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Antibodies to hepatitis B core antigen in blood donors screened for alanine aminotransferase level and hepatitis non-A, non-B in recipients

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ABSTRACT: Fourhundredandseventeen patients undergoing open-heart surgery were followed for more than 9 months after transfusion. All 2270 blood units transfused had alanine aminotransferase levels ≤ 30 U/l. Blood units positive for antibodies to hepatitis B core antigen (anti-HBc) were more frequently associated with recipient hepatitis non-A, non-B (HNANB) (13.7 %) than anti-HBc-negative units (4.2 %) ($p < 0.001$). The frequency of HNANB among recipients of at least 1 anti-HBc-positive blood unit (8/79, 10.1 %) was fivefold greater than among recipients of exclusively anti-HBc-negative blood units (7/338, 2.1 %) ($p < 0.01$). In this study the exclusion of donors positive for anti-HBc (4.2 %) might have reduced the incidence of recipient HNANB by 42 percent. These results support the introduction of anti-HBc donor screening to prevent recipient HNANB.

In various prospective studies of recent years a 2 to 14 percent frequency of hepatitis non-A, non-B (HNANB) was found among blood recipients worldwide (1-5). If chronicity is measured as persistently increased alanine aminotransferase (ALT) levels for at least 6 months, about 50 to 70 percent of the cases of post-transfusion HNANB became chronic (6-10). The seriousness of this problem is emphasized by the results of histological investigations: 44 to 90 percent of the patients with chronic post-transfusion HNANB show evidence of chronic active hepatitis or cirrhosis (7-9, 11).

Of late, some investigators have proposed the use of surrogate tests, i.e. the determination of ALT (2, 3) or antibody to hepatitis B core antigen (anti-HBc) (12, 13) of blood donors, in order to reduce the high risk of transmitting HNANB by blood transfusions.

This study was undertaken to evaluate the impact of anti-HBc-positive units transfused when ALT screening had been performed and units with elevated ALT activity eliminated.

The initial goal of the investigation was to examine the efficacy of hepatitis B immunoglobulin (HBIG) in the prophylaxis of HNANB in a controlled study (14).

Materials and Methods

Patients

In 1980 and 1981, patients between the age of 15 and 65 who underwent open-heart surgery at the University of Tübingen were entered into the investigation if they fulfilled the following conditions: no signs of liver disease, no alcohol or drug abuse, no blood transfusions within the previous 6 months, ALT ≤ 22 U/l (≤ upper limit of normal), and negative tests for hepatitis B surface antigen (HBsAg). The period of observation extended over more than 9 months. Serum samples were taken before surgery and on post-operative days 1, 7 and 14, afterwards at monthly intervals until the 38th week after surgery. Additional specimens were taken when patients developed an ALT elevation > 22 U/l. All patients participated in a randomized trial to prove the efficacy of HBIG in preventing HNANB. HBIG (Biotest Pharma, Frankfurt) was given intravenously (5 ml before the first blood transfusion and 5 ml on the day after surgery) to 209 patients, 208 patients (controls) received no HBIG treatment.

Initially 478 patients were recruited of which 417 were included for evaluation. From these patients 5309 (97.9%) of 5421 expected serum samples were available for analysis.

In all patient sera ALT (optimized method at 25 °C) as well as HBsAg, anti-HBc and antibody to hepatitis B surface antigen (anti-HBs) (Abbott RIA-kits, Dane particles derived antigens) were determined. Among patients with increased ALT levels (> 55 U/l) serum from the acute phase was tested for IgM antibody to hepatitis A virus (HAV) (Abbott RIA-kits); the pre-operative serum and acute phase samples were analysed for IgM and IgG antibodies to cytomegalovirus (anti-CMV IgM and IgG) (Behring EIA-kits) as well as for IgM and IgG antibodies to the virus capsid antigen of Epstein-Barr virus (EBV) (immunofluorescence).

Diagnosis and classification of hepatitis

Hepatitis was diagnosed when the ALT activity in 2 specimens which were taken at intervals of at least 1 week between the 14th and the 180th post-operative day had risen above 55 U/l (2.5 times the upper limit of normal) and when other nonviral causes for the increased ALT levels such as hepatotoxic medication, congestive heart failure or shock could be ruled out. HNANB was assumed when no serological evidence was found for an infection with HAV, hepatitis B virus (HBV), CMV and EBV at the time of ALT increase.

Blood donors

All transfused units came from volunteer donors. Determinations of HBsAg and ALT levels had been carried out on all donors, and only units that were negative for HBsAg and had ALT levels ≤ 30 U/l were transfused. Specimens from each of the transfused units were frozen at -20 °C and examined for anti-HBc and anti-HBs.

Statistical methods

The chi-square test with Yates' correction was used to compare frequencies between groups.

Results

In this study, 417 patients and 2270 units of transfused blood were evaluated. Sixteen cases of hepatitis were observed, none of which was associated with HAV, EBV or CMV. Anti-CMV IgM appeared at the time of the ALT increase among 4 (25 %) of the 16 patients with hepatitis, but was also detectable

in 24 percent (12/50) of patients without elevated liver enzymes. Anti-CMV IgG titers did not change in any patient. One patient with hepatitis was HBsAg positive, thus 15 (3.6 %) of 417 patients were assumed to have HNANB. Twelve of the patients with HNANB could be observed for more than 6 months after the first ALT rise. Four (33.3 %) of these 12 patients had elevated ALT values for at least 6 months and were believed to run a chronic course.

As indicated in Table 1 the proportion of anti-HBc-positive units which was associated with HNANB was three times greater than the corresponding proportion of anti-HBc-negative units ($p < 0.001$).

Table 1. Anti-HBc status of blood units transfused and association with HNANB in recipients

Anti-HBc Status of Blood Units	Blood Units Transfused	Units Associated with HNANB in Recipients
Negative	2175	91 (4.2 %)*
Positive	95	13 (13.7 %)*
Total	2270	104 (4.6 %)

* $p < 0.001$

Those patients who had received at least 1 anti-HBc-positive unit developed HNANB with a fivefold greater frequency than recipients of exclusively anti-HBc-negative units (10.1 % vs 2.1 %, $p < 0.01$) (Table 2).

Table 2. Frequency of HNANB in recipients and anti-HBc status of blood units transfused

Anti-HBc Status of Blood Units	Number of Recipients	HNANB in Recipients
All Negative	338	7 (2.1 %)*
≥ 1 Positive	79	8 (10.1 %)*
Total	417	15 (3.6 %)

* $p < 0.01$

We also examined whether the development of recipient hepatitis was an effect of the increased use of blood rather than of the transfusion of anti-HBc-positive units. Patients received an average of 5.4 (range 1 to 19) units. They could be divided into two groups, one receiving a transfusion volume of 1 to 5 units and the other 6 or more units. As shown in Table 3 development of HNANB was related to the anti-HBc status of the blood units and not to the transfusion volume.

Apart from the transfusion volume, we analysed 2 further variables that may have influenced the development of HNANB: anti-HBc status and HBIG treatment of recipients.

Anti-HBc was detectable in the pre-transfusion sample of 14.8 percent (50/338) of the recipients of solely anti-HBc-negative blood and 15.2 percent (12/79) of patients who had received at least 1 anti-HBc-positive unit (Table 4). The presence or absence of anti-HBc in the pre-transfusion sample did not affect the development of HNANB (Table 4).

Twohundredandnine patients received 10 ml HBIG, 208 patients were not treated with this product. The incidence of HNANB in the HBIG treated group (5/209, 2.4 %) was not significantly different ($p > 0.05$) to that in the controls (10/208, 4.8 %). Moreover, it is shown in Table 5 that there was not a significant influence of the HBIG treatment as to the incidences of HNANB among the patients receiving anti-HBc-negative or positive blood units.

Table 3. Frequency of HNANB in recipients, anti-HBc status of blood units transfused and transfusion volume

Transfusion Volume	Number of Recipients	HNANB in Recipients Cases	%
1-5 Units	253	10	4.0
All Anti-HBc-neg.	223	7	3.1*
≥ 1 Anti-HBc-pos.	30	3	10.0*
6-19 Units	164	5	3.0
All Anti-HBc-neg.	115	0	0+
≥ 1 Anti-HBc-pos.	49	5	10.2+

* NS, + $p < 0.01$

Table 4. Frequency of HNANB, anti-HBc status of blood units and anti-HBc status of recipients

Anti-HBc Status of Blood Units Transfused	Recipient's Pre-Transfusion Sample	Number of Recip.	HNANB Cases
All Negative	Negative	288	6 (2.1)*
	Positive	50	1 (2.0)*
≥ 1 Positive	Negative	67	7 (10.4)*
	Positive	12	1 (8.3)*

* NS Values in parentheses are percentages.

Table 5. Frequency of HNANB, anti-HBc status of blood units and HBIG treatment of recipients

Anti-HBc Status of Blood Units	HBIG Treatment of Recipients	Number of Recip.	HNANB Cases
All Negative	Yes	171	3 (1.8)*
	No	167	4 (2.4)*
≥ Positive	Yes	38	2 (5.3)*
	No	41	6 (14.6)*

* NS Values in parentheses are percentages.

According to Table 2, 8 of the 15 (53 %) observed cases of HNANB occurred among patients who had received anti-HBc-positive units. However, the effectiveness of anti-HBc donor screening on the reduction of HNANB among blood recipients requires correction for those cases of HNANB, which occurred independent of the transfusion of anti-HBc-positive blood (7/338 or 2.1 %) and would not be avoided by anti-HBc screening. This yields a corrected efficacy (3, 13) of 42 percent. This reduction of recipient HNANB would result in a donor loss of 4.2 percent, as 95 of the 2270 units transfused in this study were anti-HBc-positive.

Discussion

In this study, only blood from donors with ALT levels of less than 30 U/l was transfused, as opposed to the previous anti-HBc screening studies by Stevens et al. (12) and Koziol et al. (13) in which units with elevated ALT activity had not been deferred. In West Germany donor ALT screening has been mandatory for more than 15 years. In the studies by Stevens et al. (12) and Koziol et al. (13) the recipients of anti-HBc-positive transfusions were confronted with increased donor ALT values with signi-

ficantly higher frequency than were recipients of anti-HBc-negative units. This fact weakens evidence of the expected efficacy of anti-HBc donor screening at least in part because the correlation between donor ALT levels and the development of HNANB among recipients is known (2, 3).

The study reported here clearly indicates that the transfusion of anti-HBc-positive blood units is associated with a significantly increased risk for the recipients to acquire HNANB. The corrected effectiveness of anti-HBc screening in this study, 42 percent, is quite high. These results are in agreement with those by Stevens et al. (12) and Koziol et al. (13).

Interestingly, the number of blood units transfused did not have an obvious influence on the frequency of HNANB or an effect on the above mentioned findings.

We also considered the anti-HBc status of the recipients as a further possible variable which could affect the results in 2 ways: First, it might be possible that the sera of anti-HBc-positive individuals contain antibodies to the HNANB agents that are able to protect the individuals from infection. Second, anti-HBc-positive individuals might more often have unrecognized chronic HNANB. The analysis presented here argues against any of these 2 possibilities and renders it unlikely that the anti-HBc status of the recipient had any influence on the findings of this study.

The initial goal of this study was to evaluate the efficacy of HBIG in the prophylaxis of HNANB. As presented, the frequency of HNANB was lower when HBIG was given, however this was not statistically significant when compared to the untreated group. Larger trials are required to analyse the potential efficiency of HBIG in the prophylaxis of HNANB.

Reservations to the conclusions drawn from this study might exist: Most importantly, only few cases of HNANB occurred. Two recent European studies (16, 17) showed contradictory findings, but the number of patients evaluated in both studies might have been too small for valid and meaningful conclusions. In addition, it is impossible to judge how many anti-HBc-positive HNANB carriers are no longer a part of donor collectives due to exclusion measures to avoid human immunodeficiency virus transmission. On the other hand, the results of this study and those by Stevens et al. (12) and Koziol et al. (13) support the introduction of anti-HBc screening to reduce the risk of HNANB among blood recipients. In addition, some cases of hepatitis B may be avoided. The only patient in this study who contracted hepatitis B had received an unit from an HBsAg- and anti-HBs-negative donor with a high titer of anti-HBc, probably a chronic "low level carrier" (15). It would therefore seem that anti-HBc screening should be implemented in donor screening programs and that the benefits to the recipients should outweigh any impact on the collection facilities.

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