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U.K. multicentre study on blood donors for surrogate markers of non-A non-B hepatitis. Part I: Alanine transferase and anti-HBc testing

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SUMMARY. Blood samples from 9,215 blood donors in three U.K. centres (North London, Bristol and Manchester) were tested for their alanine aminotransferase (ALT) level and the presence of anti-HBc and anti-HCV. This paper presents the results of the ALT and anti-HBc tests. The prevalence of ALT > 45 IU/l was 3·1% overall (North London 3·06%, Bristol 4·56% and Manchester 1·97%). Manchester results were skewed by the methodology used for ALT measurement, highlighting the need for standard test methods. Anti-HBc was detected using the Wellcome enzyme-immunosorbent assay (EIA) and confirmatory testing was performed using a radioimmunoassay (RIA) and the Corecell haemagglutination assay. Repeat reactive rates were 0·9, 0·79 and 0·94% for North London, Bristol and Manchester, respectively, with an overall rate of 0·9%. The confirmed positive rate was 0·73, 0·53 and 0·65% for the three centres with an overall rate of 0·63%. Donors with an ALT > 45 IU/l, or with confirmed anti-HBc, were interviewed with a medical questionnaire for risk factors. The major contributing factors in donors with a raised ALT were alcohol consumption and obesity.

Keywords: alanine aminotransferase, anti-HBc, blood donors.

The subject of testing blood donations for surrogate markers of non-A, non-B hepatitis (NANBH) is controversial. In the U.S.A. in 1986 the FDA recommended that all blood donations should be screened for alanine-amino transferase (ALT) as a surrogate marker for NANBH, and the American Red Cross initiated a screening programme. In 1987 the American Association of Blood Banks reaffirmed its decision to require ALT and anti-HBc testing on all blood donations (American Association of Blood Banks, 1987). Prior to the introduction of universal screening of blood donations, prospective studies in the USA had shown a correlation between the presence of anti-HBc in blood donations and the risk of transmitting NANBH (Stephens *et al.*, 1984; Koziol *et al.*, 1986). However, this correlation was not found in a recent study from the Netherlands (Reesink *et al.*, 1988). The main consideration for adopting ALT screening had emerged from the Transfusion Transmitted Viruses (TTV) study (Aach *et al.*, 1981) and from an evaluation of patients who underwent open heart surgery and blood transfusion at the National Institute of Health (NIH) Clinical Centre (Alter *et al.*, 1981). The TTV study showed that 45% of recipients of at least one unit of blood with an elevated ALT (> 60 IU/l) developed NANBH.

Similarly the NIH study demonstrated that 29% of the recipients of units with an ALT activity greater than 53 IU/l developed NANBH. The TTV and NIH studies were both undertaken in the 1970s, before the introduction of more stringent donor deferral policies, including self exclusion of donors at risk of HIV infection, which might have contributed to a reduction in the incidence of PTH (Alter, 1989).

Although NANBH has traditionally been regarded as a diagnosis of exclusion, this situation may change as a result of the availability of an assay for antibodies

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to the hepatitis C virus (HCV) (Alter, 1989; Choo *et al.*, 1989; Kuo *et al.*, 1989; Esteban *et al.*, 1989; Editorial, Lancet, 1989; Kuhnl *et al.*, 1989; Van der Poel *et al.*, 1989). Prior to this, testing of blood donations for surrogate markers of NANBH was the only means of reducing the incidence of PTH and, in view of this, Alter supports the retention of ALT testing (Esteban *et al.*, 1989). Van der Poel *et al.* showed an association between HCV seropositivity, raised ALT levels and the transmission of NANBH, and they emphasized the importance of excluding blood donations with raised ALT (Van der Poel *et al.*, 1989).

Before any decision is made in the U.K. to discard blood donations with raised ALT or anti-HBc, more information is required.

This includes the determination of rates and an assessment of the aetiological factors contributing to elevated ALT values and anti-HBc positivity among the current U.K. donor population. Apart from data from the east of Scotland (Gillon *et al.*, 1988) and north London (Mijovic *et al.*, 1987) such information is not available. Our multicentre study of surrogate markers in the blood donors was conceived before the introducton of the tests for anti-HCV, and the design was then modified to incorporate these new assays. The original aims of the study, however, still pertain. The following parameters were investigated.

1 The incidence, possible aetiology and relevance of raised ALT activity (>45 IU/l).
2 The prevalence of anti-HBc.
3 The prevalence and significance of anti-HCV.

The study will be reported in two parts. This first part reports the findings on ALT and anti-HBc testing, and the second part will present the results of different anti-HCV tests.

MATERIALS AND METHODS

Three Regional Blood Transfusion Centres (RTCS) participated in this study: North London, Bristol and Manchester, with North London as the co-ordinating centre. Approval was obtained from the appropriate ethical committee for each Transfusion Centre. Blood donor sessions at each centre were selected to represent a mix of 'Industrial', 'Public' and 'College' donors. All donors at each session were provided with a leaflet requesting their participation and giving them the relevant details. An additional blood sample of 10 ml was obtained from the bleed line at the time of the donation, and collected into glass or plastic tubes without anticoagulant. Altogether 9,741 donors (3036 (51·9% males) from North London, 3,015 (52·1% males) from Bristol and 3,690 (57·7% males) from Manchester) were tested.

ALT TESTS

North London

As soon as the whole-blood samples arrived at the centre they were stored at +4°C; the serum was separated and ALT measured within 24 h of donation. An EPOS automated clinical analyser (Eppendorf range) using Merckotest reagents (according to the Scandinavian Committee on Enzymes for ALT) was used at 37°C.

Standard control sera were tested at the beginning of each run and after each subsequent 30 samples. A repeat measurement was carried out on samples with an ALT activity greater than 45 IU/l.

Bristol

Blood samples were kept at +4°C and the serum was separated within 24 h of donation. ALT testing was done within 6 days on the samples which had been stored at +4°C, except for the first 500 samples which were tested within 14 days from the date of donation. The Technicon SMAC System with an automated spectrophotometric glutamic-pyruvic transaminase method was used with a TRIS buffer at 37°C.

Manchester.

Whole blood samples were kept at room temperature and separated within 24 h. The serum samples were kept at room temperature until re-aliquoted on the following day and then stored at −30°C pending testing at the Manchester Royal Infirmary where they were stored at +4°C for up to 4 days. ALT activity was measured at 37°C using the Parallel Analyser (American Monitor Corporation) with a TRIS buffer (pH 7·9) and pyridoxal phosphate.

A further 526 samples from Manchester were separated within 8 h, and each sample was divided into two aliquots: one was stored at −30°C and sent to the North London Regional Transfusion Centre for ALT testing using the EPOS analyser detailed above. The other aliquot was stored at +4°C and tested in Manchester within 4 days.

ANTI-HBc TESTING

Initial anti-HBc testing in 9711 samples was carried out at the North London Transfusion Centre, using Wellcome EIA kits. Positive samples were re-tested in duplicate. Repeatedly reactive samples were sent to the Department of Virology, University College and Middlesex School of Medicine, London, for confirmatory testing using an in-house radioimmunoassay (RIA) (Tedder *et al.*, 1980). Seventy-eight samples from 52

donors were tested using an additional haemagglutination assay (CORECELL, Green Cross Corporation, Japan). Anti-HBs tests were also performed at the reference laboratory using an in-house RIA (Tedder *et al.*, 1980). Samples confirmed as anti-HBc positive were sent to the Academic Department of Medicine, Royal Free Hospital, London for HBV DNA analysis.

DONOR FOLLOW-UP

In all three centres, donors with a raised ALT level (>45 IU/l) or a confirmed positive anti-HBc test received a letter requesting their attendance at an interview. For each donation with an ALT >45 IU/l, the preceding sample with a normal ALT was chosen as a control, and the same letter was sent to these donors. Up to three reminders were sent to donors who failed to respond. Donors from North London who failed to reply to the first letter were contacted by telephone where possible.

At the interview, a questionnaire was administered, and data obtained on age, sex, marital status, country of birth, ethnic origin and occupation. Details of any surgical operations, serious medical illnesses, including jaundice and hepatitis, or contact with persons known to have had hepatitis were recorded. Further questions were asked relating to residence or extensive travel abroad, exposure to scarification or blood transfusion, alcohol intake, level of exercise and medications including herbal remedies. Ethnic origin, country of birth, occupation, medical history and residence abroad of the parents and the sexual partner were also recorded. A physical examination was carried out with special reference to hepatomegaly, splenomegaly, lymphadenopathy and skin examination for stigmata of chronic liver disease and tattoos.

Donors were weighed and their height was recorded. Further blood samples were taken and sera separated within 8 h for measurement of ALT, aspartate transaminase (AST), gamma-glutamyl transferase, alkaline phosphatase, bilirubin and serum albumin. These tests were carried out at the General Hospital connected with the Transfusion Centre or, in the case of North London, at the Transfusion Centre.

RESULTS

The prevalence of raised ALT (>45 IU/l) was 3·1% overall with 3·06% for North London, 4·56% for Bristol and 1·97% for Manchester. The results from

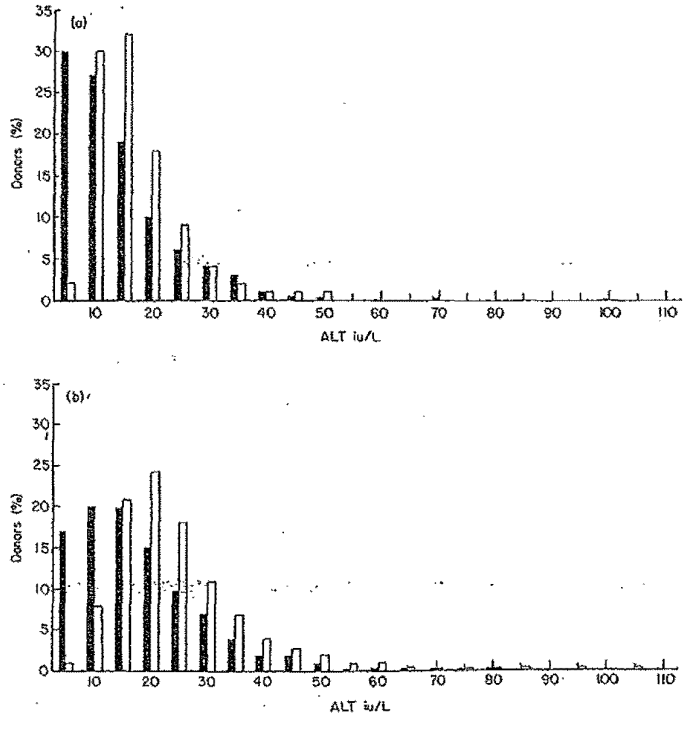

Fig. 1. (a) ALT levels in female blood donors. (■) Manchester, (□) Bristol and North London. (b) ALT levels in male blood donors. Key as for (a).

the 526 sample sub-set from Manchester have not been included in the calculation of these results. The inter-batch coefficients of variation for all centres were as follows: at 30 IU/l 7·9%, at 94 IU/l 6·4% and at 315 IU/l 2·3%. The histogram of the ALT values was not normally distributed (Fig. 1a and b). However, the logarithmic transformation of the distribution was essentially Gaussian. For female donors, 45 IU/l represents the 98·8th percentile in North London, 98·1th in Bristol and 99th in Manchester. For males, 45 IU/l represents the 95th percentile in North London, the 93rd in Bristol and the 97th in Manchester. The breakdown of results by age and gender are shown in Tables 1–3.

Follow-up of donors with ALT > 45 IU/l and controls

Three hundred and three donors with an ALT >45 IU/l, and an equal number of control donors were invited to attend follow-up interviews. Two hundred and twenty-one (72%) from the elevated ALT group (180 males) and 226 (74%) of the control group attended the interview. The mean ALT for the test group was 63·0 IU/l, (SD = 7 IU/l) and that for the control group was 17·6 IU/l (SD = 9 IU/l). One donor had an ALT level of 868 IU/l at follow-up and this result has been omitted from the calculations (Table 4). Of the donors seen at interview, 81% with a raised ALT were males, compared with 57% in the control

Table 1. Distribution of ALT values in blood donors

Centre	Gender	Number of donors	ALT IU/l		Percentage with ALT > 45 IU/l
			Mean (1 SD)	Median	
North London	Male	1583	22·2 (12·8)	12·8	4·82
	Female	1453	13·8 (8·6)	8·6	1·10
	Total	3036	18·2 (11·7)	11·7	3·06
Bristol	Male	1591	24,5 (14.1)	14,1	6·98
	Female	1424	17·1 (10·9)	10·9	1·83
	Total	3015	21·0 (13·2)	13·2	4·56
Manchester	Male	1816	16·7 (13·3)	13·3	2·75
	Female	1348	12·2 (10·9)	10·9	0·96
	Total	3164	14·8 (12·6)	12·6	1·99

Table 2. ALT test results in blood donors (percentiles)

Centre	Gender	Percentile		Mean + 2 SD	45 IU/l
		95th	98th	Percentiles	
North London	Male	45 IU/l	55 IU/l	95·7th	95·0th
	Female	26	37	96·5	98·8
	Total	40	51	96·0	96·75
Bristol	Male	51	65	95·2	93·0
	Female	33	44	96·5	98·12
	Total	44	58	95·75	95·25
Manchester	Male	39	50	96·50	97·0
	Female	30	37	96·75	99·0
	Total	35	45	96·75	98·0

Table 3. Mean ALT values according to age of donors, by centre

Centre	Age group (years)	Number of donors			Mean ALT (IU/l)		
		Male	Female	Total	Male	Female	Total
North London	18–25	229	298	527	20	12	15
	26–35	496	469	965	22	13	18
	36–45	445	378	823	23	14	19
	46–55	262	206	468	22	13	18
	56–65	137	97	234	21	16	19
Bristol	18–25	234	326	560	20	16	18
	26–35	368	294	663	24	16	20
	36–45	393	303	698	24	16	21
	46–55	239	165	405	24	18	22
	56–65	117	94	212	24	18	22
Manchester	18–25	310	344	654	16	11	13
	26–35	541	403	944	16	11	14
	36–45	521	329	850	16	11	14
	46–55	304	189	494	16	13	15
	56–65	136	80	216	16	15	15

group. This male predominance (91%) became more marked among the donors in the subgroup whose ALT remained elevated above 45 IU/l on resampling. Tables 5–7 summarize the findings elicited at the interviews. There was a significant difference in alcohol intake and body mass index between the test and control groups ($P < 0{\cdot}005$).

Alcohol intake

A daily alcohol consumption of more than 20 g was reported by 33% in the control group and 53% in the test group (Table 5). Males accounted for 98% (64/65) of the donors in the persistently raised ALT group who consumed more than 20 g of alcohol daily (Table 6).

Obesity

Moderate obesity [Grade 2, as defined by a Body Mass Index (BMI) > 29·9] was found in 27% of the donors with elevated ALT and 7% of control donors. In the persistently elevated ALT group, males constituted 85% (30/35) of those with Grade 2 obesity.

Exercise

In the raised ALT group, 18 donors (8%) were judged to be taking part in a moderately strenuous physical exercise schedule, compared with seven (3%) in the control group.

Table 4. Persistence of raised ALT in donors

	Centres			
	North London	Bristol	Manchester	Total
Donors screened	3036	3015	3140	9191
Donors with ALT level > 45 IU/l	93	137	73	303
Donors attending follow-up	65	100	56	221
Second ALT > 45 IU/l	34	42	32	108
Second ALT < 45 IU/l	31	58	24	113
'Control' donors with ALT < 45 IU/l	93	137	73	303
Donors attending follow-up	56	110	60	226
Second ALT > 45 IU/l	2	6	1	9
Second ALT < 45 IU/l	54	104	59	217

Table 5. Factors associated with raised ALT in blood donors

	Test group ALT > 45 n=221 (%)	Control group n=226 (%)	P
Alcohol intake > 20 g/day	118 (53·3)	75 (33)	< 0·0005
Body mass index > 2	60 (27·1)	17 (7)	< 0·0005
Strenuous exercise	18 (8·1)	7 (3)	0·02 (significant at 5%)
Exposure to chemicals	14 (6·3)	13 (6)	0·8
Oral contraceptives	6 (14·6)*	15 (42·5)**	0·006
Drugs	5 (2·2)	8 (4)	0·423
Herbal medicine	5 (2·2)	3 (1)	0·457

* 41 females.
** 35 females.
P values calculated by Student's t-test.

Table 6. Risk factors in donors with raised ALT

	First ALT > 45 IU/l					
	Second ALT > 45			Second ALT < 45		
	Female	Male	Total	Female	Male	Total
Number	10	98	108	31	82	113
Mean age (years)	36	36	36	40	38	39
Mean ALT of second sample (SD)	81 (39)	74* (25)	74 (28)	20 (32)	31 (9)	27 (11)
Alcohol consumption > 20 g/day	1	64	65	7	46	53
Obesity grade > 2	5	30	35	6	19	25
Exercise grade > 3	0	7	7	1	10	11

* Excluding one donor who had a second ALT = 868 IU/l.

Table 7. Hepatitis risk factors in test and control groups attending interview

	First ALT result		
	> 45 IU/l n=221		
	Second ALT > 45 n=108 (%)	Second ALT < 45 n=113 (%)	< 45 IU/l n=226 (%)
Transfusion history	6 (5·5)	2 (2)	8 (3·5)
Previous surgery	57 (52)	64 (57)	140 (61)
Tattooing	7 (6)	5 (4)	7 (3)
Occupational hazard (health workers etc.)	10 (9)	8 (7)	27 (12)
Contact with hepatitis	18 (17)	18 (16)	37 (16)
History of jaundice	3 (3)	3 (3)	16 (7)
Extensive travel to high risk areas	16 (15)	16 (14)	46 (20)
Residence abroad	22 (20)	25 (22)	50 (22)
Injection or operations abroad	10 (9)	12 (11)	24 (10)
History of i.v. drug abuse	1 (1)	0	1 (0·4)

Table 8. Anti-HBc positivity in different age groups

Donors tested			Confirmed anti-HBc positive		
Age (years)	Number tested	Percentage of total	*n*	Percentage of donors in each age group	Percentage of total anti-HBc positive
18–25	1,857	20	5	0·27	8·06
26–35	2,724	29·5	11	0·40	17·74
36–45	2,509	27	20	0·79	32·26
46–55	1,430	15·5	14	0·97	22·58
56–65	704	7·6	12	1·70	19·36
Total	9,239		62	0·67	100

Other factors

There was no marked difference in the prevalence of exposure to chemical and drugs between the raised ALT and control groups nor did hepatitis risk factors appear to be significantly different (Table 7). However, within the raised ALT group, six of the eight donors who gave a past transfusion history had an elevated ALT on the second blood sample.

Anti-HBc results

The initial screen-positive rates using the Wellcome EIA for anti-HBc were 1·8, 0·93 and 1·32% for North London, Bristol and Manchester respectively, with an overall rate of 1·3%. The overall repeat reactive rate was 0·9% (0·9% for North London, 0·79% for Bristol and 0·94% for Manchester). Confirmatory testing by RIA and haemagglutination (CORECELL) on repeatably reactive samples gave a final anti-HBc positive rates of 0·73, 0·53 and 0·65% for North London, Bristol and Manchester respectively, with an overall rate of 0·63%. The rate for Manchester may be spuriously low because of the unavailability of serum for confirmation in three cases. Of the 62 confirmed positive donors 52 were unequivocally positive and 10 were indeterminate. Males accounted for 42 (68%), seven (11%) were first time donors and two (3%) showed a raised ALT (>45 IU/l). Table 8 shows the age distribution of donors confirmed to have anti-HBc, with a cumulative acquisition of this marker with advancing years.

Although the competitive RIA and haemagglutination assays for anti-HBc were mostly in agreement, divergent results were obtained in four out of 78 samples from these 62 donors, tested by both assays. Two samples were positive only by RIA and two only by haemagglutination. In a further four samples, a positive anti-HBs result was obtained with either a negative or a divergent anti-HBc result.

In another six samples, anti-HBc status could not be confirmed because of borderline haemagglutination titres or RIA inhibition close to the 6% cut-off. In order to resolve these discrepancies, the results of confirmatory testing were divided into three groups: positive (52), indeterminate (10), and negative (16). Seven of the 52 positive samples (13·5%) were anti-HBs negative. None of the 52 confirmed anti-HBc positive samples had detectable levels of HBV DNA, at a test sensitivity of 0·5 pg HBV DNA per millilitre of serum (A. Zuckerman, personal communication).

Follow-up of donors confirmed as positive for anti-HBc

Forty-eight donors with anti-HBc by EIA and RIA and/or CORECELL were interviewed. Their risk factors for HBV infection are summarized in Table 9.

Table 9. Hepatitis risk factors elicited in 48 anti-HBc positive donors

Ethnic origin	
Southern European	2
Indian/Asian	1
Occupational hazard	7
Surgery	31
History of jaundice	6
Hepatitis contact	10
Transfusion history	6
Scarification	
Ear piercing	16
Tattooing	3
Electrolysis	2
Acupuncture	5
Travel to high risk areas	18

Some donors had more than one risk factor.

DISCUSSION

It is generally recognized that there is little overlap between anti-HBc positivity and raised ALT in blood donor populations. Hanson and Polesky quote a 0·03% combined positivity rate for surrogate markers (Hanson & Poleski, 1987), but as the TTV and NIH studies have shown, when such an overlap does occur, the likelihood of NANBH transmission is very high (Aach *et al.*, 1981; Alter *et al.*, 1981).

Re-analysis of the TTV study showed that 8·6% of anti-HBc positive donors had an elevated ALT (Stephens *et al.*, 1984). Anti-HBc positivity in past reports has varied from 53% in Kenya (Bowry & Shah, 1983) to 2% in Scottish donors (Gillon *et al.*, 1988) and in North London (Tedder *et al.*, 1980) to 1·5% in Swedish donors (Widell *et al.*, 1988). AuBuchon *et al.* reported the results of routine anti-HBc testing of 2·3 million donations in the USA (AuBuchon *et al.*, 1989b). The overall positivity rate was 2·4% with an interstate range of 1·3–4·6%. In our study, two donations were positive for anti-HBc and also had raised ALT levels; one of these donations was also anti-HCV positive (see part 2).

As far as reliability of the anti-HBc assay is concerned, although it may not be certain that the RIA produces fewer false-positive results (Troisi & Hollinger, 1987), the impression is that it is a more specific test than the EIA. In one study, less than 50% of EIA reactive samples were confirmed by RIA (Hanson & Poleski., 1987). In the present study, 80% of repeatedly reactive samples were confirmed by RIA and haemagglutination. A variable inhibition cut-off was chosen on each batch of samples based on the comparison between a weak-positive standard and the negative control, as opposed to the 50 and 70% cut-offs suggested by Hollinger (Hollinger & Poleski, 1987). A side-issue emerging from this study was the prevalence of donors with anti-HBc without other HBV markers. A report from the American Red Cross showed 17–22% of anti-HBc positive samples were 'anti-HBc' only (Akins *et al.*, 1987), while another study reported an incidence of 10% (Hanson & Poleski, 1987). In our study, 13·5% of confirmed anti-HBc positive samples were negative for other markers of HBV. These donors are potentially infective because their blood may contain subliminal levels of HBsAg.

An important finding from this study is the variability in the ALT values with respect to geography and gender (Table 1). Studies in the USA have shown that ALT is lowest below 20 years, rises to a peak at 30 years, and returns to a lower mean by 65 years (Kahn *et al.*, 1982). This pattern was recognizable for male donors from North London (Table 3), but was not detectable in the other donor populations in the study. The same pattern emerges when analysing the data from ALT measurements carried out earlier at North London (Mijovic *et al.*, 1987); while there was a peak mean ALT for males between 36 and 45 years, a gradual increase was recorded for females with a peak at 56–65 years. However, a larger donor population would need to be studied to be certain that the changes in mean ALT are not fortuitous.

Standardization of methodology

During the testing of the initial samples collected in Manchester, it became apparent that the ALT methodology in use there did not discriminate well in the lower ranges, as it was designed primarily to detect abnormally high ALT values in patients and not in healthy blood donors. Consequently, the histograms of ALT distribution were spuriously skewed, as illustrated in Fig. 1a and b. These discrepant results highlight the need for a standard test method for the healthy population, should the test be introduced for routine screening of blood donors in the U.K.

Regional cut-offs versus a national cut-off

It can be argued that were ALT testing to be adopted in the U.K., in the absence of national standardization, it would be preferable to opt for individual laboratories selecting their own cut-off values for donor deferral (Gillon *et al.*, 1988). These cut-offs could be based either on the locally derived 2·0 SD above the arithmetical mean, 2·25 SD above the logarithmic mean or preferably the value corresponding to their 95th to 99th percentile.

Donor follow-up

Gillon *et al.* recently published data on the prevalence and clinical evaluation of donors with raised ALT in the East of Scotland (Gillon *et al.*, 1988). They chose an alcohol consumption of 40 g daily as the value above which alcohol was considered to be the cause of ALT elevation. At this level, 33% of the donors with raised ALT were consuming alcohol excessively and the raised ALT was attributed to this factor. We found, however, that the level of 20 g alcohol consumption was more relevant to our sample population, in addition to being consistent with previously defined levels for excessive alcohol intake (Paton, 1988). Ten grams of alcohol is equivalent to 1 unit as defined by the Health Education Authority (Health Education

Authority, 1989). On the other hand Gillon *et al.* (1988) were less lenient with regard to obesity, using a definition of 10% above the ideal weight, which is a lower threshold than a BMI of 29·9 as selected by us.

In our study, 51% of the donors with raised ALT had a normal ALT on their second sample. This compares with 19% of plasmapheresis donors with persistently raised ALT levels in Gillon's series, and 17% in donors followed up by Alter (Alter, 1985). Our results are nearer to those of Freidman *et al.* (1987) who reported that approximately two-thirds of subjects had persistently or intermittently elevated ALT levels on follow-up. We therefore recommend that any deferral of donors, based on a raised ALT, should follow an interview with appropriate counselling and repeat ALT estimation after 6 months. For those donors whose ALT remains significantly elevated, referral to their general practitioner is indicated.

The major contributing factors to ALT elevation in the donor population are alcohol consumption and obesity. It is important to note that Gillon *et al.* (1988) emphasize that The mere documentation of obesity or alcohol abuse does not prove that these factors cause the elevation in ALT level. We are unable to confirm the significance of exposure to chemicals and drugs in causing elevated ALT, but this observation should not detract from the previously established causal relationships between hepatotoxic agents and liver damage (Sherlock, 1981). There is evidence of ALT elevation in marathon runners (Dietmar *et al.*, 1990), and our observations are consistent with the general premise that exercise increases ALT levels, but it is difficult to quantify the effect of exercise on an individual ALT result.

The issue of routine ALT and anti-HBc testing of blood donors cannot be resolved from the results of this study alone. The main arguments for adoption of these tests in the post-HCV era are the 'window of infectivity', and the possibility of other NANB viruses causing PTH.

Anti-HBc also be a surrogate marker for HIV infection, sexual promiscuity and intravenous drug abuse as well as a specific marker for residual HBV infectivity (Alter, 1989). In addition, the problems of non-specificity of the ALT test, cost, donor loss and donor counselling have to be considered, as well as the considerable input from virological reference laboratories required for confirmatory testing, with the possibility of 10% of results remaining indeterminate. The introduction of specific viral markers to narrow the 'window of infectivity' and exclude other virsuses may, in due course, render the subject of surrogate ALT and anti-HBc testing of only academic interest, particularly in countries with a low incidence of NANB post-transfusion hepatitis, such as the U.K.

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REFERENCES

Aach, R.D., Szmuness, W., Mosley, J.W., Hollinger, B., Kahn, R.A., Stevens, C.E., Edwards, V.M. & Werch, J. (1981) Serum alanine aminotransferase of donors in relation to the risk of non-A, non-B hepatitis in recipients. The transfusion transmitted viruses study. *New England Journal of Medicine*, 304, 989–994.

Akins, R., Fang, C., Mallory, D., Zelki, D., Sobol, D. & AuBuchon, J.A. (1987) Comparison of three kits for antibodies to hepatitis B core antigen (anti-HBc). *Transfusion*, 27, 522.

Alter, H.J. (1985) Post-transfusion hepatitis: clinical features, risk and donor testing, In: *Infection, Immunity and Blood Transfusion* (eds Dodd, R.Y. and Barker, S.), 47–61. Liss, New York.

Alter, H.J. (1989) Discovery of the non-A, non-B hepatitis virus: the end of the beginning or the beginning of the end. *Transfusion Medicine Reviews*, 3, 77–81.

Alter, H.J., Purcell, R.H., Holland, P.V., Alling, D.W. & Koziol, D.E. (1981) Donor transaminase and recipient hepatitis. Impact on Blood Transfusion Service. *Journal of the American Medical Association*, 246, 630–634.

Anonymous (1987) American Association of Blood Banks reaffirms decision to require ALT and anti-HBc testing. *Blood Bank Week*, 4, 1–2.

AuBuchon, J.P., Sandler, S.G., Fang, C.T. & Dodd, R.Y. (1989a) American Red Cross experience with routine testing for hepatitis core antibody. *Transfusion*, 29, 230–232.

AuBuchon, J.P., Wilkinson, J.S., Kassapian, S.J. & Edwards, G.C. (1989b) Establishment of a system to standardize acceptability criteria for alanine aminotrasferase activity in donated blood. *Transfusion*, 29, 17–22.

Bowry, T.R. & Shah, M.V. (1983) A pilot study of hepatitis B viral markers in volunteer blood donors in Kenya, East Africa. *Vox Sanguinis*, 44, 385–289.

Choo, Q-L., Kuo, G., Weiner, A.J., Overby, L.R., Bradley, D.W. & Houghton, M. (1989) Isolation of a cDNA clone

derived from a blood-borne non-A non-B viral hepatitis genome. *Science*, 244, 359–362.

Dietmar, N., Seiler, D., Franz, H. & Jung, K. (1990) Liver enzymes and long distance running. *Clinical Chemistry*, 36, 1163. (Abstract.)

Editorial (1989) Will the real hepatitis C stand up? *Lancet*, ii, 307–308.

Esteban, J.I., Esteban, R., Viladomiu, L., Lopez-Talavera, J.C., Gonzalez, A., Hernandez, J.M., Roget, M., Vargas, V., Genesca, J. & Buti, M. (1989) Hepatitis C virus antibodies among risk groups in Spain. *Lancet*, ii, 294–297.

Freidman, S.L., Dienstag, J.L., Watkins, E., Hinkle, C.A., Spiers, J.A., Rider, S.V. & Huggins, C.E. (1987) Evaluation of blood donors with elevated alanine aminotransferase levels. *Annals of Internal Medicine* 107, 137–144.

Gillon, J., Hussey, A.J., Howe, S.P., Beckett, G.J. & Prescott, R.J. (1988) Post-transfusion non-A, non-B hepatitis: significance of raised ALT and anti-HBc in blood donors. *Vox Sanguinis*, 54, 148–153.

Hanson, M.R. & Poleski, H.F. (1987) Evaluation of routine anti-HBc screening of volunteer blood donors: a questionable surrogate test for non-A, non-B hepatitis. *Transfusion*, 27, 107–108.

Health Education Authority *That's the Limit* (1989) HMSO London.

Hollinger, F.B. (1987) Specific and surrogate screening tests for hepatitis. In: *Transfusion Transmitted Viruses: Epidemiology and pathology* (eds Insalco, S.J. and Menitove, J.E.), 4: 69–86. American Association of Blood Banks, Arlington VA.

Kahn, R.A., Johnson, G., Aach, R.D., Hines, A., Ellis, F. & Miller, W.V. (1982). The distribution of serum alanine aminotransferase levels in a blood donor population. *Americal Journal of Epidemiology*, 115, 929–939.

Koziol, D.E., Holland, P.V., Alling, D.W., Melpolder, J.C., Solomon, R.E., Purcell, R.H., Hudson, L.M., Shoup, F.J., Krakauer, H. & Alter, H.J. (1986) Antibody to hepatitis core antigen as a paradoxical marker for non-A, non-B hepatitis agents in donated blood. *Annals of Internal Medicine*, 104, 488–495.

Kuhnl, P., Seidl, S., Stangel, W., Beyer, J., Sibrowski, W. & Flick, J. (1989) Antibodies to Hepatitis C Virus in German blood donors. *Lancet*, ii, 294–295.

Kuo, G., Choo, Q-L., Alter, H.J., Gitnick, G.L., Redeker, A.G., Purcell, R.H., Miyamura, T, Dienstag, J.L., Alter, M.J., Stevens, C.E., Tegtmeier, G.E., Bonino, F., Colombo, M., Lee, W-S., Kuo, C., Berger, K., Shuster, J.R., Overby, L.R., Bradley, D.W. & Houghton, M. (1989) An assay for circulating antibodies to a major etiologic virus of human non-A non-B virus. *Science*, 244, 362–364.

Mijovic, V., Contreras, M. & Barbara, J.A.J. (1987) Serum alanine aminotransferase (ALT) and gamma-glutamyltransferase (gamma-GT) activities in North London blood donors. *Journal of Clinical Pathology*, 40, 1340–1344.

Paton, A. (1988) *ABC of Alcohol* 2nd edn. British Medical Association, London.

Reesink, H.W., Leentvaar-Kuypers, A., van der Poel, C.L., Lelie, P.N., Pieth, R.N.I., Mulder-Folkerts, D.K.F., Pieters, T., van den Ende, A., Schaasberg, W. & Countinho, R.A. (1988) Non-A, non-B post transfusion hepatitis in open heart surgery patients in the Netherlands: preliminary results of a prospective study. In: *Viral Hepatitis and Liver Disease*. (ed. Zuckerman, A.J.) 558–560. Alan R Liss, New York.

Sherlock, S. (1981) *Diseases of the Liver and Biliary System*. 6th edn. Blackwell Scientific Publications, Oxford.

Stevens, C.E., Aach, R.D., Hollinger, F.B., Mosley, J.W., Szmuness, W, Kahn, R, Werch, J. & Edwards, V. (1984) Hepatitis B Virus antibody in blood donors and the occurrence of non-A, non-B hepatitis in transfusion recipients. An analysis of the transfusion transmitted virus study. *Annals of Internal Medicine*, 101, 733–738.

Tedder, R.S., Cameron, C.H., Wilson-Croome, Howell, D.R., Colgrove, A. & Barbara, J.A.J. (1980) Contrasting patterns and frequency of antibodies to surface, core and e antigens of hepatitis B virus in blood donors and homosexual patients. *Journal of Medical Virology*, 6, 323–332.

Troisi, C.L. & Hollinger, F.B. (1987) Current tests for antibody to Hepatitis B core antigen used to screen donors for non-A, non-B hepatitis are comparable to the original radioimmunoassay for Hepatitis B core antigen. *Transfusion*, 27, 438–440.

Van Der Poel, C.L., Reesink, H.W., Leslie, P.N., Leentvaar-Kuypers, A., Choo, Q.L., Koo, G. & Houghton, M. (1989) Anti-hepatitis C antibodies and non-A, non-B post transfusion hepatitis in the Netherlands. *Lancet*, ii, 297–298.

Widell, A., Sundstrom, G., Hansson, B.G., Fex, G., Moestrup, T. & Nordenfelt, E. (1988) Relation between donor transaminase and recipient hepatitis non-A non-B in Sweden. *Vox Sanguinis*, 54, 154–159.