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Serum alanine aminotransferase (ALT) and γ-glutamyltransferase (γ-GT) activities in north London blood donors

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SUMMARY Serum alanine aminotransferase (ALT) and γ-glutamyltransferase (γ-GT) activities were measured in over 2000 north London blood donors. The results were compared with those from the United States. The percentage of the total donor population with ALT activities above 40 IU/l in 1986 was greater than that found in our earlier studies in 1973 and 1982 (4·6% compared with 2·8% and 3·1%, respectively). There was a noticeable difference in the ALT distribution between male and female donors: mean +2·25 SD for male donors was 55·3 IU/l, while that for female donors was 30·8 IU/l at 37°C. In stability studies the optimal temperature for short term storage (10 days) was 4°C (6·4% loss of activity after 10 days). Surprisingly, storage at lower temperatures (−35°C and −80°C) resulted in greater loss of activity.

To try to reduce the incidence of non-A non-B hepatitis (NANB) in the United States after transfusion the American Association of Blood Banks (AABB) recently issued guidelines[1] to its member institutions for the implementation of "surrogate testing" of donor blood for alanine aminotransferase (ALT) activity and antibody to the hepatitis B core antigen (anti-HBc). Despite the fact that 60–70% of donors with abnormal surrogate markers do not transmit NANB hepatitis and that testing for these non-specific markers may reduce but not eliminate the risk of hepatitis associated with transfusion, the AABB recommended that testing of all blood donations should be implemented for both ALT and anti-HBc by June 1, 1987.

It seemed necessary, therefore, to update our previous studies[2] on the activities of alanine aminotransferase (ALT) in blood donors in the north London area. We also wished to assess the effect, if any, that the education campaign on the acquired immune deficiency syndrome (AIDS) has had on the prevalence of surrogate markers for NANB hepatitis in the donor panel. Additionally, we wanted to see whether any changes in the local ALT distribution had an obvious influence on the reported incidence of NANB hepatitis associated with transfusion. Measurement of the activities of γ-glutamyltransferase (γ-GT) was also included in this study because it seemed the ideal opportunity to widen our previous study[3] of the activity and distribution of γ-GT in a healthy adult population.

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Material and methods

Over 2000 serum samples from known donors (donors who had donated blood on at least one previous occasion) were randomly selected and tested for ALT and γ-GT. The age and sex of each donor was recorded (54% male, 46% female; age range 18–65). The serum samples were stored at 4°C and the enzyme activities determined within 24 hours of donation on an EPOS automated clinical analyser (Eppendorf range) using Merckotest reagents (according to the Scandinavian Committee on Enzymes for ALT) at 37°C. Two different standards were measured at the beginning and at the end of every run and they were also included after every series of 30 samples during the runs. The interbatch coefficient of variation for ALT and γ-GT was 4·5% and 4·4%, respectively.

A repeat measurement was made of every sample with an enzyme activity above a set value (40 IU/l for ALT, 50 IU/l for γ-GT). For all high values, there was no significant difference between the original screen and repeat measurements. A small proportion of samples was used to test the effect of storing sera at different temperatures (4°C; room temperature; −35°C; −80°C) over 10 days. The effect on ALT activities of repeatedly freezing and thawing serum samples was also investigated.

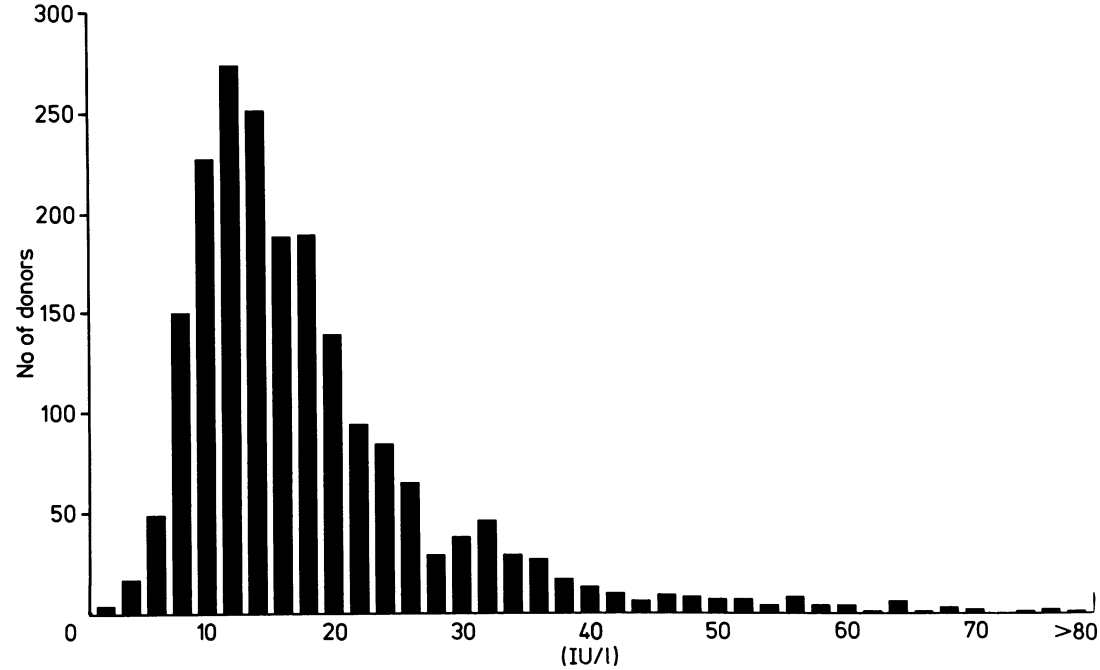

Fig 1 *Distribution of ALT activities in 2023 male and female blood donors in north London.*

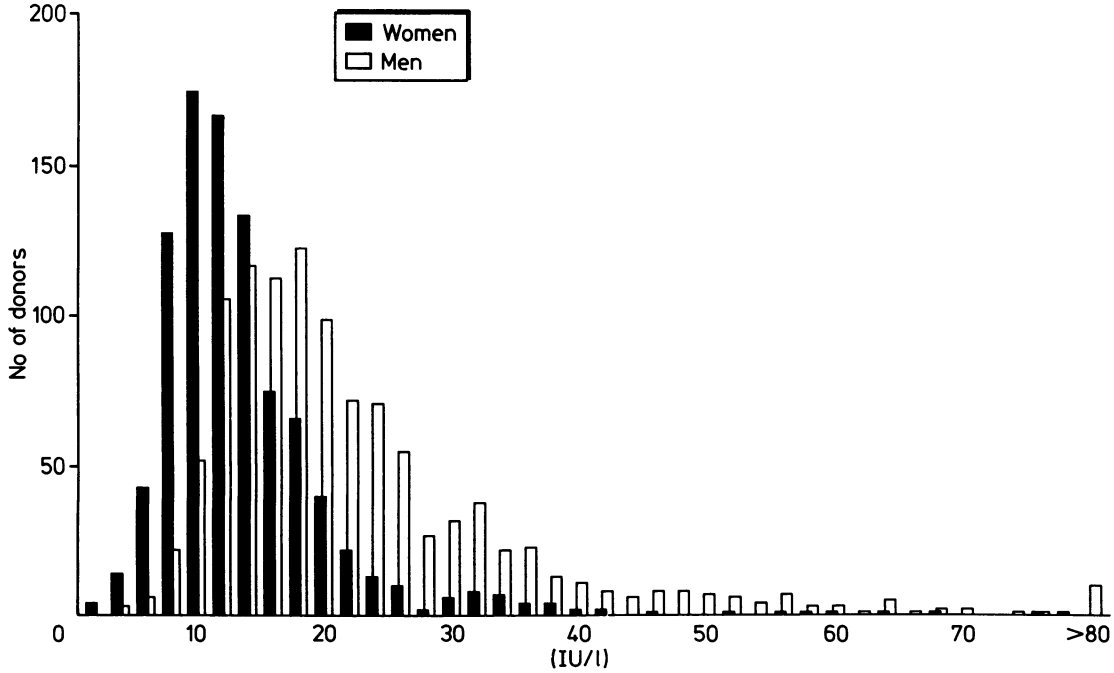

Fig 2 *Distribution of ALT activities in 1088 male and 935 female blood donors in north London.*

Results

Enzyme values obtained from the EPOS analyser were comparable with those obtained from the LKB 8600 reaction rate analyser (after temperature correction) which we used in our previous studies.[2 3] Fig 1 shows the distribution of ALT activities in a population of 2023 donors of both sexes. As expected there was considerable positive skew. When the population figures are analysed according to sex (1088 men and 935 women) the resulting histogram showed a much wider spread of higher ALT values in male donors (fig 2).

Table 1 shows the mean values and standard deviation for ALT and γ-GT in blood donors. In a skewed distribution of this kind the mean value is artificially raised, and the median gives a better estimate of the average value. Hence the median for each age group is also presented, together with the range of enzyme activities observed.

Groups of 10 serum samples were stored at four different temperatures for 10 days. The ALT values were measured daily and the percentage loss of activity over 10 days calculated. Two sets of samples were kept at −35°C: in one set the samples were kept frozen and only thawed once immediately before measurement; in the second set the samples were thawed and refrozen every day (10 freeze-thaw cycles in all).

Table 2 *Effect of storage temperature on ALT activity*

Storage temperature	*Room temperature*	*+4°C*	*−35°C*	*−35°C with 10 freeze-thaw cycles*	*−80°C*
% loss of activity after 10 days	67·2	6·4	12·3	12·2	9·1

The results show that the optimal temperature for short term storage was 4°C and that repeated freeze-thaw cycles had little adverse effect on ALT activity (table 2).

Table 3 shows the percentage of donors (male and female) in each age group with ALT activities above 45 IU/l, together with the size of each group. The group of donors most likely to have raised ALT values were men between the ages of 26 and 45.

Of the donors with raised ALT activities (>55 IU/l), 19 (0·9%) (14 men, five women) had normal γ-GT values, and 24 (1·2%) (22 men, two women) had raised (>55 IU/l) values. In this study 4·5% of the subjects (73 men, 19 women) had normal ALT activities with raised γ-GT values, suggesting that alcohol consumption could be an underlying problem.

Table 1 *Mean, (SD), and median values for ALT and α-GT according to age and sex of donors*

Age (years)		*ALT (IU/l)*		*γ-GT (IU/l)*	
		Men	*Women*	*Men*	*Women*
18–25	x (median)	20·0 (16)	12·4 (10)	25·6 (21)	18·6 (16)
	SD	13·3	6·8	13·8	7·2
	n	200	247	202	247
	range	4–114	1–59	13–110	9–65
26–35	x (median)	22·5 (18)	13·2 (11)	29·8 (23)	19·7 (16·5)
	SD	9·5	7·1	23·3	10·4
	n	292	240	299	240
	range	4–154	2–57	7–226	9–100
36–45	x (median)	24·1 (19)	13·7 (11)	34·8 (26)	21·7 (17)
	SD	17·2	9·0	30·7	16·9
	n	317	235	311	238
	range	5–168	2–77	13–307	8–148
46–55	x (median)	20·6 (18)	14·5 (12)	34·0 (25)	21·2 (18)
	SD	9·9	8·9	24·0	14·2
	n	191	126	189	124
	range	6–84	4–75	10–163	10–149
56–65	x (median)	20·3 (18)	14·9 (13)	33·8 (28)	24·8 (20)
	SD	9·7	5·9	21·0	13·5
	n	88	74	87	74
	range	8–66	5–40	14–174	13–81
All age groups	x (median)	22·3 (18)	13·4 (11)	31·4 (24)	20·6 (17)
	SD	14·7	7·7	24·0	12·9
	n	1088	935	1088	934
	range	4–168	1–77	7–307	8–149
All ages, both sexes	x (median)	18·2 (14·5)		26·4 (20)	
	SD	12·8		20·4	
	n	2023		2022	
	range	1–168		7–307	

Table 3 *Percentage of donors in each age group with ALT activities above 45 IU/l*

	Age group (years)				
	18–25	*26–35*	*36–45*	*46–55*	*56–65*
Male % (n=)	4·5 (200)	8·6 (292)	6·6 (317)	2·6 (191)	4·5 (88)
Female % (n=)	0·4 (247)	0·8 (240)	1·3 (235)	1·6 (126)	0 (74)

Discussion

The reported incidence of hepatitis associated with transfusion in the United States ranges from 5·4% to 27·1%, with over 90% of these cases designated NANB hepatitis.[5] The agent(s) responsible for this type of hepatitis have not been identified. Although most cases of NANB hepatitis run a subclinical course, the American prospective studies show that one of the most characteristic features is the propensity of about half the cases to progress to some form of chronic liver disease[6]; for this reason the board of directors of the AABB issued guidelines for the implementation of tests for "surrogate" markers for NANB hepatitis on all blood donations.

In the United Kingdom the incidence of NANB hepatitis is not well documented, and no large prospective studies have been carried out recently. For the past 16 years we have carefully monitored reports of clinically manifest hepatitis incurred after transfusion from the 52 hospitals supplied by the North London Blood Transfusion Centre (population 3·4 m); fig 3 shows the number of cases of probable NANB hepatitis reported in this period. The increase in 1981 was probably due to improved reporting by clinicians after an education campaign to heighten awareness of hepatitis associated with transfusion in hospitals. Currently, about four to six cases are reported annually. In the absence of a proper controlled trial these data are likely to represent an underestimate.

The value of ALT screening of donors as a surrogate test for NANB hepatitis in this country ought to be critically examined in case it is ever proposed as a potential extra safeguard for recipients of blood. Without accurate figures to show the incidence of NANB hepatitis incurred after transfusion in the United Kingdom, it is impossible to make realistic estimates of the cost effectiveness of screening programmes.

It is interesting to compare the percentage of the total donor population with raised ALT activities at various cut off points in the current study with those found in two earlier studies on our donors in 1973 and 1982.[2] These results (table 3) could be interpreted in one of two ways: either ALT values are rising in the population as a whole or the Transfusion Service is

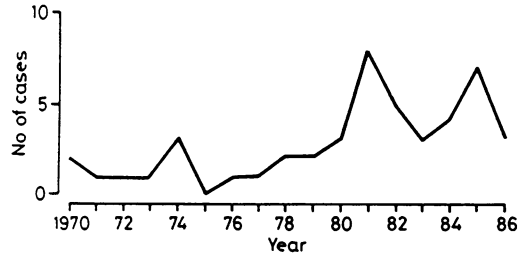

Fig 3 *Reported incidence of suspected non-A, non-B hepatitis, incurred after transfusion in recipients of blood from the North London Blood Transfusion Centre between 1970 and 1986.*

now attracting a different cross section of the community as blood donors. Although the number of blood donors with raised ALT activities has risen over the past four years, the incidence of reported NANB hepatitis associated with transfusion in our region has remained roughly constant. The increase of donors with raised ALT values has occurred despite the intensification of donor education and subsequent "self exclusion" of donors in high risk groups for human immunodeficiency virus (HIV) infection. This confirms that many other factors, apart from NANB hepatitis, affect ALT activity. Factors such as high alcohol consumption, obesity, medication, strenuous exercise, inhalation of solvents, etc, increase activities of ALT, in addition to the higher activities normally seen in male donors. It has been estimated that 50% of donor rejections in the United States due to raised ALT activity would be due to non-viral causes.[6] This is supported by reports that only 30–40% of donors with high ALT seem to be infected with NANB hepatitis agents.[7]

In the United States many transfusion centres have selected 45 IU/l as a useful cut off point for ALT. Any donation with an ALT activity above this value should be discarded. Donors should be informed or counselled, or both, if two successive donations are above the cut off point or if any single donation has an ALT value three times the upper limit of normal (about 135 IU/l). The same policy, if applied to the North London Blood Transfusion Centre, collecting about 200 000 donations annually, would result in the rejection of 7 200 donations/year (about 21 donations

Table 4 *Percentage of total donor population with raised ALT activities at various cut off points*

Cut off point (IU/l)	*1973*	*1982*	*1986*
>40	2·8%	3·1%	4·6%
>45	1·6	1·8	3·6
>50	1·2	0·9	2·7
>55	0·6	0·7	1·9

Table 5 *Normal ALT activities in male and female donors (north London)*

	Both sexes		*Male*		*Female*	
	Normal data	*Log data*	*Normal data*	*Log data*	*Normal data*	*Log data*
Mean	18·2	15·5	22·3	19·5	13·4	11·9
SD	12·8		14·7		7·7	
Mean + 2 SD	43·7	45·7	51·7	52·4	28·8	30·3
Mean + 2·25 SD	46·9	52·2	55·3	59·3	30·8	34·1

every day). It is not known what proportion of these rejections in the United Kingdom would be due to non-viral causes nor the proportion of donors who would have repeatedly high ALT activities.

Another factor which has not received much consideration in the United States is the pronounced difference in ALT distribution between male and female donors, which prompts the question, should there be two different cut off points, one for male and another for female donors and also, what should be considered a normal activity in males and females? Table 5 highlights these differences and is presented so that the American way of calculating the log mean normal value can be compared with the usual method using untransformed data, when applied to ALT activities obtained from United Kingdom donors.

Either method (and there does not seem to be any practical advantage in using logs) shows a large difference between the cut off value of mean +2·25 SD between the sexes. Once again, in the absence of some form of prospective trial, there is no evidence to show that infectivity is increased in female donors with ALT activities in the "grey area" between 30 and 60 IU/l.

Our results on the influence of storage temperature on ALT stability do not agree with those of Williams *et al*,[4] who reported large losses (46%) of activity over six days at −20°C; their results at other temperatures, however, agree with ours.

Before we even consider testing blood donors for ALT, a well designed prospective trial is needed to compare the incidence of hepatitis associated with transfusion in patients who have received blood only from donors with normal ALT activities with those receiving untested blood. As shown above, even in the United States the predictive value of ALT testing of blood donations for NANB hepatitis is very poor. The costs of testing and discarding donor blood would need to be examined as well as the costs of informing and counselling donors found to have ALT values repeatedly above the normal, or donors with excessively high values at any one time. Extrapolating data from the United States to this country without knowing the magnitude of the problem or its preventability would be ill advised.

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