Donor Transaminase and Recipient Hepatitis

Impact on Blood Transfusion Services

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• To assess the relationship of donor alanine aminotransferase (ALT) level to recipient hepatitis, 283 transfused patients were prospectively followed up after open heart surgery; hepatitis developed in 12.7%, of which 97% was non-A, non-B. The ALT tests on 3,359 donors to these patients indicated that risk of hepatitis was significantly associated with the level of donor ALT; 29% of 52 patients receiving at least 1 unit of blood with an ALT level greater than 53 IU/L had hepatitis develop (20.7 cases per 1,000 units), compared with 9% of 231 recipients of only blood with an ALT level of 53 IU/L or less (7.8 cases per 1,000 units). Calculation of corrected efficacy predicts that, at an exclusion level equivalent to 2.25 SDs above the mean log for normal subjects, ALT testing of donors could prevent 29% of posttransfusion hepatitis at the loss of 1.6% of donor units.

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THE TRANSFUSION Transmitted Virus Study (TTV), a multihospital cooperative study of posttransfusion hepatitis, has recently reported a significant association between donor serum transaminase (ALT, SGPT) and recipient non-A, non-B (NANB) hepatitis.[1] This finding has major implications for blood transfusion services and raises difficult scientific, ethical, and administrative questions. The present study, which was independently conducted, confirms the significant association of an elevated ALT level in donor blood and the development of recipient posttransfusion hepatitis; it suggests that pretransfusion screening of donor blood for ALT level can identify some carriers of the NANB hepatitis virus and possibly prevent approximately 30% of transfusion-related hepatitis.

MATERIALS AND METHODS

The conduct of the study was similar to that reported earlier[1]; 283 consecutive adult patients undergoing open heart surgery and on whom complete donor ALT data were available were entered into the study and followed up for six to nine months.

Blood donors were all volunteers. A serum sample was obtained from each donor at the time of phlebotomy and then sent to a local laboratory for ALT testing. The result of donor ALT testing was generally not known at the time the corresponding blood unit was transfused; moreover, since the implications of the ALT test were still under study, no attempt was made to withhold blood units found to have an elevated ALT level. The recipients of all blood units were followed up from the day of surgery. Weekly or biweekly serum samples were obtained from patients during the first three postoperative months; monthly samples were then obtained for an additional three months and a final sample drawn nine months after surgery. Each sample was tested for ALT, AST (aspartate aminotransferase, SGOT), bilirubin, and hepatitis B surface antigen (HBsAg); (HBsAg was tested by solid-phase radioimmunoassay). In addition, pretransfusion and 3-, 6-, and 9-month posttransfusion samples were tested for antibody to HBsAg (anti-HBs) and pretransfusion, three- and six-month samples were tested for antibody to heptitis B core antigen (anti-HBc). There were 2,952 (88%) donors also tested for anti-HBs. Both anti-HBs and anti-HBc were tested by solid-phase radioimmunoassay.

Criteria for Diagnosis of Posttransfusion Hepatitis

Hepatitis was diagnosed when, between two and 26 weeks after transfusion, a patient with a normal preoperative ALT level demonstrated a rise in the level of ALT to 2.5 times the upper limit of normal (110 IU/L), followed one or more weeks later by an elevation at least two times the upper limit of normal (88 IU/L). Nonviral causes of transaminase elevation, such as drug toxic hepatitis, anesthesia, alcoholism, anoxia, shock, congestive failure, and sepsis, had to be reasonably excluded. When viral hepatitis seemed to be the most likely cause of transaminase abnormalities, serological tests were performed to establish the responsible viral agent. Hepatitis B was diagnosed if the patient showed development of HBsAg during the acute phase of illness, and/or seroconverted for anti-HBs or anti-HBc. Hepatitis A was diagnosed if the development of antibody to the hepatitis A virus (HAV) occurred in temporal relationship to the appearance of transaminase abnormalities. Antibody to HAV was measured by solid-phase radioimmunoassay. Antibody seroconversion to the Epstein-Barr virus (EBV) was sought by immunofluorescence and to the cytomegalovirus (CMV) by indirect hemagglutination. The diagnosis of NANB hepatitis was made only when there was reproducible elevation of the ALT level, as previously described, when nonviral causes of these ALT elevations could be reasonably excluded, and when there was no serological evidence for infection with hepatitis B virus (HBV), HAV, or EBV; five cases with CMV seroconversion were considered as NANB hepatitis for the purpose of this analysis, since the possibility of simultaneous NANB and CMV infection could not be excluded and since a previous study[2] indicated that CMV seroconversions occur with equal frequency among blood recipients who do or do not have development of hepatitis. The statistical associations described in this article were similar whether or not the possible CMV cases were included in the analysis.

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Table 1.—Association of Elevation in ALT Levels in Donors and Hepatitis in Recipients*

Maximum ALT Level Among Donated Units, IU/L (SD)†	Recipients		Donors	
	No. Tested	No. (%) With Hepatitis	No. Tested	No. (%) Associated With Hepatitis in Recipients
≤33 (≤1.5)	162	14 (8.6)	3,179	422 (13.3)
34-53 (>1.5-2.25)	69	7 (10.1)	124	17 (13.7)
54-88 (>2.25-3.0)	38	10 (26.3)	42	11 (26.2)
88 (>3.0)	14	5 (35.7)	14	5 (35.7)
Total				
≤53 (≤2.25)	231	21 (9.1)‡	3,303	439 (13.3)§
>53 (>2.25)	52	15 (28.8)‡	56	16 (28.6)§

*ALT indicates transaminase.
†At least one donor with ALT level in that range; no donor with ALT greater than indicated limit.
‡χ^2=14.9; P<.001.
§χ^2=9.7; P<.01.

Table 2.—Relationship of Donor ALT Level and Transfusion Volume to Recipient Hepatitis*

Maximum Donor ALT Level, IU/L (SD)	No. of Recipients	Average No. of Units Transfused	Recipient Hepatitis	
			No. (%)	No. of Cases per 1,000 Units
≤33 (≤1.5)	162	11.2	14 (8.6)	7.7[A]
34-53 (>1.5≤2.25)	69	12.9	7 (10.1)	7.9[B]
≥54 (≥2.25)	52	14	15 (28.8)	20.7[C]

*ALT indicates transaminase; B vs A, not significant; C vs A or B, P<.001.

Transaminase Testing

Tests for ALT in donor serums were performed by a commercial laboratory, using a kinetic assay on a biochromatic analyzer. A frequency distribution was calculated for 499 consecutive donors in this study and the geometric mean, mean log (base 10), and SD determined. The geometric mean was 12.0 IU/L, the mean log 1.08, and the SD of the mean log, 0.29. The antilog of the mean log plus 2 SDs was 44 IU/L; seven donors (1.4%) exceeded this level. The range of ALT for all 3,359 donors was 1 to 195 IU/L.

Recipient serum samples were tested in the Clinical Chemistry Laboratory, National Institutes of Health (NIH), in a three-point kinetic assay employing a sequential computer-controlled biochemical analyzer. The geometric mean for this assay was 14.6 IU/L, the mean log 1.16, and the SD of the mean log, 0.65. Four of 206 normal control subjects (1.9%) exceeded an ALT of 44 IU/L, and this level was taken as the upper limit of normal for the laboratory.

So the results could be applied to other laboratories, donor transaminase limits in this study are stated in terms of SD from the mean log. The mean log and SD were used because ALT values were found to follow a log normal rather than normal distribution. Equivalent ALT values in international units per liter correspond to the antilog of each log value. The following ALT donor ranges (given as deviations from the mean log value) were examined in this study: ≤1.5 SD (≤33 IU/L); >1.5-2.0 SD (34-44 IU/L); >2.0-2.25 SD (45-53 IU/L); >2.25-2.5 SD (54-63 IU/L); >2.5-3.0 SD (64-88 IU/L); and >3.0 SD (≥89 IU/L).

Statistical Methods

Unless otherwise stated, statistical analyses were based on comparisons in contingency tables and results expressed as χ^2 and its P value.

RESULTS

Relationship of Magnitude of Donor ALT Level to Recipient Hepatitis

Of the 283 recipients in this study, 36 (12.7%) had development of hepatitis. Of the 36 hepatitis cases, 35 (97%) were classified as NANB. Table 1 depicts the risk of recipient hepatitis according to the maximum ALT level of the donated units. The majority of patients (162) received blood with an ALT level of 33 IU/L or less. Of these recipients, 14 (8.6%) had hepatitis develop. Hepatitis incidence did not change appreciably (10.1%) among 69 recipients of blood, in which at least one donor had an ALT level between 34 and 53 IU/L. There was, however, a sharp increase in hepatitis incidence among recipients of blood, in which at least one donor had an ALT level between 54 and 88 IU/L (26.3%), and the incidence increased still further (35.7%) when there was a donor with an ALT level greater than 88 IU/L. The incidence of hepatitis among recipients of blood in which all donor ALT levels were 53 IU/L or less was approximately one third that among recipients of at least 1 unit of blood with an ALT level greater than 53 IU/L (P<.001).

Table 1 also shows the relative frequency of donors associated with a case of hepatitis according to ALT level. Of 3,179 persons with an ALT level of 33 IU/L or less, 422 (13%) donated a unit of blood to a patient who subsequently had hepatitis develop. As the level of donor ALT increased, the frequency with which recipients of that blood had hepatitis develop also increased; donors with an ALT level greater than 53 IU/L were significantly more likely to be involved in a case of posttransfusion hepatitis than donors with an ALT level of 53 IU/L or less (P<.01).

Relationship of Posttransfusion Hepatitis to Transfusion Volume

Since all patients received multiple units of blood, the volume of blood administered introduces a variable that must be distinguished from the effect of donor ALT. Table 2 therefore examines transfusion volume in relation to donor ALT level. Patients who received blood with increasingly higher ALT levels were, on the average, transfused with increasingly larger volumes of blood. To equalize the effect of transfusion volume in each range of donor transaminase, the data are expressed as hepatitis cases per 1,000 units transfused. When transfusion volume was maintained constant in this manner, the number of hepatitis cases per 1,000 units transfused increased from 7.8 to 20.7 for those receiving blood with ALT levels lower and higher than 53 IU/L, respectively (P<.001).

Table 3 indicates that hepatitis incidence increased stepwise as the range of the number of units transfused increased from 1 to 6 up to 10 to 12. Thereafter, the incidence of hepatitis reached a plateau despite increasing transfusion volume. Although the risk of hepatitis did not increase significantly at any of the higher transfusion volumes, the trend suggested that transfusion volume might be a confounding variable in the interpretation of the effect of an elevated ALT level. To evaluate the variable of transfusion number fur-

Table 3.—Impact of Donor ALT Levels at Various Transfusion Volumes*

No. of Transfusions	No. of Recipients	No. of Cases of Recipient Hepatitis (%)
1-6		
ALT ≤53	46	2(4.4)
ALT >53	5	1(20.0)
Total	51	3(5.9)
7-9		
ALT ≤53	44	4(9.1)
ALT >53	5	2(40.0)
Total	49	6(12.2)
10-12		
ALT ≤53	42	4(9.5)
ALT >53	10	5(50.0)
Total	52	9(17.3)
13-15		
ALT ≤53	57	8(14.0)
ALT >53	13	2(15.4)
Total	70	10(14.3)
>15		
ALT ≤53	42	3(7.1)
ALT >53	19	5(26.3)
Total	61	8(13.1)

*ALT indicates transaminase; ALT levels measured in international units per liter; weighted mean difference (see text)=14% ($P<.001$).

Table 4.—Impact of Donor ALT Testing at Various Exclusion Levels*

Exclusion level	Mean Log+Indicated SD				
ALT Equivalent, IU/L	1.5 >33	2.0 >45	2.25 >53	2.5 >63	3.0 >88
χ^2†	5.68	7.85	14.9	4.07	7.01
P Value†	<.02	<.01	<.001	<.05	<.01
Crude efficacy‡	61	44	42	22	14
Corrected efficacy§	32	26	29	12	9
% Blood units excluded	5.3	2.6	1.6	1.0	0.4

*ALT indicates transaminase.

†Significance of association between donor ALT and recipient hepatitis at indicated exclusion level.

‡Maximum prevention based on assumption that unit with elevated ALT level was cause of hepatitis.

§Corrected for hepatitis caused by donors with normal ALT level (see text).

ther, the effect of receiving blood with an ALT level higher or lower than 53 IU/L was examined at each transfusion volume (Table 3). Among patients receiving 1 to 6, 7 to 9, or 10 to 12 units of blood, the incidence of hepatitis was strikingly higher if they received at least 1 unit of blood with an ALT level greater than 53 IU/L. Because of relatively small numbers at each ALT level, a weighted mean difference was calculated.[3] This method uses all the frequency information while preserving the difference in each subset. The weighted mean difference was found to be 14% ($P<.001$), indicating that when transfusion volume is maintained constant and, hence, removed as a variable, there is a highly significant association between donor ALT and recipient hepatitis.

Recipient Susceptibility to Infection

Analysis of demographic and serological characteristics of recipients indicated that patients who had received blood with or without an elevated ALT level did not differ significantly in their sex, age, race, history of hepatitis, history of blood transfusion, or type of cardiac surgery. They did, however, differ significantly in regard to past exposure to the HBV, as assessed by the presence of anti-HBs. Patients who received blood with an elevated ALT level had significantly less evidence of past exposure to HBV ($\chi^2=4.5$, $P<.05$). To distinguish the relative contributions of donor ALT level and recipient susceptibility, as implied by the absence of anti-HBs, the influence of elevated donor ALT level was examined in the 250 patients who did not have anti-HBs in their pretransfusion sample; of these, 199 received only donor blood with an ALT level of 53 IU/L or less. The incidence of hepatitis among the latter was 8.0%; in contrast, 51 patients without pretransfusion anti-HBs who received at least 1 unit of blood with a donor ALT level greater than 53 IU/L had a hepatitis incidence of 27%. The difference in these groups was significant ($P<.001$) and indicates that the level of donor ALT is an important determinant of recipient hepatitis when all recipients have similar susceptibility as judged by the absence of anti-HBs. The data could not be meaningfully analyzed for patients who had anti-HBs before transfusion, since only one of the 32 patients in this group received a unit of blood with an elevated ALT level.

Relationship of Donor ALT to Donor HBV Markers

Of 2,826 donors with an ALT level of 33 IU/L or less, 4.6% had anti-HBs, compared with 15.1% of 86 donors with ALT levels of 34 to 53 IU/L and 10% of 40 donors with ALT levels greater than 53. In composite, donors with an ALT level greater than 33 IU/L (1.5 SD) were significantly more likely to have anti-HBs than donors with an ALT value below this level ($\chi^2=18.6$, $P<.001$), indicating a higher frequency of past HBV exposure in the group with a higher ALT level.

Impact of Donor ALT Testing at Various Exclusion Levels

Table 4 shows the significance of the association between donor ALT and recipient hepatitis at specific ALT exclusion levels and also the percent of hepatitis that might be prevented and the number of donor units that would be sacrificed. Hepatitis prevention is expressed in two ways: (1) crude efficacy based on the assumption that in each hepatitis case where a donor had an elevated ALT level, exclusion of that donor would have prevented the hepatitis; and (2) corrected efficacy in which hepatitis incidence (I) is first calculated in those receiving only normal ALT blood. The number (N) of patients receiving blood with elevated ALT value is then multiplied by I; this establishes the number of cases that would have occurred if only blood with a normal ALT level had been transfused. This product (I×N) is subtracted from the observed number of cases in the group with elevated ALT levels (A) to estimate the number of cases presumably related to the unit with an increased ALT value. Dividing by the total number of observed cases (T) expresses the proportion of cases that might have been prevented by ALT testing: E (corrected efficacy)=100×[A−(I×N)]/T.

Table 4 indicates that as the exclusion level is increased from 1.5 to 2.25 SDs above the mean log, χ^2 increases from 5.68 to 14.9, and that beyond 2.25 SDs, the χ^2 begins to diminish. Thus, the most significant association between donor ALT and recipient hepatitis is achieved at an ALT exclusion level of 2.25 SDs, which in our laboratory was equivalent to an ALT level of 53 IU/L.

Table 5.—Frequency Distribution of ALT Values for 791 Consecutive NIH Donors*

SD	ALT Range, IU/L	No. of Donors With ALT In Range	% In Range
<2.25	0-10	184	23.3
	11-20	400	50.5
	21-30	143	18.1
	31-40	36	4.6
	41-50	17	2.1
Subtotal		780	98.6
>2.25	51-60	6	0.8
	61-70	3	0.4
	>70	2†	0.2
Subtotal		11	1.4

*ALT indicates transaminase; NIH, National Institutes of Health.
†71 and 134.

Table 4 also indicates that although crude efficacy seems distinctly better at low ALT exclusion levels, this is not true for corrected efficacy; there is no meaningful change in corrected efficacy between exclusion levels of 1.5 and 2.25 SDs. Above 2.25 SDs, corrected efficacy markedly diminishes. The number of donor units sacrificed diminishes greatly as one increases the exclusion level from 1.5 to 2.25 SDs.

Application of Donor Exclusion Rule to Other Laboratories

Since completion of the present study, ALT determinations on donor blood have been performed by the Hepatitis Testing Laboratory of the Clinical Center Blood Bank, NIH, rather than at an outside laboratory. This provided an opportunity to see whether the exclusion level chosen on the basis of the data collected in the prospective study could be applied to other laboratories. Using the solid-phase radioimmunoassay method, 791 consecutive NIH donors were tested and a new mean log, SD, and frequency distribution for ALT levels determined (Table 5). The 791 volunteer donors were bled during a single eight-week interval so that no donor was included twice. The vast majority of donors (92%) had ALT values below 30 IU/L, and 98.6% had ALT values below 2.25 SDs from the mean log. This frequency distribution would thus predict a loss of 1.4% of an all-volunteer donor population using an ALT exclusion level of 2.25 SDs. This percent of donors lost agrees closely with the corresponding percent of blood units lost (1.6) previously presented.

COMMENT

Since the sine qua non for the diagnosis of viral hepatitis in transfusion recipients is elevation of serum ALT or AST levels, and since these elevations tend to persist in patients in whom chronic hepatitis develops, it is not unreasonable to assume that some asymptomatic donors who carry a hepatitis virus might also have an abnormally high level of serum transaminase. This concept has been previously investigated,[4] but either because of the simultaneous use of commercial or HBsAg-positive donors or both, or because of insufficient numbers of recipients, incomplete follow-up, or low incidence of hepatitis, none of these studies provided compelling evidence to justify the adoption of routine donor ALT screening.

The most extensive study of the relationship of donor transaminase to recipient hepatitis was conducted by the TTV,[1] a large, prospective study involving four geographically distinct transfusion centers. Composed of more than 1,200 recipients and 4,700 transfused blood units, the TTV study showed that (1) the higher the level of donor ALT, the more likely the donor was to be associated with a case of NANB hepatitis; the relative frequency of association increased progressively from 3.4% in donors with an ALT value of 1 to 14 IU/L to 48.9% in donors with an ALT level greater than 40 IU/L ($P<.01$); (2) the hepatitis attack rate among recipients varied according to the highest donor ALT unit received, ranging from an attack rate of 4.3% in those who received only blood with an ALT level less than 14 IU/L to 50% for those receiving at least 1 unit with an ALT level greater than 60 IU/L; (3) the same relationship between recipient hepatitis and the extent of donor ALT elevation held for 225 patients who received only single-unit transfusions (among such patients, the hepatitis attack rate was ten times higher in those receiving blood with an ALT level greater than 45 IU/L than in those given blood with an ALT level less than 45 IU/L); and (4) the hepatitis risk increased dramatically if more than 1 unit of blood with an elevated ALT level was administered; ten of 11 patients receiving 2 units of blood with an ALT value greater than 45 IU/L showed development of hepatitis.

The results presented here confirm those of the TTV report, except that we could not analyze the effect of elevated ALT level in respect to single-unit transfusion. As in the TTV study, our recipients were increasingly liable to have hepatitis develop the higher the ALT level of the donor and, conversely, the higher the donor ALT level, the more likely that donor was to be associated with a case of posttransfusion hepatitis. The incidence of hepatitis among recipients of at least 1 unit of blood with an ALT value greater than 53 IU/L (2.25 SDs) was strikingly greater than the incidence among recipients of blood in which all ALT levels were less than 53 IU/L ($P<.001$).

To exclude the possibility that the observed relationship between donor ALT and recipient hepatitis was coincidental, a number of donor and recipient variables were assessed. In addition to donor ALT level, only the volume of blood transfused and the hepatitis B immune status of the recipient showed a possible relationship to recipient hepatitis. Since the more blood received, the greater the probability that at least 1 unit would have an elevated ALT value, the possibility existed that the observed association of donor ALT with hepatitis was coincidental to increased transfusion volume and the likelihood of receiving an infectious unit irrespective of donor ALT. However, this does not seem to be the case; when transfusion volume was equalized among recipient groups by expressing hepatitis risk as cases per 1,000 units received (Table 2), or by examining the level of ALT as a variable at each transfusion level (Table 3), there remained a significant increased hepatitis risk in those recipients of blood with an elevated ALT level ($P<.001$).

In the absence of specific serological tests for the agent or agents of NANB, there is no way to assess directly the hepatitis susceptibility of transfusion recipients. If, however, populations or persons with increased exposure to HBV also have increased exposure to NANB, then the presence of antibody to HBV might be used as an indirect measurement of immunity to NANB. This is of relevance to the

current study, since recipients of blood with normal ALT levels had an increased prevalence of anti-HBs in their pretransfusion sample ($P<.05$), suggesting they may have been less susceptible to both HBV and NANB hepatitis viruses than recipients of blood with an elevated ALT level. The importance of donor ALT as a hepatitis risk factor was, however, distinguished from the variable of recipient susceptibility by examining the influence of ALT only in recipients with similar pretransfusion anti-HBs status.

The essence of this study is summarized in Table 4, where hepatitis association, hepatitis prevention, and donor loss are calculated at various donor ALT exclusion levels. It can be seen that the most significant, and presumably specific, association between donor ALT and recipient hepatitis is achieved at a donor exclusion level of 2.25 SDs above the mean log ALT level. The considerably higher χ^2 is a compelling reason to choose 2.25 SDs as the appropriate exclusion level; this is further emphasized when both efficacy and donor loss are considered. When one corrects for hepatitis caused by blood units with a normal ALT level (corrected efficacy—see "Results"), the percent of hepatitis prevented does not differ appreciably using cutoffs of 1.5, 2.0, and 2.25 SDs. Beyond 2.25 SDs, there is a striking decrease in corrected efficacy, suggesting that exclusion levels above 2.25 SDs have little practical value even though they have the enticing feature of reduced donor loss. Exclusion levels below 2.25 SDs do not offer a significant advantage in corrected efficacy but result in the loss of considerably more donor units. In this study then, an exclusion level of 2.25 SDs is the most advantageous in that it correlates highly with the development of posttransfusion hepatitis (PTH) ($P<.001$), in that it potentially prevents 29% of PTH, and in that it results in the loss of only 1.6% of blood units.

The TTV study predicted that exclusion at a donor ALT level of 45 IU/L would prevent approximately 40% of PTH; however, this prediction is based on the crude, rather than the corrected, efficacy and, hence, is probably too high. Using the TTV data on single-unit transfusions, where no correction is necessary, four of the observed hepatitis cases might have been prevented if donors with elevated ALT levels were excluded. This represents a 28.5% hepatitis reduction, a figure virtually identical to the 29% derived in our study.

It is important to emphasize the negative aspect of the donor ALT-recipient hepatitis relationship, namely, that 70% of PTH will not be prevented by screening donors for ALT. In addition, 40 (72%) of the 56 donors with elevated ALT levels were not associated with a case of PTH. While some of these elevated ALT units were undoubtedly transfused to patients who were not susceptible to the NANB virus, and others may have resulted in hepatitis too mild to meet the criteria of our study, it is probable that many donors with elevated ALT levels were not, in fact, carriers of a hepatitis virus. These imperfect correlations reflect the nonspecific nature of the ALT test and emphasize that adoption of donor ALT screening will, at best, be an interim measure. Continued vigorous pursuit of a specific serological test for the agent or agents of NANB is mandatory.

The NIH and TTV studies combined provide data on more than 8,000 donors and 1,500 recipients and have important implications for blood transfusion services, raising many difficult ethical and practical issues. Paramount among these is the question of whether the findings now available are sufficient to require that routine donor screening for ALT be instituted or whether a randomized, controlled, prospective study is needed to confirm that the predicted reduction in PTH can actually be achieved. Many of the current considerations are similar to those raised by the introduction of tests for HBsAg. Indeed, even the projected extent of hepatitis prevention (30%) is similar to that predicted and then confirmed for HBsAg testing. There are, however, two major differences. First, the ALT test does not identify a specific viral marker but is a nonspecific test identifying a variety of nonviral as well as viral disorders. Second, donor loss will amount to 15 to 30 per 1,000 instead of the one to three per 1,000 that occurred with HBsAg testing.

For the blood recipient, the ALT test offers new hope for hepatitis prevention; for the donor, it offers new information, but perhaps information that is not really desired; for the blood supplier, it increases the complexity and cost of blood delivery and reduces the available amount of a product already in critically short supply. The ALT testing of donors is thus in a tenuous balance between risk and benefit. The balance shifts toward testing when one considers that approximately 30% of PTH might be prevented (90,000 cases per year in the United States), but this is tempered by the realization that 70% will not be prevented and that even the prevention of 30% is in some doubt unless confirmed by a randomized clinical trial. The balance also shifts away from testing when one considers the estimated additional $20 million in the annual cost of blood in the United States alone and the potential national loss of 45,000 donors and more than 90,000 blood units. It is a difficult equation, whose solution will require thought and planning.

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Gamma glutamyl transpeptidase

V. sensitive

Occasional Survey

POST-TRANSFUSION HEPATITIS IN AUSTRALIA

Report of the Australian Red Cross Study

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Summary Post-transfusion hepatitis developed in 2% of 842 cardiac-surgery patients surveyed in Sydney (4 cases per 1000 units of transfused blood). 3 of the 18 cases were caused by hepatitis B virus even though all units of blood which contained hepatitis B surface antigen (HBsAg) had been rejected. 1 case was caused by cytomegalovirus, and there were 14 (78%) cases of non-A, non-B hepatitis. A significantly higher proportion of the units of blood given to the patients in whom non-A, non-B hepatitis developed contained antibodies against both hepatitis B core antigen and HBsAg than the units of blood given to the other patients. Rejection of blood with these markers of past exposure to hepatitis B may reduce the incidence of post-transfusion non-A, non-B hepatitis by up to a half.

INTRODUCTION

ROUTINE testing of blood donations for hepatitis B surface antigen (HBsAg) was instituted in Australia in 1970. Radioimmunoassay (RIA) supplanted testing by counter-current electrophoresis and passive haemagglutination in 1976, and 0·06% of blood donations are now rejected because of a positive RIA result. Exclusion of donors who have been jaundiced within the previous 2 years is the only other measure used to prevent post-transfusion hepatitis.

When HBsAg screening became mandatory in the U.S.A. the prevalence of hepatitis after transfusion of blood from unpaid volunteer donors fell to about 4% among cardiac-surgery patients,[1] and the proportion of cases caused by hepatitis B virus also fell, so that now about 90% of cases are anicteric and show no serological evidence of hepatitis A or B.[2] The epidemiology of non-A, non-B hepatitis is thought to resemble that of hepatitis B, but is is not known whether countries such as Australia, with a very low hepatitis-B-carrier rate, also have a low prevalence of non-A, non-B hepatitis.

Although rejection of HBsAg-positive blood donations prevents many cases of hepatitis B, there have been several well-documented instances of transmission of hepatitis B by blood which was HBsAg negative but contained antibody to hepatitis B core antigen (anti-HBc).[3] It is not clear whether all units of anti-HBc-positive blood are infective or whether the proportion that is infective bears a constant relation to the overall HBsAg-carrier rate. It is difficult to decide whether testing for anti-HBc would prove cost-effective for the transfusion service.

PATIENTS AND METHODS

Patients

The study began in January, 1979, and the last patient was admitted to the study in December, 1980. Patients were interviewed soon after admission to the cardiac-surgery units at Royal Prince Alfred and St Vincent's Hospitals and invited to participate in the study. If they agreed, a specific hepatitis history was taken, and a preoperative blood sample was obtained. The composition of the study group is shown in table I.

TABLE I—POST-TRANSFUSION HEPATITIS STUDY PATIENTS

	RPA	St V	Total
Total no. of patients 1979–80	1009	1400	2409
Excluded:			
Domicile remote	144	1052	1196
HBsAg carrier	2	3	5
Remainder suitable	863	345	1208
Number agreeing to participate	813	322	1135
Number completing study	583	259	842

RPA = Royal Prince Alfred Hospital; St V = St Vincent's Hospital.

All the packs and bottles which had contained material transfused to a patient at operation and in the recovery wards were placed in a container labelled with the patient's name. The used packs and bottles were collected daily and checked against the transfusion records. Any further blood needed was made up from material held by the blood group serology laboratories. Blood samples were taken from each patient 2, 4, 8, 12, 16, and 24 weeks after operation. Serum bilirubin, aspartate transaminase (AST), and alanine transaminase (ALT) levels were measured locally before the specimens were sent to the hepatitis serology laboratory. First (preoperative) and last (24-week) samples from each patient were tested for HBsAg, anti-HBs, anti-HBc, and hepatitis A antibody (anti-HAV). First samples were tested for cytomegalovirus antibody (anti-CMV), and first and last samples from patients who were negative preoperatively were retested for anti-CMV. All the samples of blood and plasma were stored at −20°C.

Laboratory Methods

Biochemistry.—Serum transaminase levels were measured with automatic methods (Technicon 'S.M.A.C.' or Union Carbide 'Centrifichem').

Hepatitis B serology.—The same methods were used to test both the patients' serum samples and the material from transfusion. HBsAg and anti-HBs were measured with commercial RIA ('Ausria' and 'Ausab', Abbott, North Chicago, Illinois). Anti-HBc was measured with two methods. Patients' serum samples were tested both with commercial RIA ('Corab', Abbott) and with countercurrent electrophoresis;[4] cores extracted from liver were used as antigen. Material for transfusion was tested with the latter method only. Hepatitis B e antigen (HBeAg) and antibodies against e antigen were measured by both gel diffusion and radio-immunoassay (Abbott).

Hepatitis A antibody was measured with commercial RIA ('Havab', Abbott).

Anti-CMV was measured by means of complement fixation with antigen supplied by Behring, Marburg.

Infectious mononucleosis.—'Cellognost' (Behring) was used to demonstrate heterophile antibody.

Diagnosis of Hepatitis

The serum transaminase levels were regarded as the best indicators of liver damage. If either transaminase level was higher than 2·5 times the upper limit of the normal range (i.e., AST greater than 150 U/l, ALT greater than 88 U/l, a second sample of blood was obtained between 1 and 2 weeks later. If the result was still higher than normal the patient was assessed clinically, and further blood samples were obtained approximately every 2 weeks until the transaminase levels were normal on three successive occasions. As the study progressed we decided not to recall patients immediately if their 2-week follow-up sample was abnormal but to rely on the 4-week sample for verification of the result.

Patients whose transaminase levels were raised on two successive occasions were classified as having post-transfusion hepatitis if there was no obvious alternative diagnosis.

TABLE II—FACTORS RELATED TO PARTICIPANTS' SUSCEPTIBILITY TO HEPATITIS

—	RPA (n=583)	St V (n=259)	Total (n=842) no.	(%)
Age: mean and range (yr)	53·2 (17–84)	54·6 (21–70)	53·9 (17–84)	..
Male/female ratio	5·2/1	6·2/1	5·5/1	..
Previous transfusion	132	29	161	(19)
Previous jaundice or hepatitis	91	26	117	(14)
Born in Australia or U.K.	502	237	739	(88)
Recent travel abroad	197	64	261	(31)
Wartime service in Middle East or Pacific campaign	155	48	203	(24)
Non-medical inoculations (acupuncture, tattoo, &c).	60	7	67	(8)
Previous contact with hepatitis	129	53	182	(22)

RPA = Royal Prince Alfred Hospital; St V = St Vincent's Hospital.

TABLE III—AMOUNT OF BLOOD AND BLOOD PRODUCTS USED

Material	No. units used		
	RPA	St V	Total
Whole blood	1372	650	2022
Packed cells	768	430	1198
Autotransfusion	0	52	52
Fresh platelets	12	0	12
Platelet concentrates	620	34	654
Fresh-frozen plasma	137	3	140
Cryoprecipitate	51	5	56
Cryosupernate	195	136	331
Fibrinogen	15	0	15
Factor VIII	34	0	34
Stabilised plasma protein solution	88	8	96
'Haemaccel'	11	0	11
Cardioplegic solution	168	0	168
Total	3471	1318	4789
Average per person	6·0	5·1	5·7

RPA = Royal Prince Alfred Hospital; St V = St Vincent's Hospital.

All follow-up samples of all hepatitis patients were then tested for anti-HAV, anti-CMV, HBsAg, anti-HBc, and anti-HBs. The first samples with abnormal enzyme levels were tested by Cellognost for evidence of Epstein-Barr virus (EBV) infection.

RESULTS

Prevalence of Post-transfusion Hepatitis

Some of the epidemiological factors which might have influenced the patients' susceptibility to hepatitis are listed in table II. Most of the patients were middle-aged men of anglo-saxon descent. In all but a few patients there was no specific reason for suspecting hepatitis-B exposure, and only 3 patients (including 1 Noumean and 1 from Laos) were HBsAg-positive. Anti-HAV and anti-CMV were found in over 80% of the patients; however, these rates are not significantly different from those expected for the 50–60-year-old age group in the general population in Australia (80%).[5] In contrast only 53 (6%) had anti-HBs as evidence of immunity to hepatitis B.

The indications for surgery and the operations performed were similar in the two hospitals. 648 patients (77%) had coronary-artery grafts, and 152 (18%) had valve repair or replacement; combined coronary grafts and valve repair accounted for most of the others. All the operations required cardiac bypass and transfusion. The quantities of blood used per patient were also quite similar in the two hospitals (table III); an average of 5·7 units was used for each patient in the study. Two-thirds of all the material used was whole blood or packed cells, and the Sydney Blood Bank was the only source. Since both the patients admitted and the surgical procedures used at the two hospitals were similar and there was only one centre supplying blood, the results have been pooled for analysis.

Each finding of raised serum transaminase levels is shown in table IV. Haemolysis, shown by raised potassium levels in the same sample, accounted for only 21 out of the total of 278. Repeated samples showed raised transaminase levels in only

TABLE IV—RAISED TRANSAMINASE LEVELS AND THEIR CAUSES

—	No. patients with			
	Raised transaminase	Raised transaminase and potassium	Repeat transaminase raised	Reason for elevation in patients where repeat also raised*
Preoperative	17	0	2	1 haemophiliac with chronic liver disease. 1 with history of alcohol abuse.
Weeks after operation				
2	158	2	6	5 difficult post-operative course. 1 cholecystitis.
4	24	6	7	2 ? post-transfusion hepatitis. 4 continued postoperative problems. 1 past treatment for alcoholic liver disease.
8	24	4	12	10 ? post-transfusion hepatitis. 2 with history of alcohol abuse.
12	23	5	7	5 ? post-transfusion hepatitis. 1 reoperation. 1 pneumonia and heart failure.
18	19	2	5	1 post-transfusion hepatitis. 1 diabetic. 1 alcoholic. 2 severe heart failure.
24	13	2	0	..
Total	278	21	39	..

Only the first occasion is indicated for each patient. *18 ? post-transfusion hepatitis, 21 other causes.

TABLE V—HEPATITIS SEROLOGY ON PATIENTS WITH RAISED TRANSAMINASE LEVELS

No. of patients and category of hepatitis	Anti-HAV		HBsAg in any sample	Anti-HBs		Anti-HBc		Anti-CMV	
	First sample	Last sample		First sample	Last sample	First sample	Last sample	First sample	Last sample
18 Post-transfusion hepatitis	15	15	3*	1	3	1	4	12	15†
21‡ Patients with repeated high enzyme levels due to other causes	16	16	0	2	2	2	2	13	13
803 Patients with normal enzyme levels or with enzyme levels raised on only 1 occasion§	652/799	653/799	0	50/797	54/797¶	37/799	38/799¶	691/778	710/778
842 Total (%)	683/838 (82)		..	53/836 (6)		40/838 (5)		716/817 (88)	

First sample taken before and last sample 24 weeks after operation.
*3 cases of hepatitis B.
†Includes 1 case caused by CMV; 2 of the hepatitis B patients showed increases in anti-CMV.
‡No evidence of infection with hepatitis A or B, EBV, or CMV.
§Denominator = no. patients whose tests were technically satisfactory.
¶Retesting serial samples from all patients with seroconversions to HBs or HBc showed they were due to passive transfer of antibody by transfusion.
1 of the patients with normal enzyme levels had EBV post-pump syndrome.

39 cases. The large number of raised enzyme levels in the samples obtained 2 weeks after operation presumably reflects surgical trauma.[6] In 21 of the patients whose enzyme levels were raised in repeated samples there was no serological evidence of recent hepatitis A or B infection, infectious mononucleosis, or CMV infection (table V). Most of these patients had normal enzyme levels at the next follow-up test, but some had abnormal results after one or more normal results. Non-infective causes of the raised enzyme levels were found in all 21 (table IV).

There were 18 cases of probable post-transfusion hepatitis, a rate of 2% of study patients or approximately 4 cases per 1000 units of transfused blood. None of these were caused by hepatitis A virus or EBV. 3 were caused by hepatitis B virus and 1 by CMV. The remaining 14 have been classified as non-A, non-B hepatitis. The serological evidence for these aetiological diagnoses is shown in table V.

The preoperative and last (24-week) samples from all the patients whose enzyme levels remained normal were also tested for anti-HAV, HBsAg, anti-HBs, and anti-CMV (table V). There was one seroconversion to hepatitis A without any rise in serum transaminase, which was due to passive transfer of antibody in the transfused blood. The 4 patients who acquired anti-HBs also did so by passive transfer of antibody in the transfused blood. 19 patients acquired anti-CMV during convalescence; of these, 14 had received blood less than 4 days old. The single patient with infectious mononucleosis did not have raised transaminase levels. Her symptoms were fever and malaise, and the incubation period was 3 weeks.

Clinical Features of Post-transfusion Hepatitis

Hepatitis B virus had a longer incubation period and produced more severe illness than the other viruses, but 3 of the 14 patients classified as having non-A, non-B hepatitis still had abnormal liver-function tests at the end of follow-up. The clinical features of all the patients with post-transfusion hepatitis are summarised in table VI.

Source of Infection

The transfused material was examined in an attempt to identify the source of infection. The amounts of blood and the different blood products given to the patients in whom hepatitis developed are shown in table VII together with the results of serological tests on the transfused samples.

Hepatitis B occurred in 3 patients who had received blood screened for HBsAg. Retesting of samples of the relevant blood confirmed that each was indeed HBsAg-negative. Patient 256 received 1 unit of blood which contained anti-HBc, but no other hepatitis markers, and 6 other units which were negative in all the tests. The suspect donor was recalled, and the test results were confirmed on a new sample of blood. The donor had experienced an attack of hepatitis of uncertain type 3 years previously, so it was thought highly probable that his blood was still infectious, although it now contained e antibody. The recipient's preoperative ALT level was slightly raised, and he had undergone a cholecystectomy 1 year previously. All his serum samples gave positive results in the RIA test for HBeAG, and the result of a preoperative RIA for anti-HBs was close to the cut-off point for a positive

TABLE VI—CLINICAL FEATURES OF POST-TRANSFUSION HEPATITIS

Category of hepatitis	No. of patients	Mean incubation period (range, weeks)	Number with			Duration and outcome
			Jaundice	Malaise	Immune complex disease	
Hepatitis B	3	14 (12–18)	3	3	2	1 died, week 30 1 recovered after 24 weeks 1 liver function still abnormal after 20 weeks
CMV	1	7	0	0	0	1 liver function still abnormal after 10 weeks
Non-A, non-B hepatitis	14	7·7 (4–12)	2	3	0	10 recovered, liver function normal after 3–44 weeks (mean 19 weeks) 4 disease still active after 12, 28, 38, and 44 weeks

TABLE VII—BLOOD AND BLOOD PRODUCTS GIVEN TO PATIENTS IN WHOM HEPATITIS DEVELOPED

Category of hepatitis and patient no.	No. units				No. units containing:				*No. new donors
	Blood	Plasma	SPPS	Cryos	HBsAg	Anti-HBc only	Anti-HBc + anti-HBs	Anti-HBs only	
Hepatitis B									
256	7	0	1	0	0	1	0	0	3
319	4	0	0	0	0	0	1	0	0
326	52	12	0	0	0	0	1	2	7
CMV									
670	3	0	0	0	0	1	0	0	0
Non-A, non-B									
15	4	0	0	0	0	0	1	0	0
20	29	9	4	0	0	0	1	1	2
221	25	2	1	4	0	0	1	2	1
422	12	0	0	2	0	0	0	1	1
508	14	4	0	0	0	0	0	0	1
573	4	0	0	0	0	0	1	0	0
574	2	0	0	0	0	0	1	0	1
576	4	0	0	2	0	0	1	0	3
768	11	0	0	3	0	0	0	1	0
23	1	0	0	0	0	0	0	0	1
35	4	0	0	0	0	0	1	1	1
134	8	0	0	0	0	0	0	0	2
315	2	0	0	0	0	0	0	0	0
316	2	0	0	0	0	0	0	0	0

*Refers only to blood. SPSS = stabilised plasma protein solution. Cryos = cryosupernatant.

result. These results were attributed to an autoimmune phenomenon rather than to previous exposure to hepatitis B because of the patient's current parathyroid problem.

It was difficult to ascribe either of the other 2 cases of hepatitis B to transfusion. Although both patients received a unit of blood containing anti-HBc, anti-HBs was also present in both units. Patient 319 had her operation 2 days after patient 326 in the same theatre, so we investigated the possibility of nosocomial spread. Different surgical teams were involved, but the staff were not tested for HBsAg. The preoperative cardiac catheterisations were carried out on Jan. 11, 1980 and Nov. 26, 1979. All patients were tested for HBsAg before admission, and no hepatitis-B carriers were present in the cardiac unit during this period. The other 5 patients operated on in the same week were all included in the survey, but post-transfusion hepatitis did not develop in any of them.

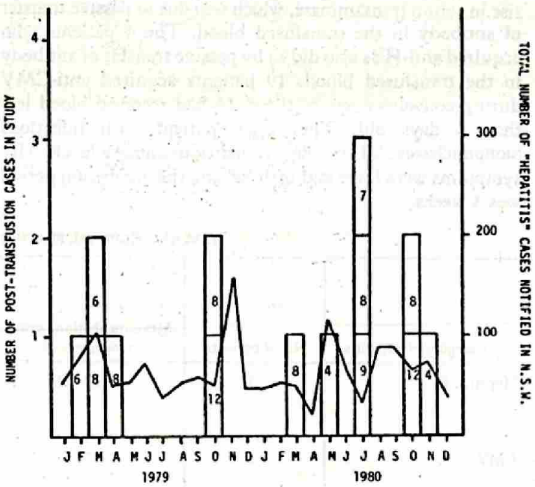
Patient 326 was 55 years old and had had severe rheumatoid arthritis for many years. It was thought that both his coronary-artery disease and his arthritis might be manifestations of hepatitis B immune-complex disease, but this could not be substantiated by laboratory testing. His preoperative sample and samples of serum taken 2 years previously and 2 and 4 weeks after operation were negative for HBsAg and anti-HBc; 18 weeks after transfusion HBsAg and anti-HBc appeared, and the patient became jaundiced. The background of patient 319 contained nothing to suggest any exposure to hepatitis B other than her operation in the same theatre as patient 326.

The cases of non-A, non-B hepatitis also showed some temporal clustering (see accompanying figure). The operations on patients 573, 574, and 576 were performed on 3 successive days in one hospital and those on patients 315 and 316 4 days apart in the other hospital, but no suspected hospital contacts could be found. There was no obvious relation of the clusters of cases to periods of increased notification of hepatitis in New South Wales as a whole (figure).

Testing of the blood given to the patients in whom non-A, non-B hepatitis developed showed that a significantly higher proportion of units (table VIII) contained both anti-HBs and anti-HBc than units given to patients in whom hepatitis did not develop ($p<0.005$).

DISCUSSION

Post-transfusion hepatitis is still a problem in Sydney. It affected 2% of the study patients. 1 of the 3 patients with hepatitis B died, and 3 of the 14 patients with non-A, non-B hepatitis still had abnormal liver-function tests at the end of the survey, although their acute infection was mild and asymptomatic. Both the relative proportion of hepatitis B and the clinical features of the two diseases were similar to those reported from other cardiac-surgery centres with volunteer donor panels.[2,7]



Seasonal incidence of non-A, non-B post-transfusion hepatitis.

Incubation period for each study case is shown in weeks.
Total notified excludes known cases of hepatitis B.

TABLE VIII—RESULTS OF HEPATITIS B SEROLOGICAL TESTING OF TRANSFUSED MATERIAL

	Total number	% positive samples (*no. positive/no. tested*)*			
		Anti-HBs only	Anti-HBc only	Anti-HBs+ anti-HBc	Any hepatitis B markers
Donations given to 824 patients in whom hepatitis did not develop (CMV patient excluded)	4552	2·7 (*35/1301*)	0·2 (*5/3142*)	0·5†(*20/4443*)	3·4
Donations given to 3 patients in whom hepatitis B developed	79	2·6 (*2/79*)	1·3 (*1/78*)	2·6 (*2/78*)	6·5
Donations given to 14 patients in whom hepatitis non-A, non-B developed	155	4·8 (*6/154*)	0 (*0/154*)	5·2† (*7/154*)	10·0

*All donations for Royal Prince Alfred Hospital were first tested for anti-HBc by countercurrent immunoelectrophoresis and the positives were retested for anti-HBs by RIA. The converse order was used at St. Vincent's Hospital. There was no significant difference in the proportion of donations positive for both antibodies in the two hospitals. Percentages for anti-HBs are based on St. Vincent's Hospital only and those for anti-HBc on Royal Prince Alfred Hospital only.
†% positive for anti-HBs and anti-HBc: donations given to patients in whom hepatitis did not develop *vs* those given to non-A, non-B hepatitis patients; $p<0·0005$.

Clearly better control of both infections is needed, and various alternatives have been investigated to decide what can be achieved with current technology and within reasonable financial contraints.

Hepatitis B

Hepatitis B developed in 3 patients despite rejection of all blood donations giving positive results in the RIA test for HBsAg. Re-examination of all the units of blood involved also gave negative results. Since this test is now highly developed,[8] it seems improbable that increasing its sensitivity would be worth while.

Anti-HBc tests were proposed for blood-donor testing as soon as a satisfactory method was devised.[9] This still seems logical since anti-HBc is present in high titre in both acute hepatitis and in carriers and it remains detectable in convalescence, whereas HBsAg may disappear from the circulation weeks or months before anti-HBs is formed. Reactivation of infection has been observed[10] when patients are immunosuppressed during the phase of convalescence when HBsAg has disappeared but the patient has not yet produced anti-HBs. It is not surprising that infectivity of anti-HBc-positive blood has also been reported.[3] Although the infection of patient 256 would have been prevented by anti-HBc screening, the relevant blood donation would also have been rejected if the period of exclusion of donors after acute hepatitis had been longer.

Ranque[11] studied the consequence of transfusing blood obtained from donors at various intervals after acute hepatitis and found that the risk was greatest within the 1st year and declined slowly until the 5th year, after which the blood of donors with a history of hepatitis actually became safer than that of donors who had never been jaundiced. This work was done before HBsAg was recognised as a marker of hepatitis-B infection, but it correlates well with the time course of HBsAg clearance.

It is not certain that either screening for anti-HBc or extending the period of donor exclusion would have prevented the other 2 cases of hepatitis B in our survey, since we found no unequivocal evidence relating these infections to blood received at operation. It may be fortuitous that each of these patients received 1 unit of blood which contained both anti-HBs and anti-HBc, but similar observations have been reported by Katchaki et al.[3]

Anti-HBs is produced a variable time after the disappearance of HBsAg, and its presence in the patient correlates well with immunity to reinfection.[12] In general, studies of the transfusion of anti-HBs-positive blood[13,14] have shown that it is unlikely to transmit hepatitis B. These studies were carried out before anti-HBc screening tests were available, and they do not differentiate between units of blood containing both anti-HBs and anti-HBc and those containing anti-HBs alone. It may even become necessary to specify the class of anti-HBc, since the persistence of anti-HBc-specific IgM appears to indicate continuing activity of hepatitis B.[15]

Non-A, Non-B Hepatitis

The prevention of non-A, non-B hepatitis may be impossible while specific markers of infection are lacking. At present it is not even clear how many agents are involved or what their natural history might be. We hoped to obtain better insight into this problem by confining our study to one city because pooling of data from several centres could easily obscure significant epidemiological features. Some temporal clustering of cases of non-A, non-B hepatitis did occur (figure), which suggested that transfusion-associated non-A, non-B hepatitis might be the tip of an iceberg representing some common infection in the community. There was no relation of our clusters to peaks of notification of either hepatitis or gastroenteritis in Sydney.

Serological tests showed that many more of the donations given to patients in whom non-A, non-B hepatitis developed were positive for hepatitis-B markers than donations given to patients whose transaminase levels remained normal. This is likely to be an indirect relation reflecting the donors' occupational or environmental exposure to blood or blood products and hence to both hepatitis B and non-A, non-B hepatitis. The presence of anti-HBc as well as anti-HBs may be significant because anti-HBc remains detectable for a shorter time after acute hepatitis B than does anti-HBs. The presence of both antibodies could indicate exposure to blood-borne infection in the relatively recent past and so correlate with an increased risk of non-A, non-B infection.

None of our patients received single-unit transfusions, so we cannot be absolutely confident that we have identified the icterogenic units despite the statistical significance of the results. A similar difficulty was encountered by Aach et al.[16] when they analysed the consequence of transfusing blood with raised ALT levels. They found only 12 recipients of single units of such material among 1513 patients. Nevertheless they concluded from their overall results that the statistical association between elevated ALT in donor blood and recipient non-A, non-B hepatitis is sufficient to justify its biochemical testing as a routine. Our findings suggest that anti-HBc screening might have a similar effect of reducing by about half the number of cases of post-transfusion non-A, non-B hepatitis (table VII).

It may be that the units of blood with raised transaminase levels are the same as those containing anti-HBc. This

information should be sought without delay so that the value of introducing these tests can be assessed.

A major uncertainty in deciding on any policy change is the lack of long-term prospective studies of the outcome of non-A, non-B hepatitis. The progression from mild acute disease to chronic active hepatitis and the long persistence of abnormal serum-transaminase results have both been documented,[14,17] but there are as yet no reports of 10-year or even 5-year follow-up studies on patients in whom asymptomatic non-A, non-B hepatitis developed after transfusion. Our results suggest that the findings of such studies are unlikely to be as sinister as the first accounts suggested.

At present the commercial RIA tests for detecting anti-HBc are more expensive than those for HBsAg; the method is also more time-consuming, and some samples give equivocal results. The availability of substantial amounts of HBcAg made in *Escherichia coli* by means of genetic-engineering techniques[18] should now permit the development of a fourth generation of methods better adapted to the current needs in the transfusion service.

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Public Health

THE RISING PRICE OF MUSHROOMS

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HARD on the heels of reports on the rapid rise in heroin addiction,[1] the increasing drugs problem,[2] and continuing solvent inhalation[3,4] in Glasgow there has been an unprecedented epidemic of the abuse of indigenous hallucinogenic fungi. In September and October, 1981, 49 teenagers and young adults (44 males, 5 females; age range 12 to 28 years, mean age 17·5 years) presented to the accident-and-emergency department of four Glasgow teaching hospitals after deliberate ingestion of varying quantities of raw, freshly picked *Psilocybe semilanceata* (liberty cap), known colloquially as "magic mushrooms". 41 (83·7%) had evidence of sympathomimetic stimulation including mydriasis and tachycardia, while 47 (95·9%) had experienced or were experiencing euphoria and/or visual hallucinations. 4 patients had also ingested alcohol, but no other intoxicants had been taken. There was incomplete documentation of previous drug or alcohol abuse, but none had eaten "magic mushrooms" before, and none admitted to practising solvent inhalation. Gastric lavage was carried out in 39 (79·6%) patients. 35 (71·4%) of the 49 were admitted for observation, and all of these made a rapid and uneventful recovery without further therapy. Of the remainder, 13 were discharged after assessment and 1 refused to be admitted. At one of the hospitals 14 patients attended during September and October for the effects of "mushroom" abuse, compared with 6 for manifestations of the abuse of other hallucinogenic agents or narcotics (2 cannabis, 2 toluene-containing compounds, 1 lysergic acid diethylamide, 1 heroin). The figures for the latter group of substances are representative for any two-month period in 1981.

Ps. semilanceata is a gill fungus, commonly found growing in troops among grass in parklands, gardens, fields, and heaths in Britain, particularly in western regions. It has a sharply pointed pale-yellow cap 8 to 14 mm wide and up to 18 mm tall, supported by a tall cream-coloured wavy stem.[5,6] Its gills are purple/black with a white edge. A few hours after picking, the base of the fungus turns greenish-blue, especially the part which was below ground. This is due to an oxidation reaction[7] which is characteristic of *Psilocybe* genus. Liberty caps appear in autumn (mainly September to November), and cropping is heavy when the season is wet. The fruiting-bodies contain indoles, 4 phosphoryloxy-N, N-dimethyl-tryptamine (psilocybin) and the demethylated equivalents of this (baeocystin and norbaeocystin), as well as the more unstable psilocin.[8] All these are psychoactive compounds of varying potency and are found in varying quantities within the fungus. Psilocin is also produced by the hydrolysis of psilocybin after ingestion, and it is the more potent hallucinogenic agent.[9] The effect of these substances on brain biochemistry is very complex and ill understood.[8] They are thought to act by altering the concentrations of indoles, including serotonin, in the central nervous system, and thus interfering with the transmission of stimuli regulating the