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Infectivity of blood seropositive for hepatitis C virus antibodies

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Stored serum samples from 5150 blood product transfusions and 383 recipients were tested for antibodies to hepatitis C virus (anti-HCV) by a recombinant enzyme-linked immunosorbent assay (ELISA) as part of a prospective study on post-transfusion non-A, non-B hepatitis (NANBH). Donor cofactors associated with HCV infectivity of anti-HCV-positive blood products were raised alanine aminotransferase concentrations (6 of 9 infective *vs* 1 of 26 not infective); a mean ELISA optical density/cut-off ratio ⩾2 (7 of 9 *vs* 9 of 26); both preceding factors (together in 6 blood products, all of which transmitted infection); and persistent donor anti-HCV seropositivity. Use of anti-HCV screening to prevent post-transfusion NANBH was compared with measurement of alanine aminotransferase concentrations: a corrected efficacy of 63% and 65%, a specificity of 93% and 64%, and a positive predictive value of 16·2% and 3·6% were found, respectively; 0·7% or 3·8% of blood donations, respectively, would be discarded. Blood donor screening for anti-HCV is recommended to reduce the incidence of post-transfusion NANBH.

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Introduction

Antibodies to hepatitis C virus (HCV) in blood products are associated with post-transfusion non-A, non-B hepatitis (NANBH) in recipients.[1-3] The recombinant HCV antigen (C100-3) of the prototype anti-HCV radioimmunoassay developed by Chiron (Emeryville, CA, USA)[2-4] is now available as an anti-HCV enzyme-linked immunosorbent assay (ELISA),[5] which can be used for screening of blood products. However, use of the ELISA in large populations with a relatively low prevalence of infection, such as blood donors, may yield false-positive results. A confirmatory assay, indicative of HCV infection in anti-HCV-positive blood donors is needed, but not yet available,[6,7] although viral sequences of HCV have been detected in patients with non-A, non-B hepatitis.[8] We have investigated the in-vivo infectivity of anti-HCV-positive blood products to determine the risk of such infection and whether cofactors can be identified to discriminate between infective and non-infective specimens, and to assess the risk/benefit ratio of prevention of post-transfusion NANBH by screening blood donors for anti-HCV, compared with routine measurement of alanine aminotransferase.[1,9-11]

Materials and methods

A prospective study to establish the incidence of post-transfusion NANBH in 393 patients who underwent cardiac surgery was conducted in Amsterdam from 1984 to 1986.[1] 383 sets of stored serum samples obtained before and at 2, 4, 6, 8, 10, 12, 16, 20, 26, and 32 weeks after transfusion were available. 10 patients could not be followed because of incomplete data (eg, move, death, clerical error, incomplete donor records, incomplete testing). Serum samples from 4900 of the 4906 whole blood donations involved were also available for investigation. None of the donations for which the serum was not available was associated with post-transfusion anti-HCV seroconversion or NANBH. The 4906 blood donations had been processed into 5150 implicated blood products.

Post-transfusion NANBH was defined, as previously described,[1,3] by raised alanine aminotransferase concentrations in the recipient after transfusion in the absence of evidence of hepatitis A and B, or acute Epstein-Barr virus or cytomegalovirus infection. Information on post-transfusion NANBH in recipients, and on cofactors in blood donations, was obtained from the original study as previously described.[1,3] In blood donations, an alanine aminotransferase concentration of or greater than 2 SD above the mean log was considered to be raised.[1,10,11]

All 4900 serum samples of implicated blood donations were tested for anti-HCV, as were serum samples from all recipients obtained before, and at 12 and 26 weeks after, transfusion. All sequential serum samples were tested in recipients who had a positive anti-HCV result, 1 or more anti-HCV-positive blood product transfusions, or post-transfusion NANBH. Anti-HCV seroconversion in the recipient was diagnosed when the serum sample obtained before transfusion was anti-HCV-negative, and 2 or more sequential serum samples obtained more than 6 weeks after transfusion were anti-HCV-positive. When passive antibody transmission was likely (sample before transfusion anti-HCV-negative, samples at 2 and 4 weeks anti-HCV-positive) seroconversion was diagnosed when 2 or more samples obtained more than 12 weeks after transfusion were anti-HCV-positive. The term recipient HCV infection is used to indicate all recipients with post-transfusion NANBH, anti-HCV seroconversion, or both conditions.

Anti-HCV was detected with an ELISA system[5] that used recombinant HCV C100-3 antigen[2-4] on the solid phase (Ortho, Raritan, New Jersey, USA), at a 1:10 dilution according to the manufacturer's instructions. Reactive samples were repeated in duplicate, and were only considered positive if at least 2 of 3 test determinations were reactive. To compare ELISA results of

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TABLE I—ANTI-HCV ELISA RESULTS IN RECIPIENTS AND BLOOD PRODUCTS

Recipients	Anti-HCV seroconversion (n = 383)	Anti-HCV-positive blood products (n = 5150)
Post-transfusion NANBH	6/9 *(67%)*	6/151 *(4%)*
No NANBH	9/374* *(2·4%)*	31/4999 *(0·6%)*
	p < 0·001 (χ^2)	p < 0·001 (χ^2)

*33 recipients were anti-HCV-positive before transfusion.

different blood donation samples, the mean optical density (OD) reading/cut-off ratio (ELISA ratio) of the triplicate ELISA test results was calculated.

A frozen serum sample of every blood donation to the Amsterdam blood bank is stored. For 28 of 35 anti-HCV-positive blood products, sequential serum samples, obtained from the donors between 1987 and 1989, were available for analysis. The donors of 7 anti-HCV-positive blood products did not donate blood during this time. 147 (range 1–8) serum samples thus obtained from the donors of 28 anti-HCV-positive blood products (in 1984–86) were also tested for anti-HCV, and were classified as negative if all follow-up serum samples were anti-HCV-negative; persistently positive if all follow-up samples were anti-HCV-positive, interrupted by no more than 1 negative serum sample; or intermittently positive. Calculation of corrected efficacy, sensitivity, and specificity of blood donor screening for anti-HCV and raised alanine aminotransferase concentration toward prevention of post-transfusion NANBH was done according to Koziol et al.[9] The χ^2 test with Yates' correction and 2-tailed Fisher's exact test were used for statistical analysis.

Results

6 of 9 (67%) recipients with post-transfusion NANBH, and 9 of 374 (2·4%) without post-transfusion NANBH, seroconverted to become anti-HCV-positive (χ^2, $p < 0·001$). (33 recipients, all without post-transfusion NANBH, were anti-HCV-positive before transfusion, and were excluded from the seroconversion analysis.) 37 of 5150 (0·7%) blood product transfusions were anti-HCV-positive. 6 of 151 (3·9%) blood products transfused to recipients with post-transfusion NANBH and 31 of 4999 (0·6%) blood products transfused to recipients without post-transfusion NANBH were anti-HCV-positive (χ^2, $p < 0·001$; table I). Of the 34 recipients who received 1 or more anti-HCV-positive blood products, 2 were anti-HCV-positive before transfusion. 35 of 37 anti-HCV-positive blood products were transfused to 32 recipients who were anti-HCV-negative before transfusion. 6 of these 35 (17%) anti-HCV-positive blood products were associated with post-transfusion NANBH, 7 (20%) were associated with anti-HCV seroconversion in the recipient, and 9 (26%) were associated with either or both outcomes (overlap, 4).

7 of 35 (20%) anti-HCV-positive blood products had a raised alanine aminotransferase concentration; 6 of the 7 (86%) were associated with post-transfusion NANBH in the recipient compared with 0 of 28 with a normal enzyme concentration (Fisher's exact test $p = 0·00004$). 16 of 35 (46%) anti-HCV-positive blood products had an ELISA ratio ≥ 2, 7 (44%) of which were associated with post-transfusion NANBH and/or anti-HCV seroconversion in the recipient, compared with 2 of 19 (11%) who had an ELISA ratio below 2 (Fisher's exact test, $p = 0·049$). The correlation of raised alanine aminotransferase and an ELISA ratio ≥ 2 as cofactors for recipient HCV infection is shown in table II. None of the 37 anti-HCV-positive blood products was anti-HBc-positive. 5 of 35 (14%) anti-HCV-positive blood products were associated with a donor history of jaundice after the age of 18 years. 3 of 5 (60%) anti-HCV-positive blood products in which the donor had had jaundice were associated with post-transfusion NANBH and/or anti-HCV seroconversion in the recipient, compared with 6 of 30 (20%) not associated with a history of jaundice (not significant).

TABLE II—ALANINE AMINOTRANSFERASE (ALT) CONCENTRATION AND ANTI-HCV ELISA RATIO OF ANTI-HCV-POSITIVE BLOOD PRODUCTS AS COFACTORS FOR INFECTIVITY

	Anti-HCV-positive blood product transfusions implicated in recipient infection					
	Post-transfusion NANBH		Seroconversion		NANBH/ seroconversion	
Cofactors	I/T	p*	I/T	p*	I/T	p*
ALT						
Normal	0/28		3/28 *(11%)*		3/28 *(11%)*	
Raised	6/7 *(86%)*	<0·0001	4/7 *(57%)*	<0·05	6/7 *(86%)*	<0·0005
ELISA ratio						
< 2	0/19		2/19 *(11%)*		2/19 *(11%)*	
≥ 2	6/16 *(38%)*	<0·01	5/16 *(31%)*	NS	7/16 *(44%)*	<0·05
Total	6/35 *(17%)*		7/35 *(20%)*		9/35 *(26%)*	

*2-tailed Fisher's exact test.
I = implicated; T = total.

The mean period of serological follow-up of donors of 28 anti-HCV-positive blood products after their anti-HCV-positive donation in the original study was 45 months (range 17–61). Serological follow-up of donors was negative in 9 of 28 (32%), intermittently positive in 5 (18%), and persistently positive in 14 (50%) donors. 7 of 14 (50%) anti-HCV-positive blood products from persistently positive donors were associated with post-transfusion NANBH and/or anti-HCV seroconversion in the recipients, compared with 0 of 14 anti-HCV-positive blood products from donors with negative or intermittently positive follow-up (Fisher's exact test, $p = 0·006$; table III).

In 6 of 35 (17%) anti-HCV-positive blood products the alanine aminotransferase concentration was raised and the ELISA ratio ≥ 2. All 6 were associated with post-transfusion NANBH and, in the 5 for whom serological follow-up of the donor was possible, all were persistently anti-HCV-positive.

TABLE III—PERSISTENCE OF DONOR ANTI-HCV-POSITIVITY AS COFACTOR OF INFECTIVITY TO RECIPIENTS

	Anti-HCV-positive blood product transfusions implicated in recipient infection					
	Post-transfusion NANBH		Seroconversion		NANBH/ seroconversion	
Donor anti-HCV positivity during follow-up	I/T	p*	I/T	p*	I/T	p*
Negative or intermittent + ve	0/14		0/14		0/14	
Persistent + ve	5/14 *(36%)*	<0·05	5/14 *(36%)*	<0·05	7/14 *(50%)*	<0·005
Total	5/28 *(18%)*		5/28 *(18%)*		7/28 *(25%)*	

*2-tailed Fisher's exact test.
I = implicated; T = total; + ve = positive.

TABLE IV—CALCULATED PREVENTIVE EFFECT ON POST-TRANSFUSION NANBH OF BLOOD DONOR SCREENING FOR ANTI-HCV OR ALANINE AMINOTRANSFERASE (ALT)

Calculated preventive effect	Donor screening	
	Anti-HCV	ALT
Crude efficacy = sensitivity*	67%	78%
Maximal corrected efficacy*	63%	65%
Specificity*	93%	64%
Positive predictive value*	16·2%	3·6%
Risk of NANBH/1000 transfusions negative for marker	0·65	0·65
% blood donations positive for marker discarded	0·7%	3·8%

*Calculated according to Koziol et al.[9]

34 of 383 (8·9%) recipients were transfused with one or more of the total of 37 anti-HCV-positive blood products. 6 of 34 (18%) recipients of one or more anti-HCV-positive blood products, and 3 of 349 (0·86%) recipients of anti-HCV-negative blood developed post-transfusion NANBH (χ^2, $p < 0·001$; odds ratio 20·5, 95% CI 5·4–78·4). 142 of 383 (37%) recipients were transfused with one or more blood products with raised alanine aminotransferase concentrations. 7 of 142 (5%) recipients of one or more such blood products, and 2 of 241 (0·83%) recipients of blood with normal alanine aminotransferase concentrations, developed post-transfusion NANBH (χ^2, $p < 0·05$; odds ratio 5·9, 95% CI 1·3–28·2). Recipients of anti-HCV-negative blood only were transfused with 4613 blood products, recipients of blood with normal alanine aminotransferase concentrations received 3068 blood products. Post-transfusion NANBH occurred after 0·65 per 1000 anti-HCV-negative blood product transfusions and after 0·65 per 1000 blood product transfusions with normal alanine aminotransferase (table IV). 37 of 4906 (0·7%) whole blood donations were anti-HCV-positive, and 184 of 4906 (3·8%) had a raised alanine aminotransferase concentration (≥ 2 SD above the mean log). Table IV shows calculations, according to Koziol et al,[9] of crude and corrected efficacy, specificity, and positive predictive value of blood donor screening for anti-HCV or alanine aminotransferase for prevention of post-transfusion NANBH.

Discussion

In our study population, recipients of one or more anti-HCV-positive blood products were 20 times more likely to have evidence of post-transfusion NANBH than recipients of anti-HCV-negative blood. Of the anti-HCV-positive blood products, 26% were associated with HCV infection, shown by post-transfusion NANBH or anti-HCV seroconversion. Assays to prove HCV infection in an anti-HCV-positive blood donor are not widely available,[6,7] and false-positive results may occur in mass screening programmes.

We have identified several cofactors in anti-HCV-positive blood donors that indicate a high risk of HCV infectivity. A raised serum alanine aminotransferase (over 2 SD above the mean log in anti-HCV-positive blood products) was a highly significant cofactor for recipient HCV infection, with a positive predictive value of 86%. An anti-HCV ELISA ratio ≥ 2 was also significantly associated with recipient HCV infection.

An unexpected finding was that some 50% of the anti-HCV-positive blood donors lost antibody reactivity or were only intermittently seropositive during follow-up. This can be interpreted as resolution of HCV infection, latent infection without infectivity, or an artifact yielding false-positive results. Virus isolation or RNA amplification techniques may resolve this question,[8] but our findings indicate that infectivity of anti-HCV-positive donors was significantly associated with persistence of antibody reactivity.

The calculated efficacy of anti-HCV screening of blood donors for prevention of post-transfusion NANBH was 63%—similar to that found for alanine aminotransferase surrogate screening in our study population. However, screening for alanine aminotransferase would have caused a 5-fold higher rejection rate of donor blood.

Post-transfusion NANBH is an important complication of blood transfusion,[12] and we strongly recommend blood donor screening for anti-HCV. By analogy with the early days of donor screening for anti-HIV, it may be considered appropriate to discard blood seropositive by ELISA for anti-HCV, but to postpone notification of donors until after a confirmatory test becomes available. Meanwhile, if a blood sample is anti-HCV-positive, our findings indicate an especially high risk of HCV infectivity if the donor sample has a mean ELISA ratio ≥ 2 or a raised alanine aminotransferase concentration, and if the donor is persistently anti-HCV-positive.

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achieved complete healing of trophic changes on iloprost treatment were observed at 6 months, 13 of whom were still completely healed. 3 of the 5 patients who achieved complete healing of trophic changes on aspirin treatment were observed at 6 months, and were still completely healed.

During treatment 2 patients from both groups required amputation; during the next 5 months, 1 patient who received iloprost, and 6 who were treated with aspirin, required amputation.

Independent assessment showed good agreement with that of the physician in charge of the care: for example, in assessment of ulcer healing and necrosis there was disagreement in only 2 patients treated with iloprost and 4 patients who received aspirin.

Discussion

Intravenous iloprost was significantly more effective than low-dose oral aspirin for relief of rest pain and healing of trophic lesions in patients with thromboangiitis obliterans. These results are more encouraging than those reported for prostanoids in arteriosclerosis obliterans,[8-13] where placebo response rates can be as high as 60%.[12,13] Of the few reports of prostanoids in the treatment of thromboangiitis obliterans, Olsson observed a woman with ischaemic ulcers which healed after treatment with prostaglandin E_1,[7] and Nyzankowski and co-workers,[8] in a randomised study in 30 patients—15 of whom had Buerger's disease—found a beneficial effect after intra-arterial prostacyclin infusion.

Several observations should be made about the study design. Thromboangiitis obliterans is unusual in Europe, and many centres were required to obtain enough patients; although 1 centre included 26 patients, only 2 others had more than 10. To reduce any potential bias, patients were centrally randomised within each centre. Although the trial was designed to be double-blind, and parallel treatments were given, the side-effects of a prostanoid infusion may be obvious to both patient and physician, and the definition of response was partly subjective. To reduce the risk of physician bias, a second evaluation was made by an independent medical observer who only saw the patient before and after the course of treatment: there was good agreement between these medical opinions.

The rate of response to aspirin was unexpectly low (16·9% at 28 days), the reasons for which are unclear. It is possible that aspirin might enhance the inflammatory process of thromboangiitis obliterans by an effect on leukotriene production, but as aspirin and other antiplatelet drugs are commonly prescribed in patients with arteriopathy, one would suspect such an effect to have already been observed. Moreover, Bollinger et al[14] have described a beneficial effect of high-dose aspirin in thromboangiitis obliterans. Another explanation could be the stringent classification that we used: to be considered a responder, a patient had to have reduced pain and analgesic requirement, an overall clinical improvement, and healing of trophic lesions, when present. In both groups, however, the amputation rate is lower than in most published series.[2-5]

Only 95 patients were available for follow-up at 6 months, so 38 (29%) patients were lost to long-term review. If it is assumed that all 17 missing patients who received iloprost died, whereas all 21 who received aspirin achieved full response, the final response rates at 6 months would become 66% for iloprost versus 51% for aspirin. This eventuality seems very unlikely, but should be considered.

In thromboangiitis obliterans, requirement for major amputation is the nearest to an entirely objective guide to therapeutic efficacy. In this study, the need for major amputation was low compared to most other reports and was not statistically different between the treatment groups. However, in the 2 patients on iloprost and 2 on aspirin who required amputation during treatment (days 1–28), it is possible that amputation was inevitable because of the initial severity of ischaemia. During the next 5 months, only 1 patient treated with iloprost required amputation, compared with 6 who received aspirin—a tendency that may become statistically significant with longer follow-up or more patients.

Our findings indicate that intravenous iloprost for 21–28 days is well tolerated and has a greater effect than low-dose aspirin on symptoms and signs in patients with thromboangiitis obliterans. These encouraging results require further investigation, perhaps with amputation rate as a primary endpoint.

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