Prevalence of antibodies to hepatitis C virus after blood transfusion in heart surgery

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Summary: We studied the frequency and time of appearance of antibodies to the hepatitis C virus (HCV) retrospectively in the sera of 127 patients who underwent heart surgery between 1983 and 1986. They received blood from volunteer donors hepatitis B surface antigen (HBsAg) negative with normal serum alanine-aminotransferase levels. A prospective follow-up was carried out every 15 days for at least 6 months from the moment of the transfusion.

Of the ten patients who developed biochemical criteria of post-transfusional non-A non-B hepatitis, six seroconverted to anti-HCV (60%). Of the other 117, two were already positive before transfusion (1.51%), one patient showed antibodies only in the first post-transfusional serum (passive transfer), and another two patients with no evidence of post-transfusional hepatitis developed HCV antibodies on the 90th day, remaining indefinitely (afterwards seroconversion without hepatitis); both patients' earlier sera were anti-HCV negative.

Four (40%) of the ten patients with post-transfusional hepatitis did not develop any serum markers to known hepatotropic agents. Although these findings do not exclude a viral infection by these viruses, they are consistent with the involvement of an unidentified non-A, non-B, non-C agent.

Introduction

Post-transfusional hepatitis has been and continues to be one of the most serious complications of blood transfusions.¹ Before the discovery of the hepatitis C virus (HCV) and donor screening the incidence was very high, approximately 10–15%. In an attempt to lower the incidence, all donor blood with high alanine-aminotransferases (ALAT) was excluded.² Two retrospective studies showed that this measure could help reduce the incidence by at least 30%.^{3,4} We carried out a prospective study of the incidence of post-transfusional hepatitis before and after the exclusion of high ALAT donor blood, and found a significant reduction of almost 50%.^{5,6} However, the incidence still remained high at 7.6%.

The discovery more than 2 years ago of the HCV genome using a recombinant complementary DNA,⁷ and the development of a diagnostic test specifically designed to detect circulating HCV antibodies (anti-HCV) by means of a viral purified polypeptide derived from recombinant yeasts that express a small part of the HCV genome,⁸ has shown that this virus is the major cause of post-transfusional hepatitis.^{9–11} Nevertheless, and in

spite of screening donors for anti-HCV, the incidence of post-transfusional hepatitis still remains at 3–4% in Spain and Japan.^{12,13} In the USA the incidence of post-transfusional hepatitis has fallen sharply to 1%.¹⁴ It is also known that there are patients with non-A non-B post-transfusional hepatitis who do not develop anti-HCV.⁹

We studied retrospectively sera samples stored prospectively while carrying out a study on the incidence of post-transfusional hepatitis after heart surgery between 1983 and 1986. Our aim was to see how frequently HCV antibodies appeared in transfused patients and what their relationship was to the development of post-transfusional hepatitis.

Material and methods

Between 1980 and 1983 we studied the incidence of post-transfusional hepatitis in patients transfused with blood from hepatitis B surface antigen (HBsAg) negative donors.⁵ Between 1983 and 1986 we continued studying post-transfusional hepatitis incidence in patients who had undergone heart surgery but who had received blood from HBsAg-negative volunteer donors with normal ALAT levels.

There were 130 patients in the study, although pretransfusional serum was only available in 127 of

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Accepted: 9 March 1994

the 130 patients. We took post-transfusal serum for 6 months if hepatitis did not develop, or for at least 3 years if it did develop. This study deals with the analysis of the serum from these 127 patients.

The criteria for inclusion in the study were: age between 18 and 70 years, no known liver disease, normal transaminases, no transfusion or hepatitis in the 12 months prior to the study. The criteria for post-transfusal hepatitis were established based on two consecutive ALAT levels two and a half times the normal level, taken between the second and 28th post-transfusion week and separated by at least 7 days. Excluded were non-viral liver disease such as anaesthesia, alcoholism, heart failure, sepsis and drug-induced toxic hepatitis. The hepatitis was diagnosed as non-A non-B, if the serological tests for IgM antibody to hepatitis A, IgM antibody to B core antigen, HBsAg, antibody responses to cytomegalovirus by complement fixation and Epstein-Barr virus by the heterophil antibody were negative.

The sera were kept at -30°C and were not thawed until the moment they were used. In all sera HCV antibodies were analysed by enzyme immunoassay II (ELISA II) (Ortho Raritan, NJ). Patient's serum with post-transfusal hepatitis and serum positive for HCV antibodies by ELISA II were analysed by RIBA II (Chiron Corporation, Emeryville, CA) to confirm the results. There was no difference in the results obtained with the two techniques. The average age of our patients was 47.5 ± 10.48 years. Sixty-five were men and 62 were women.

Results

Of the 127 patients, ten developed non-A non-B post-transfusal hepatitis and 117 did not. Of these ten patients, six developed anti-HCV in the 6 months following the transfusion (two after 30 days, one at 50, one at 40, one at 76 and another after 145 days). Four did not develop antibodies during the 3 year follow up.

Of the 117 patients who did not develop post-transfusal hepatitis two showed HCV antibodies in the pretransfusal samples and in the post-transfusal serum (previous carriers). One patient showed anti-HCV only in the serum extracted 15 days after the transfusion, but was negative before transfusion as well as in the ten following serum samples. This was considered a case of passive antibody transfer. Two patients whose pretransfusal sera were anti-HCV negative as well as on days 15, 30, 45 and 60 after the transfusion, showed antibodies on day 90 and remained positive. These patients were considered as seroconverters without clinical or biochemical hepatitis.

Those patients who developed non-A non-B post-transfusal hepatitis received a significantly higher number of blood units (10.3 ± 5.8 versus 5.3 ± 3.9 , $P < 0.01$).

With respect to non-A non-B post-transfusal hepatitis clinical evolution (Table I), just three patients who seroconverted were symptomatic. There were no differences in the age, sex, incubation period, serum transaminase levels between the post-transfusal hepatitis patients who seroconverted and the four who did not.

Of the former, five developed chronic hepatitis and of the latter one did. This difference was not significant. There were also no significant differences in the number of blood units transfused between the patients who developed chronic hepatitis and those who did not.

Discussion

In this study two patients were anti-HCV positive before the transfusion (1.57%), a percentage similar to the incidence of 1.05% of anti-HCV found in present-day blood donors.¹⁵

In our series 60% of the patients who developed biochemical criteria for non-A non-B post-transfusal hepatitis seroconverted to anti-HCV. The frequency of anti-HCV is close to 90% in post-transfusal chronic hepatitis,^{9,15,16} but is always less in the acute phase.^{17,18} This difference could be explained by late seroconversion but patients in most of the studies were followed for long periods of time, up to 2 and 3 years, as in our study. There was no increase in the number of seroconversions after the sixth and seventh month, at most 1 year after the beginning of the hepatitis.¹⁷⁻²¹

Another explanation could be that those patients who did not seroconvert had non-viral hepatitis.

Table I Clinical and evolution characteristics of ten patients with post-transfusal non-A non-B hepatitis (n = 10)

	Anti-HCV (+) (n = 6)	Anti-HCV (-) (n = 4)	
Age (years)	48 ± 11	49 ± 8	NS
Blood units transfused	11.5 ± 4.9	7.5 ± 6.2	NS
Hepatitis symptoms	3	0	NS
ALAT (U/l)	560 ± 390	257 ± 85	NS
Incubation period (days)	44 ± 21	36 ± 16	NS
Non-resolved	5	1	NS

We studied one patient who developed non-A non-B post-transfusional hepatitis after surgery to replace the aortic valve. Clinically detectable heart failure was never manifested and his ALAT levels remained high for more than 9 months. A liver biopsy revealed ischaemic hepatitis and the serum was negative for HCV antibodies.

An alternative explanation for the different frequency of anti-HCV in acute and chronic post-transfusional hepatitis is the role of one or more non-A non-B non-C virus. Supporting this hypothesis are the different incidence of seroconversion to anti-HCV in the different prospective post-transfusional hepatitis studies. In the Northern European series seroconversion in the acute phase does not reach 50%.^{17,20,22,23} In Japan, Italy and Spain it is almost 90%^{13,21,24,25} and in Taiwan and the USA it is between these extremes.^{18,24} These figures could indicate different prevalences of HCV and of a potential non-A non-B non-C virus in the different countries. If, as seems to be the case, there is a high cure rate for non-A non-B non-C hepatitis^{18,19,25,26} while a high percentage of C hepatitis becomes chronic,^{18,19,25,26} then in all countries most chronic post-transfusional hepatitis would be due to HCV which seems to be the case.^{9,15,16,24}

In a recent study, Aach *et al.*²⁶ found 111 cases of post-transfusional hepatitis among 1,232 transfusion patients and 37 of non-A non-B hepatitis

among 1,230 non-transfused control patients. Curiously, none of the 37 control patients with hepatitis converted to anti-HCV and, among the post-transfusional hepatitis, 44 did not seroconvert (40%). As these authors suggest, this could be due to non-viral hepatitis or C hepatitis with immunological responses not detected by the tests used. However, as long as we do not have more specific markers the existence of one or more non-A non-B non-C viruses cannot be discarded as responsible for these cases.

We did not determine HCV infection by the polymerase chain reaction (PCR) in the four patients with hepatitis but without HCV antibodies. We believe that this diagnostic method would have not been useful because sera storage conditions and previous unfreezing for ELISA II and RIBA II serum antibodies determinations could affect RNA virus detection. RNA determination in the present sera of these patients would also not be useful, as at least in three of the four patients, the hepatitis had resolved, and the absence of RNA HCV particles would not eliminate the possibility they had suffered from an hepatitis C virus. Moreover, although RNA-HCV detection has been described in sera from patients without detectable HCV antibodies,²⁷⁻²⁹ these findings should be interpreted cautiously because of the low reliability of PCR for detection of hepatitis C virus.³⁰

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