria for hepatitis were reviewed by a four-member panel (see Acknowledgments). Hepatitis classification was made without knowledge of the donors' test results or of the patient's transfusion status. The final diagnosis represented the consensus of the panel.

STUDY METHODS

All available serum samples from donors, transfusion recipients, and untransfused patients were saved and stored at -20 to -70 °C. The present study involved retrieving donor serum samples and testing them for the presence of anti-HBs and anti-HBc. Analysis of the relationship between donor anti-HBc and anti-HBs status and recipient hepatitis was restricted to those patients for whom all donor samples were available for testing, as indicated in Table 1. Overall, blood samples from 6293 donors to 481 recipients were assayed for anti-HBs and anti-HBc. The 481 recipients represented 66% of the total population. A subset of blood samples from 2549 donors was also tested for ALT with a kinetic assay on a biochromatic analyzer. The level of ALT in donor samples was considered elevated if it exceeded 53 IU/L, representing 2.25 SD above the mean log ALT level.

Using manufacturer's criteria for interpretation of the anti-HBc qualitative (screening) test, we calculated a cutoff value by averaging the negative control mean and positive control mean. A 10% "gray zone" around the cutoff was then imposed to identify borderline results that required retesting in duplicate. Specimens whose counts per minute were greater than 110% of the cutoff value were considered negative for anti-HBc. Specimens with counts per minute lower than 90% of the cutoff value were considered positive for anti-HBc. Specimens whose rates fell within the 10% range of the cutoff value were retested in duplicate, and the three results were averaged to establish a definitive result.

STATISTICAL METHODS

The ordinary chi-square test (1 degree of freedom) was used to compare frequencies entered in 2×2 contingency tables. Kendall's V -statistic (14) was calculated to measure the strength of association between a pair of classifications. Frequencies entered in larger contingency tables were compared with a chi-square test with an appropriate number of degrees of freedom. When statistical significance was found, partitioning of the chi-square statistic was applied to show the principal source of variation (15). When indicated, chi-square tests with multiple degrees of freedom were modified so as to assess the presence of linear trends in the proportion of positive findings (15). Association between quantitative variables was measured by the Spearman rank correlation coefficient (r_s). Student's t -test was used to compare means, and results were expressed as a p value.

Results

COMPARISON OF STUDY POPULATIONS

The hepatitis events of the included subpopulation (those with a complete set of donor samples available) and those of patients excluded from the analysis are compared in Table 1. No instance of hepatitis A or Epstein-Barr virus hepatitis occurred in this study. Cases of hepatitis were significantly ($p < 0.02$) overrepresented in the study population, although differences in inclusion among the various types of hepatitis events were not sta-

Table 2. Association of Donor Hepatitis B Virus Antibodies and Recipient Hepatitis Events

	Any Hepatitis B Virus Event	Cytomegalovirus Hepatitis	Non-A, Non-B Hepatitis	Total Hepatitis Events
	n (%)			
Donor status of transfused patients*				
Donor anti-HBc present (n = 193)	7 (3.6)	0 (0)	23 (11.9)	30 (15.5)
Anti-HBs present (n = 148)†	5 (3.4)	0 (0)	18 (12.2)	23 (15.5)
Anti-HBs absent (n = 45)‡	2 (4.4)	0 (0)	5 (11.1)	7 (15.5)
Donor anti-HBc absent (n = 288)	5 (1.7)	7 (2.4)	12 (4.2)	24 (8.3)
Anti-HBs present (n = 67)	2 (3.0)	1 (1.5)	2 (3.0)	5 (7.5)
Anti-HBs absent (n = 221)	3 (1.4)	6 (2.7)	10 (4.5)	19 (8.6)
Total (n = 481)	12 (2.5)	7 (1.5)	35 (7.3)	54 (11.2)
Source of variation				
Donor anti-HBc present vs. absent (df = 1)				
Chi square	1.70	4.76	10.29	6.03
p Value	0.19	< 0.03	0.001	< 0.02
Donor anti-HBs present vs. absent, within each anti-HBc category (df = 2)				
Chi square	0.72	0.54	0.24	0.07
p Value	> 0.50	> 0.50	> 0.50	> 0.50

* n refers to the number of recipients; df = degrees of freedom; anti-HBc = antibody to hepatitis B core antigen; anti-HBs = antibody to hepatitis B surface antigen.

† All anti-HBc-positive donors to a given recipient are also anti-HBs-positive.

‡ At least one anti-HBc-positive donor to a given recipient lacked concomitant anti-HBs.

tistically significant ($p > 0.50$). The reason for this apparent selection is unknown but might have been due to a coincidental higher incidence of hepatitis during the study years when overall donor sample retention was maximal ($r_s = 0.76$, $p < 0.025$). Demographic and serologic characteristics of recipients included in the analysis were compared with those of recipients excluded from analysis; no significant difference in age, sex, or prior exposure to hepatitis B virus was noted. Similarly, no significant difference in age, sex, or prior hepatitis B virus exposure was noted for patients who did or did not receive anti-HBc-positive blood. Because availability of all donor samples was increasingly less likely for recipients with increasingly large numbers of donors, recipients excluded had a significantly higher transfusion volume than the recipients included in this study (15.7 and 13.1 donors/recipients, respectively; $p < 0.001$, Student's *t*-test). Transfusion volume is considered separately in the Results section as an important independent variable in our analysis.

RELATIONSHIP OF DONOR ANTIBODY TO HEPATITIS B CORE ANTIGEN TO RECIPIENT HEPATITIS EVENTS

The proportions of recipients found to have non-A, non-B hepatitis, cytomegalovirus hepatitis, or a hepatitis B event are shown in Table 2 according to the anti-HBc and anti-HBs (antibody to HBsAg) status of the individual donors. Of 481 patients, 288 received only blood negative for anti-HBc. Of these 288 recipients, 5 (1.7%) developed hepatitis type B or a serologic response for the hepatitis B virus, 7 (2.4%) developed cytomegalovirus hepatitis, and 12 (4.2%) developed non-A, non-B hepatitis. Thus, 24 (8.3%) recipients of only anti-HBc-negative blood had a hepatitis event. Of the 193 recipients of at least 1 unit positive for anti-HBc, 7 (3.6%) developed hepatitis type B or a serologic response for hepatitis B virus, 0 developed cytomegalovirus hepatitis, and 23 (11.9%) developed non-A, non-B hepatitis. Thus, 30

(15.5%) recipients of anti-HBc-positive blood developed a hepatitis event.

There was no statistically significant difference in the incidence of hepatitis B or serologic responses for hepatitis B virus among recipients who did or did not receive anti-HBc-positive blood. The occurrence of cytomegalovirus hepatitis was inversely associated with receipt of anti-HBc-positive blood ($p < 0.03$). In contrast, the incidence of non-A, non-B hepatitis was almost threefold higher among recipients of anti-HBc-positive blood than among recipients of only anti-HBc-negative blood (11.9% and 4.2%, respectively; chi square = 10.29, $p = 0.001$, 1 degree of freedom [df]).

Because the presence of anti-HBc is usually associated with that of anti-HBs, the analysis of anti-HBc status was stratified by the presence or absence of anti-HBs, and the source of variability of the chi-square statistic was partitioned by the method of Cochran (15). As shown in Table 2, within anti-HBc categories, recipients of donor blood positive for anti-HBs were at no higher risk for a hepatitis event than recipients of blood negative for anti-HBs (15.5% and 15.5%, respectively, in those with donors positive for anti-HBc and 7.5% and 8.6%, respectively, in those with donors negative for anti-HBc; chi square = 0.07, $p > 0.50$). When we examined hepatitis B virus events, cytomegalovirus, and non-A, non-B hepatitis separately, the partitioning of the chi-square variability clearly showed that within anti-HBc categories the presence or absence of anti-HBs was not significantly associated with the development of any hepatitis events and that the presence or absence of anti-HBc was the most important contributor to recipient hepatitis. This difference was most pronounced in the non-A, non-B hepatitis group ($p = 0.001$). If the recipients listed in Table 2 were classified first according to their donor anti-HBc status and then subclassified according to donor anti-HBs status, a similar partition of chi-square variability was obtained. The major contribution to non-A, non-B

Table 3. Frequency of Hepatitis and Hepatitis B Virus Events Among Recipients According to Transfusion Volume and Donor Status for Antibody to Hepatitis B Core Antigen (Anti-HBc)

	Any Hepatitis B Virus Event	Cytomegalovirus Hepatitis	Non-A, Non-B Hepatitis
	n (%)		
Transfusion volume and donor anti-HBc status			
1-5 units transfused			
Anti-HBc present (n = 7)*	1 (14.3)	0 (0)	1 (14.3)
Anti-HBc absent (n = 24)	0 (0)	0 (0)	1 (4.2)
6-10 units transfused			
Anti-HBc present (n = 21)	0 (0)	0 (0)	2 (9.5)
Anti-HBc absent (n = 60)	1 (1.7)	2 (3.3)	1 (1.7)
11-15 units transfused			
Anti-HBc present (n = 96)	3 (3.1)	0 (0)	14 (14.6)
Anti-HBc absent (n = 151)	3 (2.0)	5 (3.3)	7 (4.6)
16-20 units transfused			
Anti-HBc present (n = 56)	2 (3.6)	0 (0)	5 (8.9)
Anti-HBc absent (n = 48)	1 (2.1)	0 (0)	3 (6.2)
> 20 units transfused			
Anti-HBc present (n = 13)	1 (7.7)	0 (0)	1 (7.7)
Anti-HBc absent (n = 5)	0 (0)	0 (0)	0 (0)
Total			
Anti-HBc present (n = 193)	7 (3.6)	0 (0)	23 (11.9)
Anti-HBc absent (n = 288)	5 (1.7)	7 (2.4)	12 (4.2)
Statistical analysis			
Weighted mean difference (\pm SE) [†]	1.69 \pm 1.50	-3.32 \pm 1.69	7.86 \pm 2.51
z Statistic	1.13	1.98	3.14
p Value	> 0.10	< 0.05	< 0.001
Slope of regression (\pm SD), $\times 10^{-3}$ [‡]			
Anti-HBc present	0.4 \pm 3.1	0 \pm 0	-3.6 \pm 5.4
Anti-HBc absent	0.8 \pm 1.8	0 \pm 5.5	1.9 \pm 2.7

* refers to the number of recipients.

† At each transfusion level, the incidence of hepatitis among recipients of anti-HBc-positive blood was compared with that among recipients of anti-HBc-negative blood. The results were pooled in a weighted mean difference test.

‡ Slope of regression was performed with frequency of disease event as the dependent (or outcome) variable and number of units transfused as the independent variable; 0.5 for each of the six regression coefficients.

hepatitis was still that due to anti-HBc (present compared with absent, chi square = 8.16, 2 df, $p < 0.025$) as compared with anti-HBs (present compared with absent, chi square = 2.37, 1 df, $p = 0.12$). Because donor anti-HBs status was not associated with a higher incidence of recipient hepatitis, subsequent analyses were restricted to donor anti-HBc status.

The data in Table 2 were used to assess the potential efficacy of screening blood donors for anti-HBc to reduce the incidence of transfusion-associated non-A, non-B hepatitis. If one assumes that 23 of the 35 cases of non-A, non-B hepatitis were due to the anti-HBc-positive blood given, then rejection of these donors might have prevented 23 (66%) of the non-A, non-B cases (crude efficacy). However, because the receipt of anti-HBc-negative blood also carried a 4.2% risk of non-A, non-B hepatitis, 8 of the 193 recipients of anti-HBc-positive blood might have developed non-A, non-B hepatitis even if anti-HBc-negative blood had been substituted for each anti-HBc-positive unit. This would have resulted in 15 (23 minus 8) cases of non-A, non-B hepatitis potentially prevented by screening for anti-HBc for a maximal corrected efficacy of 43%. No correction for the background incidence of non-A, non-B hepatitis among nontransfused controls (0.5%) was imposed.

The efficacy of the donor screening procedure can also be judged in terms of recipient outcome. Of the 35 patients with non-A, non-B hepatitis, 23 received at least 1

unit positive for anti-HBc, for a test sensitivity of 66%; among the 446 patients who remained free of non-A, non-B hepatitis, 276 received only anti-HBc-negative blood, for a test specificity of 62%. Eighty-eight percent of recipients of anti-HBc-positive blood did not develop non-A, non-B hepatitis, resulting in a positive predictive value for this test of only 11.9%.

RELATIONSHIP OF TRANSFUSION VOLUME TO RECIPIENT HEPATITIS EVENTS

The percentage of patients who developed non-A, non-B hepatitis, cytomegalovirus hepatitis, or a hepatitis B virus event did not change significantly as the transfusion volume increased in either recipients of anti-HBc-positive blood or in recipients of only anti-HBc-negative blood (see regression analysis, Table 3). Table 3 shows that at each transfusion level, recipients of anti-HBc-positive blood had a consistently higher incidence of non-A, non-B hepatitis than recipients of anti-HBc-negative blood. Moreover, as the number of units transfused increased, there was a corresponding increase in the proportion of recipients with anti-HBc-positive units transfused, from 7 of 31 recipients (22.6%) anti-HBc positive at 1 to 5 units to 13 of 18 recipients (72.2%) anti-HBc positive at greater than 20 units ($r_s = 1.00$, $p < 0.01$). When the incidence of hepatitis among recipients of anti-HBc-positive blood was compared with that among recipients of anti-HBc-negative blood at each transfusion volume and the

Table 4. Relationship Between Donor Status for Antibody to Hepatitis B Core Antigen (Anti-HBc) and Donor Alanine Aminotransferase (ALT) Level*

Donor ALT Level	Donor Anti-HBc Status		Total
	Positive	Negative	
Elevated	3	33	36
Normal	100	2413	2513
Total	103	2446	2549

* Kendall coefficient of association = 0.03; chi square = 1.74; degrees of freedom = 1; $p > 0.10$.

results pooled in a weighted mean difference test (15), non-A, non-B hepatitis was significantly more common among recipients of donor blood positive for anti-HBc ($p < 0.001$).

NUMBER OF DONOR UNITS POSITIVE FOR ANTIBODY TO HEPATITIS B CORE ANTIGEN AND RECIPIENT NON-A, NON-B HEPATITIS

With a spectrum of transfusion volumes ranging from 1 to 41 units, some patients received blood from more than one donor who was positive for anti-HBc. Of the 141 recipients of only 1 donor unit positive for anti-HBc, 17 (12.1%) developed non-A, non-B hepatitis compared with 6 (14.3%) of the 42 recipients who received two donor units positive for anti-HBc. Ten patients received blood from three donors positive for anti-HBc, and 0 of these 10 developed hepatitis. Regression analysis (15) resulted in an estimated slope that was virtually 0 (-0.02) and a 95% confidence interval ($-0.10, 0.06$) that indicated the true slope was also very likely close to 0. Hence, a dose-response relationship is unlikely with receipt of increasing numbers of anti-HBc-positive blood units.

RELATIONSHIP OF DONOR ANTIBODY TO HEPATITIS B CORE ANTIGEN AND DONOR ALANINE AMINOTRANSFERASE LEVEL

Previous studies have shown a correlation between donor alanine aminotransferase (ALT) level and the development of non-A, non-B hepatitis in the recipient (16, 17). In this study, donor ALT values were determined only during the latter half of the study period; thus, ALT data were available for only 2549 (40%) of the 6293 donors analyzed for anti-HBc. Table 4 shows that among the 36 donors with elevated ALT levels, only 3 (8.3%) also had anti-HBc, a proportion that is similar to the corresponding percentage with anti-HBc among donors with normal ALT levels (100 of 2513, 4.0%; $p > 0.10$). The similarity in these two percentages is consistent with a lack of association between donor ALT level and anti-HBc status. This conclusion is supported by the very low value of Kendall's coefficient of concordance, $V = 0.03$. Presence of anti-HBc and an elevated ALT level appeared to be events occurring in two unrelated donor populations.

The frequency of non-A, non-B hepatitis among the 230 recipients whose donors were tested for both anti-HBc and ALT is shown in Table 5. The highest incidence

(33.3%) occurred in recipients of both a unit of blood with anti-HBc and a unit of blood with an elevated ALT level (not necessarily in the same unit); the lowest incidence (4.5%) occurred in recipients of only blood negative for both markers. The risk of non-A, non-B hepatitis was significantly greater for the 80 recipients of anti-HBc-positive blood (20%) than for the 150 recipients of anti-HBc-negative blood (5.3%), regardless of whether there was the concomitant administration of a unit of blood with an elevated ALT level ($p < 0.001$). Partitioning of the chi-square variability reveals that anti-HBc is the primary contributor to hepatitis outcome (chi square = 12.01). However, within the anti-HBc-positive group, donor ALT status made a significant but smaller contribution to the demonstrated hepatitis risk of anti-HBc-positive blood (chi square = 4.42, $p < 0.04$).

In Table 5, if the data were classified according to donor ALT status first and then according to anti-HBc status, a similar partition of the chi-square variability was obtained. The major contribution was still that due to anti-HBc (present compared with absent, chi square = 10.09, 2 *df*, $p < 0.01$), and most of this effect was associated with the group of patients receiving blood with normal ALT levels (chi square = 6.16, 1 *df*, $p < 0.02$). However, the contribution due to presence or absence of an elevated ALT level was also significant (chi square = 7.32, 1 *df*, $p < 0.01$). These findings emphasize that although donor anti-HBc status is the principal marker associated with recipient non-A, non-B hepatitis, the contribution of an elevated ALT level is also significant.

RELATIONSHIP OF DONOR ANTIBODY TO HEPATITIS B CORE ANTIGEN TO THE BIOCHEMICAL SEVERITY AND PERSISTENCE OF RECIPIENT HEPATITIS

Among the 23 patients who developed non-A, non-B hepatitis after the receipt of anti-HBc-positive blood, 14 (61%) had a maximum ALT level of greater than ten times the upper limit of normal and 7 (30%) had a bilirubin level of greater than 5 mg/dL. In the 12 patients who developed non-A, non-B hepatitis without receiving

Table 5. Frequency of Non-A, Non-B Hepatitis Among Recipients Grouped According to Donor Status for Antibody to Hepatitis B Core Antigen (Anti-HBc) and Donor Alanine Aminotransferase Level

Donor Status	Recipient Non-A, Non-B Hepatitis <i>n</i> (%)
Anti-HBc present (<i>n</i> = 80)	16 (20.0)*
ALT elevated (<i>n</i> = 18)†	6 (33.3)‡
ALT normal (<i>n</i> = 62)	10 (16.1)‡
Anti-HBc absent (<i>n</i> = 150)	8 (5.3)*
ALT elevated (<i>n</i> = 16)	2 (12.5)§
ALT normal (<i>n</i> = 134)	6 (4.5)§
Total (<i>n</i> = 230)	24 (10.4)

* Anti-HBc present as compared to anti-HBc absent; chi square = 12.01; degrees of freedom = 1; $p < 0.001$. *n* refers to the number of recipients.

† Except in three instances, the donor with anti-HBc is different from the donor with elevated ALT (see Table 4).

‡ ALT elevated as compared to ALT normal in group with anti-HBc present; chi square = 4.42; degrees of freedom = 1; $p = 0.04$.

§ ALT elevated as compared to ALT normal in group with anti-HBc absent; chi square = 0.98; degrees of freedom = 1; $p > 0.30$.

an anti-HBc-positive unit, the corresponding values were (67%) and 3 (25%), respectively. Chi-square analysis revealed no significant association between donor anti-HBc status and the magnitude of either the ALT or bilirubin elevation in the patient.

The development of chronic hepatitis, as defined by ALT elevations persisting for greater than 1 year, was analyzed in a similar manner. In the 23 patients with non-A, non-B hepatitis who received anti-HBc-positive blood, 14 (61%) developed chronic hepatitis as compared with 9 (75%) of 12 who received anti-HBc-negative blood ($p > 0.50$).

Discussion

In this study, 729 patients having open-heart surgery were prospectively followed for at least 6 months after transfusion. Biochemical or overt hepatitis occurred in 59 patients (8.1%), and an additional 9 patients (1.2%) developed a serologic response to the hepatitis B virus without concomitant evidence of hepatitis. Seventy-six percent of the cases were classified as non-A, non-B hepatitis, 17% as cytomegalovirus hepatitis, and 7% as hepatitis B. The high proportion of cytomegalovirus hepatitis was related to a cluster occurring in 1978. Data not included in this report indicate that since 1978, cytomegalovirus hepatitis has not occurred in this open-heart surgery population. A subpopulation of 481 patients, for whom samples of all donor blood were available, was extensively investigated to define further the relationship of donor anti-HBc status to the occurrence of hepatitis in the recipient.

The salient features of this analysis were as follows. The incidence of non-A, non-B hepatitis among recipients of anti-HBc-positive blood was almost three times that of recipients of only anti-HBc-negative blood (11.9% compared with 4.2%, $p = 0.001$); in contrast, hepatitis B events did not significantly correlate with donor anti-HBc status. The presence of anti-HBs in the donor did not correlate with the development of recipient hepatitis. The occurrence of non-A, non-B hepatitis was not associated with the volume of blood transfused, but at each transfusion volume there was a positive association between donor anti-HBc status and recipient non-A, non-B hepatitis. There was a significant association between elevated donor ALT level and recipient hepatitis ($p < 0.01$), but partitioning of the chi-square variability showed that donor anti-HBc was the primary contributor to the occurrence of hepatitis. Among individual donors there was no correlation between the presence of anti-HBc and the presence of an elevated ALT level, and hence, these two surrogate markers for the non-A, non-B carrier state appeared to act as independent variables. The receipt of anti-HBc-positive blood did not correlate with the biochemical severity or persistence of the ensuing hepatitis, and finally, the predicted efficacy for the exclusion of anti-HBc-positive donors, when corrected for the hepatitis risk of anti-HBc-negative donors, was 43%.

The Transfusion-Transmitted Viruses Study Group (11) has previously reported data showing a strong rela-

tionship between the presence of anti-HBc in the donor and the development of non-A, non-B hepatitis in the recipient. In an accompanying editorial, Alter and Holland (18) have raised concerns about this study, not in terms of the data presented, but in terms of the interpretation of these data. The results of the current study allow for a reexamination of these issues. The primary concern expressed was that the determination of efficacy in the Transfusion-Transmitted Viruses Study involved a correction factor for the background incidence of hepatitis in the nontransfused control population which necessitated exclusion from analysis of 31 cases of hepatitis or almost half the cases in one study arm. It was suggested that deletion from analysis of such a large number of cases might introduce a bias into the efficacy calculation. In our current study, the background incidence of hepatitis in the nontransfused population was sufficiently low (0.5%) that a correction for background was not necessary. Our predicted efficacy for anti-HBc testing (43%) is similar to that obtained in the Transfusion-Transmitted Viruses Study and, hence, concern regarding the correction factor that was imposed appears to have been unjustified.

The purported mechanism by which anti-HBc identifies non-A, non-B carriers is that such carriers might be sequentially or concomitantly exposed to both hepatitis B and non-A, non-B viruses. If this were true, then donor status for anti-HBs, another sensitive serologic marker of past hepatitis B virus infection, should also correlate with the occurrence of non-A, non-B hepatitis in the recipient. This finding was not the case in the Transfusion-Transmitted Viruses Study (11, 19) and was a second point of concern expressed in the editorial (18). Our study confirms the lack of association between donor anti-HBs status and subsequent occurrence of non-A, non-B hepatitis in the recipient, and it suggests that the association of anti-HBc with the non-A, non-B carrier state may involve more than the coincidental exposure to multiple hepatitis viruses. In this regard, it has been suggested that the non-A, non-B virus and the hepatitis B virus may have a common origin, as shown by the finding of hepatitis B virus DNA in the serum and liver tissue of some patients diagnosed as having non-A, non-B hepatitis (20). The possibility of a direct relationship between the non-A, non-B and hepatitis B viruses, however, remains a controversial issue (21).

A third concern in Alter and Holland's editorial (18) was that previous studies of the relationship between donor ALT level and recipient hepatitis (16, 17) and the more recent Transfusion-Transmitted Viruses Study analysis of anti-HBc (11) represented predictions of efficacy based on the assumption that the anti-HBc-positive or ALT-elevated donor in any given case was the donor to transmit hepatitis and that exclusion of that particular donor would have prevented the ensuing hepatitis. The editorial stressed that predicted efficacy was not the equivalent of established efficacy and recommended that these predictions be tested in a randomized, prospective, controlled trial which compared directly the incidences of hepatitis after the receipt of blood tested and untested

for ALT and anti-HBc. However, it has become increasingly unlikely that such a study will ever be initiated. The anticipated reduction in the incidence of transfusion-related hepatitis as an indirect consequence of donor testing for antibody to the human T-lymphotropic virus type III and other trends in blood donation practice would necessitate a substantial increase in the number of study participants to confirm the predicted efficacy of anti-HBc testing. The consequent increased time, cost, and complexity of such a study do not appear to be logistically, financially, and perhaps ethically feasible in the perspective of current research priorities.

A fourth concern was that the presence of anti-HBc and the presence of an elevated ALT level behaved as independent variables, even though both appear to serve as surrogate markers for the non-A, non-B carrier state. This lack of concordance, first noted by the Transfusion-Transmitted Viruses Study (11), has been confirmed by our finding of virtual independence between the outcome of the two tests (Kendall's $V = 0.03$). Although most donors were both anti-HBc negative and had a normal ALT level, donors who were positive for anti-HBc only rarely had an elevated ALT level (3 of 103). If, indeed, the ALT and anti-HBc tests are detecting distinct populations of non-A, non-B virus carriers, then the potential impact of adopting indirect screening measures would be greatly magnified.

In the absence of a prospective controlled study, the existing database must be used to decide whether or not to adopt the ALT test, the anti-HBc test, or both. In considering these options, we must keep in perspective the surrogate nature of both assays. Both have a relatively low level of predicted efficacy in preventing non-A, non-B hepatitis, and 60% to 70% of non-A, non-B transfusion-associated hepatitis will probably continue to occur despite implementation of either of these tests. Equally disturbing, both tests have a high rate of false positivity: 70% to 88% of recipients of blood with anti-HBc or an elevated ALT level do not develop non-A, non-B hepatitis (11, 16, 17), and approximately 60% of donors with elevated ALT levels have nonviral factors as the most likely cause of their transaminase elevation (22). An additional major concern is the resultant loss of blood donors, estimated to be 1% to 3% for ALT elevations and 4% to 8% for anti-HBc (11, 16, 17, 23). In this discussion we do not wish to deal with the relative merits of one surrogate marker over the other, but rather to deal with the key issue of whether to adopt any indirect screening measure for detection of the non-A, non-B virus carrier state. Two important variables enter into this decision: the likelihood that a specific test for the non-A, non-B virus carrier state will become available in the near future, and the clinical significance of non-A, non-B hepatitis. Despite numerous reports of putative non-A, non-B assays, none has been independently confirmed (24), none has been able to distinguish proven non-A, non-B infectious sera from noninfectious control sera in coded panels (25), and none has withstood the test of time. Currently, no specific assay is available for the agent(s) of non-A, non-B hepatitis, and none appears imminent;

anticipation of specific assays should not defer the need for a prompt decision regarding the adoption of surrogate tests.

The severity of non-A, non-B hepatitis has been a controversial issue. The disease tends to be mild at onset with only a quarter of patients being jaundiced and with other clinical symptoms being generally minor (22). Most of the transfusion-related cases that have been identified in prospective studies are based primarily on biochemical rather than clinical evidence of disease. Questions have arisen repeatedly about the need to introduce costly screening procedures to prevent this seemingly benign disease. The answer resides in an analysis of the chronic sequelae of non-A, non-B hepatitis. Virtually every study (25-27) that has investigated this aspect of non-A, non-B hepatitis has confirmed that an inordinately high percentage of patients develop chronic hepatitis as shown by persistent ALT elevation; that liver biopsies in patients with chronic ALT elevation show chronic active hepatitis as the predominant lesion; and that 10% to 20% of those who have biopsies have evidence of cirrhosis. Although the cirrhosis tends to be clinically mild, deaths related to non-A, non-B-associated cirrhosis are being reported (25, 27-29). Prospective studies indicate that at least 5% of transfusion recipients develop biochemical or clinical evidence of non-A, non-B hepatitis. For an estimated 3 million blood recipients, this percentage represents 150 000 cases of transfusion-associated non-A, non-B hepatitis in the United States annually. If half these patients have chronic ALT elevation and 10% of these develop cirrhosis, then up to 7500 cases of non-A, non-B-related cirrhosis might be induced annually as a result of blood transfusion. If, as predicted, surrogate screening of blood donors could prevent approximately one third of these cases, then this could represent an annual reduction of 50 000 cases of hepatitis and 2500 cases of cirrhosis. The potential to achieve this degree of disease prevention now appears to outweigh the disadvantages inherent in the adoption of surrogate tests for the non-A, non-B virus carrier state.

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