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Post-Transfusion Non-A, Non-B Hepatitis: Significance of Raised ALT and Anti-HBc in Blood Donors

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Abstract. The FDA has recommended that all blood collected in the USA should be screened for antibody to hepatitis B core antigen (anti-HBc) and for raised alanine aminotransferase (ALT) as possible indicators of non-A, non-B hepatitis carriage. As part of an assessment of the medical and economic implications of such a screening programme, we have screened 1,742 regular blood donors for ALT and 2,086 (including the same 1,742) for anti-HBc. 42 (2.4%) of the 1,742 donors had ALT levels above 45 units/l. Clinical assessment of 33 of these revealed that 26 exceeded their ideal body weight by more than 10% and 15 by more than 20%. 11 admitted to an alcohol intake of over 40 g daily. In all, 82% of donors with raised ALT had a 'non-viral' clinical explanation for this abnormality. Anti-HBc was detected in 42 (2.0%) of the 2,086 donors screened. 27 (64%) also had anti-HBs, and 11 (26%) had anti-HBe. There was no overlap between donors with raised ALT and those with anti-HBc. Combined screening would lead to a loss of at least 4.4% of donations in the population studied. In view of the medical and economic implications of the introduction of these screening tests, and the poverty of data on the clinical significance of post-transfusion non-A, non-B hepatitis, we conclude that such a screening programme cannot be justified at present. Further studies are required, including a prospective controlled trial of the effects of screening.

Introduction

Since the advent of specific serological tests for hepatitis A and B, it has been recognised that around 90% of all post-transfusion hepatitis is due to the agent(s) of non-A non-B (NANB) hepatitis [1]. In the Transfusion Transmitted Viruses (TTV) study [2], the incidence of post-transfusion NANB hepatitis was found to vary in different parts of the USA, ranging from 4% in St. Louis to 18% in Houston. Other studies of transfusion recipients in the USA have described incidences varying from 5.4 to 27.1% [3]. Reported incidences in Europe are equally varied: 19% in Sweden [4], 13.8% in Italy [5], 3.4% in the Netherlands [6] and 3.9% and 2.4% in the UK [7, 8].

Many laboratories have sought a serological test system for NANB hepatitis, but to date all such putative antigen-antibody systems have proved impossible to substantiate [9, 10]. In the absence of specific markers for the virus, the TTV study was designed to examine the possibility that donors with a raised level of alanine aminotransferase (ALT) might be more likely to transmit NANB hepatitis than those with normal ALT [2]. The overall incidence of post-transfusion NANB hepatitis was 10%. A direct relationship was found between ALT levels in the donations received and incidence of NANB hepatitis, and the authors concluded that 40% of post-transfusion NANB hepatitis could be prevented by excluding donations with ALT levels above 45 units/l. This, according to their data, would lead to a loss of 3% of donations. Holland et al. [11] disputed this calculation but conceded that the data suggested that at least 21% of post-transfusion NANB hepatitis could be prevented by ALT screening.

An unexpected finding in the TTV study was an apparently similar relationship between antibody to hepatitis B core antigen (anti-HBc) and recipient NANB hepatitis [12]. The authors estimated that between 21.4 and 34.9% of post-transfusion NANB hepatitis might be prevented by screening donations for anti-HBc. Koziol et al. [3] at NIH reported similar findings from their study of 729

adult patients undergoing cardiac surgery. NANB hepatitis developed in 11.9% of 193 recipients of at least one unit of blood positive for anti-HBc while 4.2% of recipients of blood negative for anti-HBc had evidence of NANB hepatitis. Having calculated a 'maximal corrected efficacy', they predicted that anti-HBc screening would prevent 43% of post-transfusion NANB hepatitis at a cost of 4% of donations. These data were, however, obtained before the introduction of measures to exclude donors at high risk for HIV infection, and it is not known whether the same results would be obtained now.

These studies therefore suggest that combined ALT and anti-HBc screening of blood donors might reduce the incidence of post-transfusion NANB hepatitis by up to 60% at a cost of around 7% of donated blood. In view of the current anxieties about transfusion-related infections, it might be expected that these tests would be implemented by transfusion services with alacrity, and indeed the FDA last year issued a recommendation that both tests should be carried out by all blood-collecting agencies. There are, however, several reasons to question the validity of this recommendation. The clinical value of screening has not been proved by adequate prospective controlled trials to demonstrate a reduction of hepatitis in transfusion recipients. As far as the donor population is concerned, few studies have been done to examine whether donors identified by screening have clinical or epidemiological features suggesting chronic NANB hepatitis.

We have screened regular blood donors in Edinburgh for both ALT and anti-HBc in the period after the introduction of screening for HIV, in order to estimate the implications of combined testing. The study allowed us to carry out a clinical and laboratory assessment of donors with raised ALT, and thus to estimate the frequency with which a non-viral cause can explain a raised ALT, and to assess the probable consequences of screening for our donor population.

Methods

Between April and November 1986, ALT levels were measured in 1,742 regular blood donors attending the Regional Blood Donor Centre. Prior informed consent to additional blood testing, and further call-up if necessary, was obtained from all donors. The study was approved by the Ethics Committee of the Scottish National Blood Transfusion Service.

Serum samples were taken from the specimens used for virology testing, and were analysed for ALT in the Department of Clinical Chemistry, Royal Infirmary of Edinburgh within 24 h. The assays were performed using a Cobas-FARA centrifugal analyser (Roche Diagnostics, Welwyn Garden City), with kit reagents obtained from Boehringer Mannheim Diagnostics, Lewes, Sussex. For the purposes of this study we chose an upper limit of 'normal' of 45 units/l, in order to allow comparison with published studies from the USA in which 3% of donors had ALT levels >45 units/l. All donors with ALT levels equal to or above this cut-off were requested to attend for further evaluation including a medical history and examination. Further blood samples were obtained for full blood count, ESR, repeat ALT, bilirubin, alkaline phosphatase, gamma glutamyl transferase (GGT), total protein, albumin, ferritin, caeruloplasmin, alpha-1 antitrypsin, thyroxine, blood alcohol and antibodies to hepatitis B core and surface antigens (anti-HBc and anti-HBs).

All donors attending these sessions were also tested for anti-HBc using an enzyme-linked immunoassay (Wellcome Diagnostics, Beckenham, England). Donors positive for anti-HBc were not recalled for clinical assessment, but stored serum samples from all of the positives were retested for repeat anti-HBc, and for anti-HBs and anti-HBe.

We also examined the records of 708 plasmapheresis donors, in order to assess the frequency of raised ALT at initial interview, and subsequent fluctuations in level (liver function tests are routinely carried out at 6-monthly intervals in these donors).

Results

Donors with Raised ALT

Forty-two (2.4%) of the 1,742 donors screened had ALT levels greater than 45 units/l (range 45–248, mean 69.8 units/l). Thirty-three of these 42 (78.5%) attended for further evaluation. Twenty-five were male and 8 female. Most of them expressed anxiety at the request to attend the Donor Centre, but all agreed readily to participate in the study.

The most striking finding was that 26 of the 33 donors with raised ALT exceeded their ideal body weight by more than 10%, and 15 by more than 20%. There was no correlation between the degree of obesity and the extent to which ALT was raised. At the time of initial clinical assessment (usually about 1 month after donation) 5 of these 26 had ALT levels within the normal range. 10 of the 26 obese donors admitted to an alcohol intake greater than 40 g daily. 2 of these had detectable blood alcohol at the time of the assessment, compared with none of the donors claiming an alcohol intake of less than 40 g daily. Alcohol may have been the reason for a raised ALT in one other donor, a 21-year-old non-obese female who admitted to drinking over 40 g daily at the time of donation. Thus alcohol intake of over 40 g daily was present in 33% of the 33 donors with raised ALT.

Six of the 33 donors assessed (18%) were of normal body weight, did not abuse alcohol and had no clinical abnormality to account for the elevated ALT found at the time of donation. When they returned for initial clinical

Table I. Clinical and laboratory findings in 33 regular blood donors with raised ALT at time of donation

Clinical feature or abnormality	n	Comment
Obesity	26	>10% over ideal body weight
	15	>20% over ideal body weight
Alcohol >40 g daily	11	10>10% over ideal body weight
Hypertension (diastolic >100 mm Hg)	6	All males, all obese 3 alcohol >40 g daily
Contraceptive pill	4	2 normal ALT at clinical assessment 1 remained abnormal 1 reverted to normal on discontinuing pill all >10% over ideal body weight
Heterozygous alpha-1-antitrypsin phenotype	3	2MS, 1MZ – frequency as expected for normal population [13]
Anti-HBs	1	male, obese, anti-HBc negative – no history of exposure to hepatitis B
Anti-HBc	0	

assessment 4 of these 6 had ALT levels within the normal range; at 6 months 1 of these 4 had again become abnormal, the sequence of ALT values being 48, 33, and 55 units/l.

These findings and other clinical and laboratory features of interest in this group of 33 donors with raised ALT are summarised in table I.

Abnormalities of liver function other than raised ALT were found in 9 of the 33. In 6 of these the abnormalities were minor and not considered significant on follow-up. One 39-year-old obese man had an ALT of 248 units/l, with GGT and alkaline phosphatase similarly raised and he had mild hepatomegaly. He admitted to an alcohol intake greater than 80 g daily. After 3 months of abstinence his liver function tests were completely normal and the liver was no longer palpable. One 33-year-old non-obese female had an ALT 203 units/l, GGT 257 units/l, alkaline phosphatase 362 units/l, ESR 34 mm/h and a positive anti-smooth muscle antibody test. No clinical evidence of chronic liver disease was found, but liver biopsy showed chronic active hepatitis.

Of the 708 plasmapheresis donors whose records were examined, 26 (3.7%) had raised ALT prior to commencing plasmapheresis (mean 59.5, range 46–82 units/l). Of these, 5 were subsequently normal. Of 682 with initially normal levels 41 (6%) subsequently became abnormal at some time.

Donors with Anti-HBc

2,086 donors were screened; the same 1,742 who were screened for ALT plus all of the first-time donors attending the same sessions. 42 (2.0%) were positive for anti-HBc. 25 were male, 17 female. Among the 1,742 regular donors 38 had anti-HBc. None of these had raised ALT. Among the new donors 4/344 had anti-HBc. Twenty-seven of the 42 (64%) were positive for anti-HBs, and 11 (26%) were positive for anti-HBe.

Discussion

In our regular donors a raised ALT as defined by the American studies occurs in 2.4%. The chosen cut-off value of 45 units/l corresponds closely to 2 standard deviations above the mean, the usual laboratory definition for the upper limit of normal. The number of donors identified by this cut-off level as having a raised ALT would therefore be similar in Edinburgh to that in the American studies [2, 14].

We have found a strong association between a raised ALT and both obesity and alcohol ingestion. This confirms the findings of Alter's [15] study of American donors. In his series he concluded that 45% of his donors with raised ALT could be carriers of NANB hepatitis, but he also stated that this was likely to be a spuriously high proportion because many of the donors were selected as having been implicated in cases of post-transfusion NANB hepatitis, and had been enrolled into the study on that basis. In the present series, using a definition of obesity of 10% above ideal weight for height and of alcohol excess as the ingestion of more than 40 g daily a simple explanation for a raised ALT was found in 82% of donors, though in the absence of a control group it is impossible to be certain about the relationship between weight and the frequency of raised ALT.

Six donors had raised ALT without obesity or any other clinical abnormality, and 4 of these had normal levels at follow-up. In Alter's series only 17% of donors initially found to have a raised ALT had normal levels at 6 months.

In our plasmapheresis donors 19% of those with ini-

tially abnormal values subsequently became normal, while 6% of those with initially normal values subsequently had a raised ALT at some time. Fluctuations in ALT level are characteristic of chronic NANB hepatitis, though it seems unlikely that such high percentages of the donor populations studied are carriers of NANB hepatitis. It is important for those who advocate ALT testing to recognise that this tendency to fluctuation in ALT levels would produce a cumulative loss of donors far in excess of that suggested by studies already published and that most of these donors would not be NANB hepatitis carriers.

The high rate of associated anti-HBs and anti-HBe suggests that the test for anti-HBc which we employed is highly specific for previous exposure to hepatitis B. There was no overlap between the donors identified by ALT and anti-HBc testing respectively. Combined testing would lead to an initial loss of 4.4% of donations. The American studies also found that ALT and anti-HBc screening seemed to identify different populations of donors [3, 12].

The American studies of transfusion recipients concluded that combined testing would eliminate around 60% of post-transfusion NANB hepatitis [2, 3, 12], each test accounting for half of this total. It is worth noting, however, that such testing would almost certainly have no impact on NANB hepatitis in recipients of clotting factor concentrates since each batch of factor VIII or factor IX is made from the plasma from over 4,000 donors. Furthermore, it is expected that recently introduced methods of heat treatment will substantially eliminate the problem of NANB hepatitis due to clotting factor concentrates. The impact of ALT and anti-HBc testing would therefore be restricted to recipients of cellular blood products, fresh frozen plasma and cyroprecipitate.

The only recent study in the UK suggests that the incidence of post-transfusion NANB hepatitis is around 2.5% in cardiac surgery patients receiving on average approximately 6 units of blood each. Thus the transmission rate per donation is around 0.5%. 'Look-back' studies of HIV transmission by blood or blood products have revealed that over 50% of transfusion recipients die of their original disease within 2–3 ears [16]. According to published series [1, 15], NANB hepatitis would be expected to become chronic in 50% of those recipients infected by blood transfusion and surviving the original illness; that is, in 0.5–1% of all heavily transfused patients. The best estimates suggest that 10% of these patients i.e. 0.05–0.1% may manifest cirrhosis, though the eventual morbidity and mortality attributable to this is likely to be small [15]. Potentially serious liver disease might therefore be expected to occur in up to 1 in 1,000 patients heavily transfused, and in no more than 1 in 5,000 recipients of single donations. On the basis of the US data, combined ALT and anti-HBc testing may reduce this incidence of long-term liver disease by up to two-thirds, i.e. to 1 in 3,000 of heavily transfused patients. However, the effectiveness of the surrogate markers in identifying carriers may not be the same in the UK as in the USA, since it will depend on the relative NANB hepatitis transmission rates amongst those with and without the markers. It is not known to what extent the donor populations differ in this regard, nor how they might have changed in the wake of self-exclusion and testing for HIV infection. It is likely that the donor population in the USA is already quite different from the populations studied in the TTV and NIH studies, and in fact the incidence of NANB hepatitis in the NIH study rose significantly during its second half.

Nevertheless, if we were to assume that this degree of benefit is achievable, the Blood Transfusion Services in the UK would be required to test around 2.5 million blood donations each year at an estimated cost of £2–3 per donation – an annual bill well in excess of £5 million.

In addition to this raw cost of screening all donations, account must be taken of the clinical consequences of identifying up to 5% of the donor population as being potential carriers. The assessment of each of these donors, with further laboratory testing, will generate substantial costs. If the Blood Transfusion Service were to take the same responsible attitude to the medical assessment and counselling of these donors as it has to HIV antibody-positive donors, staff and resources would be required to deal with 4–6 donors daily in a medium-sized centre. At least as important as the financial consequences of combined testing is the potential morbidity generated in these donors as a result of informing them of these abnormalities. Our experience in this study has convinced us that such donors suffer significant anxiety, though it could be argued that the identification of obesity, alcohol abuse and perhaps significant liver disease could be of potential benefit.

These calculations assume that there is a valid scientific basis for the anticipated reduction in NANB hepatitis as a result of donor screening. The case for testing is based entirely on the TTV and NIH studies [2, 3, 12], both of which were retrospective studies as far as the donors were concerned, and one of which used highly selected and heavily transfused patients. The presentation of data in both studies introduces a major statistical bias which is

not adequately offset by the 'correction factors' applied. The tests of significance used to show that recipients of at least one unit of blood from a donor with high ALT are more likely to get NANB hepatitis than recipients of blood from donors with normal ALT, could equally well show that donors who brush their teeth more than three times daily are more likely to transmit NANB hepatitis than those who do not. This bias occurs because recipients of a small number of units are less likely to develop NANB hepatitis than recipients of, say, 15 units. Equally, recipients of a few units are less likely to receive blood from donors who brush their teeth three or more times daily than recipients of 15 units. Spurious effects are, therefore, inevitable when data from recipients of different transfusion volumes are amalgamated. Nevertheless, in the TTV study the data from recipients of single unit transfusions are adequate to demonstrate a statistically significant association between donor ALT level and recipient NANB hepatitis. Thus this study establishes a relationship, but does not quantify that relationship adequately. Estimation of hepatitis transmission rates for donors with and without the markers is technically possible from the raw data, but this has not been done. A particular failing is the omission of detailed results from each of the four centres in the TTV study (St. Louis, Los Angeles, New York and Houston), which experienced NANB hepatitis attack rates ranging from 4 to 18%. If effective transmission rates showed a consistent pattern between those with and without markers in all four cities, this would be extremely valuable information.

The Americans have concluded that a prospective randomised trial to test these hypotheses will never be carried out, for logistical and ethical reasons [3]. To have adequate statistical power such a study would need to be very large and would indeed pose substantial logistical problems. However, there can surely be no ethical objection to such a study, given the very doubtful benefits which can be predicted on the basis of the available data. Four small prospective studies have been carried out, two using ALT screening and two using anti-HBc [15, 17–19]. Three of these studies, including Alter's study of ALT testing at NIH, failed to demonstrate any reduction in post-transfusion NANB hepatitis as a result of donor screening. In the most recently reported study, using methods similar to the TTV and NIH studies, an apparent association between anti-HBc in donor units and recipient hepatitis is reported [19]. Interestingly, the incidence of NANB hepatitis in recipients was high (10.1% in recipients of at least one anti-HBc positive unit, 2.1% in recipients of anti-HBc negative units) in spite of the fact that ALT screening had been carried out and only blood with ALT<30 units/l was transfused. These data were collected in 1980–81, and so may not be representative of the current West German population.

We conclude that the introduction of these screening tests cannot at present be justified. Further studies of recipient NANB hepatitis and the natural history of the disease are necessary, and a properly conducted prospective trial of screening for surrogate markers is essential. More extensive studies of the donor population would be valuable, with a particular need for elucidation of the apparent relationship between body weight and ALT level. Such studies would prove useful in the management of donors, should the case for screening ever be well enough established for its introduction to be considered necessary.

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