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Alanine Aminotransferase, Gamma-Glutamyltransferase, Antibodies to Hepatitis B Core Antigen and Antibodies to Hepatitis C Virus in Blood Donor Screening

A Prospective Study in Finland

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Abstract. Alanine aminotransferase (ALT), γ -glutamyl-transferase and hepatitis B core antibodies were evaluated as donor markers in a prospective study of 685 open-heart surgery patients. Of these three surrogate markers, only an ALT level greater than or equal to 2 SD above the log mean had a significant association with recipient non-A, non-B hepatitis (NANBH, $p=0.02$). Antibodies to the hepatitis C virus (anti-HCV) were detected by an enzyme immunoassay in 7 of the 136 units transfused to the 11 NANBH patients and 29 of 3,650 not associated with hepatitis ($p<0.001$). Calculated from this subgroup of donors, the anti-HCV test would have a 15.6% positive predictive value with 0.92% donor loss and thus is superior as a primary screening marker to all the three surrogate tests. The predictive value could be substantially increased by subsequent ALT testing or by the use of a recombinant immunoblot anti-HCV assay.

Introduction

Several markers have been proposed or used for the screening of blood donors to diminish the risk of posttransfusion non-A, non-B hepatitis (NANBH) in recipients. Before the availability of specific serologic assays, donor serum alanine aminotransferase (ALT) was found in several prospective studies to have some predictive value [1-6], and it has been adopted in donor screening as a surrogate marker in the USA and also in some European countries. There are, however, also studies where no reduction in NANBH risk could be demonstrated as a result of donor ALT screening [7-9]. Because of its nonspecificity, lack of a reference standard for establishing or determining the cut-off value for screening, costs and problems associated with donor counselling, its usefulness has remained controversial [10, 11]. Another liver enzyme, γ -glutamyltransferase (GGTP) has also been proposed as a marker on the strength of results from animal studies [12]. Also, antibodies to the hepatitis B virus core antigen (anti-HBc) have been associated with NANBH risk in the recipient [13-16], though some more recent studies have failed to show this link [4, 17-20].

The introduction of an enzyme immunoassay for detecting antibodies to the hepatitis C virus (HCV) in 1989 [21] marked a new era in the prevention of posttransfusion hepatitis. The assay, though still not optimal in sensitivity or specificity, has already been adopted in routine donor screening in several countries. A countrywide prospective study of posttransfusion hepatitis was conducted by the Finnish Red Cross Blood Transfusion Service in 1987-1989 in which 685 open-heart surgery patients received 8,436 units of blood products [22]. One goal of the study was to determine the preventive value in donor screening of surrogate marker candidates. They might be useful during the window period of infected persons who are still seronegative for the specific marker. These data are presented in this paper together with the results of anti-HCV testing on frozen donor samples from the study.

Materials and Methods

Blood Donors

The donors in this study were random voluntary, nonremunerated donors from all parts of the country. Fifty-nine per cent of all donors on the register of the Finnish Red Cross Blood Transfusion Service are

male. The mean age of all donors is 36.3 years. The routine screening included hepatitis B surface antigen, cardiolipin and antibodies to human immunodeficiency virus during the study period.

Donor Samples

Six hundred and eighty-five patients who underwent coronary artery bypass in the five university central hospitals of Finland during the period from December 1987 to November 1988 were studied. An extra plasma sample from all blood donations was collected by the Finnish Red Cross Blood Transfusion Service. When the participating patients had received their transfusions, laboratory determinations were performed on the corresponding donor samples. The 685 patients received altogether 8,436 units of blood products (red blood cell concentrate, whole blood, fresh frozen plasma or platelet concentrate) prepared from 8,346 blood donations, the mean being 12.3 units per patient (range 1-72). Patient samples were drawn preoperatively and 2, 4, 6, 8, 10, 12, 16, 20 and 24 weeks postoperatively. Posttransfusion NANBH was defined by an ALT elevation to at least 2.5 times the upper limit of normal (≥ 100 U/l) in one sample and at least twice the limit (≥ 80 U/l) in another sample in the absence of evidence of hepatitis A and B, acute cytomegalovirus, Epstein-Barr or herpes simplex virus infection or evident nonviral causes. Eleven (1.6%) of the patients developed posttransfusion hepatitis during the 6-month follow-up; all of them were of the NANBH type, 6 showed anti-HCV seroconversion [23]. These 11 patients had received 136 units of blood products (mean 12.4, range 5-17), the average transfusion volume thus did not differ from the patients without hepatitis.

Laboratory Methods

The ALT and GGTP determinations were performed with a Cobas Fara centrifugal analyzer at 37°C according to the recommendations of the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology [24, 25] on donor plasma samples stored at +4°C [26, 27]. As ALT in the general population has a non-Gaussian, skewed distribution, a log transformation was used in analysis [28]. In blood donations, an ALT and GGTP concentration greater than or equal to 2 SD above the mean log was considered to be raised. The samples were tested for anti-HBc antibodies by an enzyme immunoassay (Corzyme; Abbott, North Chicago, Ill., USA). After these initial determinations the samples were stored at -30°C. When an assay for anti-HCV became available, thawed samples were tested by an enzyme-linked immunosorbent assay (Ortho HCV Antibody ELISA Test System, Raritan, N.J., USA) based on the recombinant HCV antigen C-100-3 produced in yeast. Samples repeatedly reactive in this assay were considered ELISA positive and were tested by an experimental recombinant immunoblot assay (Human Hepatitis C Virus Chiron RIBA HCV Test System, Ortho, Raritan, N.J., USA), which has the antigen C-100 and its subsequence 5-1-1 (produced in yeast and *Escherichia coli*, respectively) each coated as discrete bands on nitrocellulose strips. Samples reacting with both bands were considered positive by RIBA.

Statistics

Calculation of corrected efficacy, sensitivity and specificity of blood donor screening for raised ALT, GGTP, anti-HBc and anti-HCV for the prevention of posttransfusion NANBH was done according to Kozioł et al. [14]. No correction for the background incidence of NANBH was considered necessary because of the low prevalence of NANBH in the nontransfused control group (0/89). The χ^2 test with Yates' correction was used for statistical analysis.

Table 1. The distributions of plasma ALT and GGTP levels among 8,346 blood donations

ALT, U/l	n	%	GGTP, U/l	n	%
1-20	5,975	71.6	1-20	5,309	63.6
21-40	1,913	22.9	21-40	2,068	24.8
41-60	308	3.7	41-60	530	6.4
61-80	84	1.0	61-80	196	2.3
81-100	30	0.4	81-100	108	1.3
101-120	13	0.2	101-120	47	0.6
121-140	9	0.1	121-140	28	0.3
>140	14	0.2	>140	60	0.7

Results

ALT, GGTP and anti-HBc

A raised ALT concentration (≥ 58 U/l) was detected in 166 (1.99%), raised GGTP (≥ 79 U/l) in 255 (3.06%) and anti-HBc antibodies in 152 (1.82%) of all the 8,346 blood donations. The geometric mean for ALT levels was 15 U/l (range 1-274) and for GGTP 17 U/l (range 3-640). The distributions of various levels of these two enzymes among the blood donations is shown in table 1. Raised ALT was significantly associated with raised GGTP in the donor units ($p < 0.00001$), whereas it had no significant association with anti-HBc positivity.

Six of the 145 recipients of at least one unit with raised ALT (≥ 58 U/l) developed NANBH in contrast to 5 of the 540 recipients who only received blood with normal ALT levels ($p = 0.02$). Five of the 198 recipients of a unit with elevated GGTP and 6 of the 482 recipients who only received blood with normal GGTP levels developed NANBH ($p = \text{NS}$). Four of the 136 recipients of anti-HBc-positive blood and 7 of the 549 recipients who only received anti-HBc-negative blood developed NANBH ($p = \text{NS}$). Two of 6 recipients of blood with elevated ALT and positive anti-HBc in the same unit developed NANBH ($p < 0.00001$).

The NANBH risk at various donor ALT levels was calculated in subgroups of patients who had received at least one unit with an ALT ≥ 1.5 , ≥ 2.0 , ≥ 2.25 , ≥ 2.5 or ≥ 3 SD above the mean log. Of these, only the ALT level equal to or above the mean log + 2 SD (≥ 58 U/l) in donor blood significantly predicted NANBH risk in the recipient (table 2). When the frequency of elevated ALT values was calculated in the blood product units transfused to patients with and without NANBH, none of these limits reached statistical significance, though the mean log + 2 SD also gave the best p value (table 3).

Table 2. The NANBH risk at or above various donor ALT levels among recipients of at least one such unit, its statistical significance and the percentage of donors excluded by each ALT limit

Donor ALT	Recipient with NANBH/ all recipients	p	Donors, %
\geq log mean + 1.5 SD (\geq 41 U/l)	8/313 (2.6)	0.13	5.49
\geq log mean + 2.0 SD (\geq 58 U/l)	6/145 (4.1)	0.02	1.99
\geq log mean + 2.25 SD (\geq 68 U/l)	3/100 (3.0)	0.44	1.33
\geq log mean + 2.5 SD (\geq 80 U/l)	2/65 (3.1)	0.64	0.84
\geq log mean + 3.0 SD (\geq 112 U/l)	2/26 (7.7)	0.09	0.32

Figures in parentheses indicate percentages.

Table 3. Frequency of elevated ALT values in blood products transfused to patients with and without posttransfusion NANBH and its statistical significance

ALT level of transfused blood product	Blood products transfused		
	no NANBH (n=8,300)	NANB (n=136)	p
\geq log mean + 1.5 SD (\geq 41 U/l)	452 (5.4)	11 (8.1)	0.25
\geq log mean + 2.0 SD (\geq 58 U/l)	162 (2.0)	6 (4.4)	0.08
\geq log mean + 2.25 SD (\geq 68 U/l)	110 (1.3)	3 (2.2)	0.14
\geq log mean + 2.5 SD (\geq 80 U/l)	70 (0.8)	2 (1.5)	0.75
\geq log mean + 3.0 SD (\geq 112 U/l)	26 (0.3)	2 (1.5)	0.12

Figures in parentheses indicate percentages.

Anti-HCV

All 136 'implicated' samples from the donations to the 11 NANBH patients were tested by ELISA for anti-HCV. Seven of them were ELISA positive, though one repeatedly gave a borderline reading. Seven of the NANBH patients had each received one such ELISA-positive blood product. Altogether 3,650 (44%) of the 8,300 'nonimplicated' donor samples (of units transfused to patients who did not develop hepatitis) were also tested for anti-HCV. Twenty-nine of

them were positive, and the corresponding blood products had been transfused to 27 recipients. Thus, anti-HCV antibodies were detected by ELISA in 7/136 (5.2%) implicated but only in 29/3,650 (0.8%) nonimplicated products ($p < 0.001$).

The 6 implicated donor samples with clearly positive anti-HCV ELISA results were also reactive for both antigens in the RIBA test (the age of these donors was 23-36 years, 5/6 were male). The borderline ELISA-positive sample did not react with either of them. Seven of the 29 nonimplicated donor samples reacted with antigen C-100, one with antigen 5-1-1 but none with both antigens. Thus, reactivity for both antigens of the RIBA was found in 6/7 implicated but in 0/29 nonimplicated ELISA-positive samples ($p < 0.00005$). There was one additional factor which distinguished between the implicated and nonimplicated ELISA-positive samples: 5/7 of the implicated but 0/29 of the nonimplicated ELISA-positive samples had raised ALT levels ($p = 0.0001$).

Only 1 of the 129 (0.8%) implicated but anti-HCV ELISA-negative units had elevated ALT (59 U/l); it had been given to one of the patients who developed hepatitis. This patient also received the borderline ELISA-positive but RIBA-negative unit and did not develop anti-HCV seroconversion. Eighty-two of the 3,621 (2.3%) nonimplicated and ELISA-negative samples had elevated ALT levels (58-190 U/l).

Table 4 summarizes the characteristics of ALT, GGTP, anti-HBc, anti-HCV ELISA alone, anti-HCV ELISA + ALT and anti-HCV ELISA + RIBA as markers for donor screening in NANBH prevention.

Discussion

Sixty-four percent of the NANBH cases in the bypass patients received a donation which was anti-HCV positive. The corresponding figure for ALT was 55%, and this was the most predictive of the 'old' surrogate markers of ALT, GGTP and anti-HBc. When the proportion of marker-positive units not transmitting NANBH is also taken into account, the positive predictive value (the percentage of NANBH cases among all recipients of marker-positive blood) of anti-HCV testing by ELISA alone (15.6%) is almost 4 times that of ALT (4.1%). Combination with subsequent RIBA makes it 100% for this study. The positive predictive values found by van der Poel et al. [29] in their prospective study in Amsterdam were quite similar: 16.2% for anti-HCV ELISA and 3.6% for ALT. The percentage of donors in this study that would be excluded would be 1.99%

Table 4. Calculated preventive effect on posttransfusion NANBH of blood donor screening for ALT, GGTP, anti-HBc, anti-HCV ELISA alone and anti-HCV ELISA together with ALT or RIBA

	ALT	GGTP	Anti-HBc	Anti-HCV		
				ELISA only	ELISA + ALT	ELISA + RIBA
Crude efficacy = sensitivity ¹ , %	55	45	36	64	45	55
Maximal corrected efficacy ¹ , %	45	18	18	68 ²	71 ²	71 ²
Specificity ¹ , %	79	71	80	90 ²	100 ²	100 ²
Positive predictive value ¹ , %	4.1	2.5	2.9	15.6 ²	100 ²	100 ²
Risk of NANBH/1,000 transfusions negative for marker	0.80	1.10	1.12	0.69 ²	0.61 ²	0.61 ²
Blood donations positive for marker discarded, %	1.99	3.06	1.82	0.92 ²	0.14 ²	0.14 ²

¹ Calculated according to Koziol et al. [14]: crude efficacy = percentage of NANBH cases with a marker-positive donor among all NANBH cases; maximal corrected efficacy = the calculated number of NANBH cases occurring in spite of marker-negative substitution for each positive unit is subtracted from the number of NANBH cases with a marker-positive donor, efficacy then calculated as above; specificity = percentage of recipients of only marker-negative blood among all patients who remained free from NANBH; positive predictive value = percentage of NANBH cases among all recipients of marker-positive blood.

² Calculated from the first part of the study where donor anti-HCV ELISA testing was performed.

for ALT, 0.92% for anti-HCV ELISA and 0.14% for anti-HCV ELISA + RIBA.

One of the most difficult problems connected with ALT screening is the determination of the cutoff level. As it is raised, the test becomes more specific but less sensitive. The prevalence of elevated ALT has clear geographic variation and is significantly associated with gender, age and body weight [30–33]. We found slightly lower prevalences of raised ALT values among our donor population than reported from the USA, the UK and the Netherlands, 1.99% of our donor samples being at or above the log mean ALT + 2.0 SD as compared with 2.6 and 2.2% in the USA, approximately 3.6% in the UK and 3.8% in the Netherlands [1, 26, 29, 34]. The ALT exclusion level with maximal significance of association with recipient hepatitis was 2.0 SD above the log mean in our material, and +2.0 or +2.25 SD in the two American materials [1, 34]. A difference was found in this significance itself, the *p* value being only 0.02 in this study, <0.05 in that of van der Poel et al. [29] but <0.001 and <0.00001 in the reports from the USA [1, 34]. This might mean that in Finland and the Netherlands there are relatively more nonviral reasons for elevated ALT.

In Finland, blood donation has always been voluntary and unpaid. Donors come from a population with a low risk for hepatitis B; the prevalence of hepatitis B surface antigen positivity among new blood donors in 1985–1988 was only 0.05%. The low prevalence of anti-HBc antibodies (1.8%) among random blood donors in the present study also supports this.

It was found in this study that the markers ALT and anti-HBc are not usually associated and detect different donor populations, which had also been established by others [13, 14, 30, 31]. Anti-HBc antibodies had no significant association with recipient NANBH risk, as in other recent studies [4, 17, 18] also performed after widespread exclusion of the human immunodeficiency virus risk groups from blood donation. The risk of the rare coexistence of both elevated ALT and anti-HBc reached statistical significance, but both such donors to the hepatitis cases were also positive for anti-HCV ELISA (and RIBA). Elevated GGTP and elevated ALT overlapped considerably in donor populations, as has also been found by others [26], but GGTP did not reach predictive significance as an independent donor marker.

The conclusion from this study is that anti-HCV ELISA was the best single primary donor screening marker for the prevention of posttransfusion NANBH; it also causes least donor exclusions. In further evaluation of the anti-HCV ELISA-positive donors, ALT and RIBA seen to be almost equally effective in identifying infectivity, RIBA having slightly better sensitivity.

On the basis of these results surrogate testing by ALT, GGTP or anti-HBc does not seem effective in the prevention of NANBH as compared to specific anti-HCV testing, and introducing surrogate testing after commencement of anti-HCV is in my opinion not recommendable. The question of abolishing an already instituted screening protocol with some surrogate tests is more complicated and associ-

ated with several other problems. In Finland, the full-scale national donor screening by anti-HCV ELISA was started in April 1990. Donors reactive by ELISA but completely nonreactive with the RIBA antigens (more than 50% of the ELISA-reactive donors) are considered anti-HCV negative, and their blood is used normally for transfusion purposes. The ELISA-reactive donors positive by RIBA are excluded from donation irrespective of their ALT level and invited to a follow-up protocol. The rapidly changing scope of available tests may give rise to reconsideration of policies adopted so far.

It must, however, be noted that the anti-HCV test here neither detected all donations associated with NANBH in recipients nor became positive for every NANBH patient even during a year's follow-up. Anti-HCV ELISA-positive, indeterminate RIBA donors have in rare cases been associated with recipient NANBH [35, 36], and anti-HCV ELISA-negative blood has been shown to transmit NANBH and contain HCV RNA detected by the polymerase chain reaction [37, 38]. The ELISA and RIBA used in this study and in our routine screening are first-generation assays. Several newer versions with more antigens to increase the sensitivity and specificity are under development and testing, and will help the distinction of true anti-HCV positivity. Obviously, a sensitive test which can detect the virus and is suitable for mass screening would be the optimal solution. This would also clarify the possibility of other blood-borne NANBH viruses.

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References

- Alter HJ, Purcell RH, Holland PV, Alling DW, Koziol DE: Donor transaminase and recipient hepatitis. Impact on blood transfusion services. *JAMA* 1981;246:630-634.
- Aach RD, Szmunn W, Mosley JW, Hollinger FB, Kahn RA, Stevens CE, Edwards VM, Werch J: Serum alanine aminotransferase of donors in relation to the risk of non-A, non-B hepatitis in recipients. The transfusion-transmitted viruses study. *N Engl J Med* 1981;304:989-994.
- Widell A, Sundström G, Hansson BG, Fex G, Moestrup T, Nordenfelt E: Relation between donor transaminase and recipient hepatitis non-A, non-B in Sweden. *Vox Sang* 1988;54:154-159.
- Reesink HW, Leentvaar-Kuypers A, van der Poel CL, Lelie PN, Pietersz RNI, Mulder-Folkerts DKF, Pieters T, van den Ende A, Schaasberg W, Coutinho RA: Non-A, non-B posttransfusion hepatitis in open heart surgery patients in the Netherlands: Preliminary results of a prospective study; in Zuckerman AJ (ed): *Viral Hepatitis and Liver Disease*. New York, Liss, 1988, pp 558-560.
- Marugan RB, Zamora C, Moreno A, Erdozain Sosa JC, Hernandez FP, San Roman AL: Serum alanine aminotransferase (ALT) assay and incidence of posttransfusion non-A, non-B hepatitis. *Transfusion* 1989;29:751.
- Mariniere AM, Smilovici W, Ducos J, Duffaut M, Puel J, Vadhat P, Berthoumieu F, Fournial G, Sauer M, Trepo C: NANB post-transfusion hepatitis. A French prospective study in open heart surgery patients (abstract). 20th Congr Int Soc Blood Transfus in association with Br Blood Transfus Soc, London, July 1988.
- Alter HJ: Post-transfusion hepatitis: Clinical features, risk and donor testing; in Barker LF, Dodd RY (eds): *Infection, Immunity and Blood Transfusion*. New York, Liss, 1985, pp 47-61.
- Steinbrecher UP, Korvacs TOG, Gelly A, Touriquy M: Abnormal alanine aminotransferase level in blood units from donors in Montreal does not indicate high risk of transmitting hepatitis. *Clin Invest Med* 1983;6:327-330.
- Katchaki JN, Siem TH, Brouwer R: Post-transfusion hepatitis and serum alanine aminotransferase in blood donors. *N Engl J Med* 1981;305:403-404.
- Klein HG: Controversies in transfusion medicine. Alanine aminotransferase screening of blood donors: Pro. *Transfusion* 1990;30:363-367.
- Spurling CL, Saxena S: Controversies in transfusion medicine. Alanine aminotransferase screening of blood donors: Con. *Transfusion* 1990;30:368-373.
- Brotman B, Prince AM: Gamma-glutamyltransferase as a potential surrogate marker for detection of the non-A, non-B carrier state. *Vox Sang* 1988;54:144-147.
- Stevens CE, Aach RD, Hollinger FB, Mosley JW, Szmunn W, Kahn R, Werch J, Edwards V: 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 viruses study. *Ann Intern Med* 1984;101:733-738.
- Koziol DE, Holland PV, Alling DW, Melpolder JC, Solomon RE, Purcell RH, Hudson LM, Shoup FJ, Krakauer H, Alter HJ: Antibody to hepatitis B core antigen as a paradoxical marker for non-A, non-B hepatitis agents in donated blood. *Ann Intern Med* 1986;104:488-495.
- Sugg U, Schenzle D, Hess G: Antibodies to hepatitis B core antigen in blood donors screened for alanine aminotransferase level and hepatitis non-A, non-B in recipients. *Transfusion* 1988;28:386-388.
- Cossart YE, Kirsch S, Ismay SL: Post-transfusion hepatitis in Australia. Report of the Australian Red Cross study. *Lancet* 1982;i:208-213.
- Hoyos M, Sarrion JV, Pérez-Castellanos T, Prieto M, Marty ML, Garrigues V, Berenguer J: Prospective assessment of donor blood screening for antibody to hepatitis B core antigen as a means of preventing posttransfusion non-A, non-B hepatitis. *Hepatology* 1989;9:449-451.
- Aymard JP, Janot C, Gayet S, Guillemin C, Canton P, Gaucher P,

- Streiff F: Post-transfusion non-A, non-B hepatitis after cardiac surgery. *Vox Sang* 1986;51:236-238.
- 19 Ohto H, Ninomiya K: Donor anti-HBc and ALT and non-A, non-B hepatitis. *Transfusion* 1989;29:277-278.
- 20 Hanson MR, Polesky HF: Evaluation of routine anti-HBc screening of volunteer blood donors: A questionable surrogate test for non-A, non-B hepatitis. *Transfusion* 1987;27:107-108.
- 21 Kuo G, Choo QL, Alter HJ, Gitnick GL, Redeker AG, Purcell RH, Miyamura T, Dienstag JL, Alter MJ, Stevens CE, Tegtmeier GE, Bonino F, Colombo M, Lee WS, Kuo C, Berger K, Shuster JR, Overby LR, Bradley DW, Houghton M: An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science* 1989;244:362-364.
- 22 Ebeling F, Naukkarinen R, Hanhela R, Jalonen J, Kaukinen L, Salmenperä M, Suistomaa M, Leikola J: Posttransfusion hepatitis after open heart surgery in Finland - A prospective study. *Transfus Med*, in press.
- 23 Ebeling F, Naukkarinen R, Leikola J: Posttransfusion hepatitis C in Finland. *Transfus Med*, in press.
- 24 Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology: Scandinavian standardizations of enzyme determination. *Scand J Clin Lab Invest* 1974;33:287-306.
- 25 Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology: Recommended method for the determination of γ -glutamyltransferase in blood. *Scand J Clin Lab Invest* 1976;36:119-125.
- 26 Mijovic V, Contreras M, Barbara JAJ: Serum alanine aminotransferase (ALT) and γ -glutamyltransferase (γ -GT) activities in north London blood donors. *J Clin Pathol* 1987;40:1340-1344.
- 26 Williams KM, Williams AE, Kline LM, Dodd RY: Stability of serum alanine aminotransferase activity. *Transfusion* 1987;27:431-433.
- 28 Saxena S, Korula J, Shulman IA: A review of donor alanine aminotransferase testing. Implications for the blood donor and practitioner. *Arch Pathol Lab Med* 1989;113:767-771.
- 29 Van der Poel CL, Reesink HW, Schaasberg W, Leentvaar-Kuypers A, Bakker E, Exel-Oehlers PJ, Lelie PN: Infectivity of blood seropositive for hepatitis C virus antibodies. *Lancet* 1990;335:558-560.
- 30 Driss F, Boboc B, Zarski JP, Cals MJ, Pol S, Eme D, Ekindjian OG, Courouge AM, Brechot C, Berthelot P, Nalpas B: An epidemiological and clinical study of transaminase levels and hepatitis B antibodies in 1,100 blood donors. *Vox Sang* 1989;57:43-48.
- 31 Gillon J, Hussey AJ, Howe SP, Beckett GJ, Prescott RJ: Post-transfusion non-A, non-B hepatitis: Significance of raised ALT and anti-HBc in blood donors. *Vox Sang* 1988;54:148-153.
- 32 Sherman KE, Dodd RY: Alanine aminotransferase levels among volunteer blood donors: Geographic variation and risk factors. *J Infect Dis* 1982;145:383-386.
- 33 Wejstål R, Hansson G, Lindholm A, Norkrans G: Persistent alanine aminotransferase elevation in healthy Swedish blood donors - mainly caused by obesity. *Vox Sang* 1988;55:152-156.
- 34 Hollinger FB, cited in Zuck TF, Sherwood WC, Bove JR: A review of recent events related to surrogate testing of blood to prevent non-A, non-B posttransfusion hepatitis. *Transfusion* 1987;27:203-206.
- 35 Van der Poel CL, Reesink HW, Lelie PN, Exel-Oehlers P, Winkel I, Schaasberg W, Polito A, Houghton H: Anti-HCV and transaminase testing of blood donors. *Lancet* 1990;336:187-188.
- 36 Bellobuono A, Mozzi F, Petrini G, Zanella A, Sirchia G: Infectivity of blood that is immunoblot intermediate reactive on hepatitis C virus antibody testing. *Lancet* 1990;336:309.
- 37 Weiner AJ, Kuo G, Bradley DW, Bonino F, Saracco G, Lee C, Rosenblatt J, Choo QL, Houghton M: Detection of hepatitis C viral sequences in non-A, non-B hepatitis. *Lancet* 1990;335:1-3.
- 38 Zanetti AR, Tanzi E, Zehender G, Magni E, Incarbone C, Zonaro A, Primi D, Cariani E: Hepatitis C virus RNA in symptomless donors implicated in post-transfusion non-A, non-B hepatitis. *Lancet* 1990;336:448.

Addendum: Since the submission of this paper, a second-generation recombinant immunoblot assay (4-RIBA) for anti-HCV (Ortho Diagnostic Systems Ltd, Raritan, N.J., USA) has become available and was used to evaluate donor samples from this study. With the two new recombinant HCV antigens incorporated in this assay, c33c and c22-3, 3 additional seropositive donors to 3 hepatitis C patients were detected; these donors and patients had been nonreactive by the first-generation ELISA. An assay detecting antibodies to the HCV antigens c33c and c22-3 would thus have a sensitivity of 82% in NANBH prevention calculated according to table 4.

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