ANTI-HEPATITIS C ANTIBODIES AND NON-A, NON-B POST-TRANSFUSION HEPATITIS IN THE NETHERLANDS

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Summary In a prospective study carried out in the Netherlands (1984–86) to establish the incidence of post-transfusion hepatitis non-A, non-B (PTH-NANB) in patients undergoing open heart surgery, 393 patients received 5315 blood product transfusions. PTH-NANB developed in 9 patients (index cases); stored serum samples from these patients and from 9 control patients, matched for age, sex, and number of blood product transfusions, as well as serum samples of all implicated blood products, were selected retrospectively. Sera were tested under code with a radioimmunoassay for the detection of antibodies to hepatitis C virus (anti-HCV). PTH-NANB patients received 151 blood product transfusions and control patients 140. 4 of 9 PTH-NANB patients (3/5 chronic, 1/4 acute resolved hepatitis) and 0/9 controls seroconverted. 7 of the transfusions given to PTH-NANB patients but none of those given to control patients were anti-HCV positive. In 7 of 9 serum sets from PTH-NANB index cases plus implicated donors, either a donor or the recipient was anti-HCV positive. Among the donors implicated in transmission of PTH-NANB there was a strong correlation between raised alanine aminotransferase levels and the presence of anti-HCV antibodies.

Introduction

RESEARCHERS at the Chiron Corporation in the USA have lately isolated a cDNA clone from a parenterally transmitted non-A, non-B (NANB)-hepatitis viral genome. This virus has been named hepatitis C (HCV), and appears to be a lipid-enveloped, single-stranded RNA virus.[1] In addition, a polypeptide antigen (C100-3) has been expressed, with which antibodies can be detected in a solid-phase radioimmunoassay (RIA). The specificity of the anti-HCV RIA was established by studying sera from patients with chronic post-transfusion hepatitis NANB (PTH-NANB) and from donors who had been implicated in the transmission of PTH-NANB.[2] We have now used this assay to study preserved (frozen) serum samples from a prospective study of PTH-NANB conducted in Amsterdam from 1984 to 1986.[3] In that study PTH-NANB was diagnosed in patients undergoing open-heart surgery according to the following criteria: (a) increase in alanine aminotransferase (ALT) level of 2·5 times the upper limit of normal 2–26 weeks post-transfusion in a patient with a normal preoperative ALT value; (b) an ALT level of at least twice the upper limit of normal within 3 weeks of the first determination of an increased level; and (c) exclusion of non-viral causes of an increased ALT, and of acute hepatitis B, hepatitis A, Epstein-Barr virus infection, and cytomegalovirus infection. Chronic PTH-NANB was diagnosed when ALT values were still increased more than 6 months post-transfusion. 393 patients received 5315 blood product transfusions from 5054 donations; PTH-NANB developed in 9 patients.

Materials and Methods

Serum samples from the 9 PTH-NANB patients in the 1984-86 study[3] (5 chronic, 4 acute resolved; index cases) and from 9 retrospectively matched control patients (matched for age, gender, and number of transfusions), obtained before, and 3 and 6 months after transfusion, were tested for anti-HCV antibodies. Serum samples from all blood products implicated in the index cases (n = 151), and from blood products that had been given to the matched controls (n = 140), were similarly tested. Information about ALT values and anti-HBc antibodies was obtained from the original study.[3] ALT levels were measured by a spectrophotometric method at 20°C,[4] and anti-HBc was tested with a radioimmunoassay (Abbott, Chicago, USA). Radioimmunoassay for anti-HCV antibodies was done on coded serum samples at the Chiron Corporation, Emeryville, California.[2] Briefly, wells of microtitre plates were coated with 0·1 μg of purified C100-3 HCV antigen before incubation for 1 h at 37°C with 100 μl of diluted (1 in 100) serum. Wells were washed, and bound antibody was detected by incubation for 1 h at 37°C with 100 μl of ^{125}I-labelled sheep anti-human immunoglobulin. Values above the mean of uninfected controls plus 3 SD were considered positive.[2] Statistical methods included χ^2, one-tailed Fisher's exact test, and Bartholomew's trend test.

Results

The results of anti-HCV testing are shown in table I. 4 of the 9 PTH-NANB patients seroconverted *vs* none of the 9 controls ($p < 0·05$). Anti-HCV seroconversion was observed 6–7 weeks after onset of hepatitis in 3 of 5 patients with

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TABLE I—ANTI-HCV TEST DETERMINATIONS IN PTH-NANB AND CONTROL PATIENTS AND IMPLICATED BLOOD PRODUCTS

Recipients	Recipients: anti-HCV seroconversion	Implicated blood products: anti-HCV positive
PTH-NANB	4/9	7/151
Controls	0/9	0/140
p	<0·05*	<0·05†

*Fisher's exact; †χ^2.

chronic PTH-NANB, and 4 weeks after onset of hepatitis in 1 of 4 patients with acute resolved PTH-NANB. Passive transmission of anti-HCV antibody was observed in 1 chronic PTH-NANB patient, with anti-HCV positivity 2 weeks after transfusion, disappearing 4 weeks later. This patient became anti-HCV positive again 8 weeks after transfusion and remained positive at the end of follow-up. 7 of 151 blood products transfused to the 9 PTH-NANB patients (4·6%), and 0 of 140 blood products transfused to the controls were anti-HCV positive ($p < 0·05$). 3 of the 4 PTH-NANB patients who seroconverted received 1 or more anti-HCV-positive blood products transfusions, whereas 3 of 5 anti-HCV-negative PTH-NANB patients received an anti-HCV positive blood product. 1 PTH-NANB patient who seroconverted received 2 anti-HCV positive blood products. 2 other anti-HCV positive blood products were from 1 donor, given on two occasions and transfused into 2 recipients who both got PTH-NANB. Overall, in the serum sets of index cases of PTH-NANB plus implicated donors, anti-HCV seroconversion was found in the recipient, or an implicated donor was found to be anti-HCV positive, in 7 of 9. None of the control sets had anti-HCV antibodies, either in the recipients or in the donors.

More recipients in the PTH-NANB group than in the control group had received at least 1 anti-HCV positive blood product ($p < 0·005$, table II). Other cofactors such as raised ALT levels and anti-HBc antibodies were not significantly increased in the PTH-NANB group. However, in the original study with a larger control group ($n = 384$) the significance of the correlation between raised donor ALT and recipient PTH-NANB was established.[3] None of the 7 anti-HCV positive blood donations was anti-HBc positive. Table III shows that among blood products implicated in PTH-NANB, there is a strong correlation between anti-HCV positivity and raised ALT levels ($p < 0·0001$).

TABLE II—COFACTORS IN BLOOD PRODUCTS TRANSFUSED TO PTH-NANB PATIENTS AND CONTROLS

Cofactor	Patients receiving ≥1 blood product with cofactor: PTH-NANB (n=9)	Patients receiving ≥1 blood product with cofactor: No PTH-NANB (n=9)	p*
Anti-HCV positive	6	0	0·0045
ALT > mean log + 2 SD	7	3	0·08 NS†
Anti-HBc positive	2	3	0·5 NS

*Fisher's exact; †in the original study,[3] with a larger group of recipients ($n = 384$) and blood products ($n = 5315$), the correlation between donor ALT and recipient PTH-NANB was significant ($p < 0·03$).

TABLE III—CORRELATION BETWEEN ALT LEVEL AND HCV ANTIBODIES IN BLOOD PRODUCTS IMPLICATED IN PTH-NANB

Donor ALT level	Anti-HCV positive/total
< Mean log + 1·5 SD	0/144
Mean log + 1·5–2 SD	0/5
Mean log + 2–2·25 SD	1/4 *(25%)*
Mean log + 2·25–3 SD	6/8 *(75%)*
> Mean log + 3 SD	0/0
Total	7/151
p (Bartholomew's trend)	<0·0001

Discussion

We have shown that in a Dutch blood donor population the new anti-HCV RIA developed by Chiron specifically identifies blood products associated with NANB hepatitis in patients who have received transfusions. In recipients, seroconversion to anti-HCV was found only in patients with PTH-NANB. Sensitivity of the assay for detecting probable NANB infective blood products was 67% in our study population. 44% of recipients with PTH-NANB seroconverted within 6 months; since seroconversion in PTH-NANB may take longer than this,[2] extended follow-up might have shown a higher conversion rate in our series. In 10 well-defined cases of chronic PTH-NANB in the USA, all seroconverted within 12 months of transfusion.[2] In our study the seroconversion rate was higher in patients with chronic PTH-NANB (3 of 5 *vs* 1 of 4 with acute resolved hepatitis). This observation accords with the findings of Kuo et al[2] in Japanese PTH-NANB cases. Nevertheless, in at least 7 of 9 sets of sera from PTH-NANB index cases, either a donor or the recipient was found to be anti-HCV positive. NANB hepatitis developed in 2 recipients who had received an anti-HCV-positive blood product but remained anti-HCV negative. The strong correlation ($p < 0·0001$) between anti-HCV positivity and raised ALT values in blood products implicated in PTH-NANB cases emphasises the importance of excluding blood donations with raised ALT values to prevent PTH-NANB in recipients. There was no correlation between anti-HBc antibodies and anti-HCV antibodies in our study. We reported previously that anti-HBc antibodies were of no significance for the prevention of PTH-NANB in the Netherlands,[3] perhaps because our study was conducted after protocols of voluntary withdrawal of blood donors at increased risk of AIDS had been implemented; these donors have a high frequency of anti-HBc antibodies (C. L. van der Poel, unpublished). Despite its limited sensitivity, the high specificity of this first generation anti-HCV assay should permit greatly improved donor screening procedures for the prevention of PTH-NANB.

We thank Mrs P. J. Exel-Oehlers and Mr W. Schaasberg for technical and statistical help; Mrs D. K. F. Mulder and Mrs T. Pieters for collection of the serum panel; Dr A. van der Ende for ALT testing; Mrs N. Schram for preparation of the typescript; and Dr R. N. I. Pietersz, Dr M. van Oers, and Dr R. A. Coutinho for assistance with the organisation of the post-transfusion hepatitis study.

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