Original Article

Antibodies to Hepatitis C Virus and Chronic Liver Disease among Finnish Patients with Haemophilia

Freja Ebeling, Vesa Rasi, Ruth Naukkarinen and Juhani Leikola

Antibodies to hepatitis C virus, hepatitis B serology and liver enzymes were examined in 137 Finnish haemophiliac patients to detect signs of chronic viral hepatitis and its possible aetiological associations. The prevalence of raised alanine aminotransferase values was 37 %. These were significantly associated with hepatitis C seropositivity but not with hepatitis B antibodies, severity of haemophilia or the type of clotting factor used in replacement therapy. The prevalence of hepatitis C seropositivity was 50 %; it was significantly associated with severe haemophilia and with the use of large pool concentrates. The hepatitis C virus seems to be the major cause of chronic liver disease transmitted by clotting factors also in Finland, despite a somewhat lower seroprevalence than described elsewhere so far.

Key words: hemophiliacs; hepatitis B; hepatitis C; non-A, non-B; plasma factor concentrates.

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Introduction

Until recently patients using clotting factor concentrates prepared from large pools of donated blood were exposed to a substantial risk of contracting hepatitis, especially chronic non-A, non-B hepatitis (1). After the cloning and sequencing of a hepatitis C virus (HCV) antigen, an ELISA test was developed for detecting anti-HCV antibodies and for evaluating the possible aetiological role of the hepatitis C virus in patients (2, 3). Several studies have already shown that antibodies to HCV are common in patients treated with commercially prepared, large-pool factor concentrates (4—10).

Finland is one of the few countries that have been self-sufficient in the production of clotting factors. The raw plasma comes from a population of donors with a low risk of hepatitis (11). In addition, until 1984 small-pool (two or eight donors) lyophilized cryoprecipitate was exclusively used to treat haemophilia A, von Willebrand's disease and factor XIII deficiency. We report the prevalence of anti-HCV and its associations with hepatitis B markers, liver enzymes and clotting factor treatment in Finnish patients with bleeding disorders.

From the Finnish Red Cross Blood Transfusion Service, Helsinki, Finland.

Address and reprint requests: Freja Ebeling, M.D., Finnish Red Cross Blood Transfusion Service, Kivihaantie 7, 00310 Helsinki, Finland.

Patients and Methods

Patients

Since 1957 patients with bleeding disorders have been diagnosed and centrally registered in the coagulation laboratory of the Finnish Red Cross Blood Transfusion Service. In January 1988 all 230 patients with a coagulation disorder using clotting factor concentrates were asked for a blood sample for testing liver parameters as part of a yearly round of testing for anti-HIV. A sample was received from 137 patients (60 %), these formed the study group (Table 1). To detect the transiently abnormal transaminase values, two additional samples were collected in 1988. A complete three sample series taken three months apart was received from 107 patients. Data about the use of concentrate was obtained by questionnaire.

Laboratory Methods

The anti-HCV testing was performed with the Ortho anti-HCV ELISA in June to August 1989 on thawed specimens. The other measurements were done on fresh samples, which were then stored at —30°C. The hepatitis B surface antigen (HBsAg) and antibodies against it (anti-HBs) were tested using the FRC-RIA and FRC-AB (12) radioimmunoassay methods. The antibodies to the hepatitis B core antigen (anti-HBc) were tested with EIA (Corzyme, Abbott). Tests for alanine aminotransferase (ALT), aspartate aminotransferase

Table 1. Characteristics of the studied patients with a bleeding disorder.

Type and severity of disease (residual factor activity)	No. of patients studied	Sex (M:F)	Mean age (range)
Haemophilia A			
Severe (<1 %)	68	68:0	29 (1—65)
Moderate (1 %—5 %)	11	11:0	29 (3—60)
Mild (5 %—25 %)	20	20:0	34 (7—64)
Haemophilia B			
Severe (<1 %)	5	5:0	38 (29—51)
Mild (>1 %)	23	23:0	32 (1—66)
von Willebrand's disease	7	3:4	36 (7—55)
F XIII deficiency	3	2:1	18 (14—25)
All	137	132:5	31 (1—66)

(AST) and gamma glutamyl transferase (GGT) were performed according to the recommendations of the Scandinavian Committee on Enzymes at 37°C (13, 14).

Statistics

The statistical methods used included the *t* test for comparing means between two groups and the chi square test with Yates's correction.

Results

Anti-HCV

Sixty-eight patients (50 %) were anti-HCV positive (Table 2), most of the samples showing strong reactivity.

HBV markers

One of the patients (0.7 %) was HBsAg-positive; 69 (50 %) were anti-HBs positive and 63 (46 %) anti-HBc positive; 51 (37 %) had both anti-HBs and anti-HBc; and 55 patients (40 %) were negative for the three HBV serological markers.

ALT

Fifty-one (37 %) of the 137 patients had raised values of ALT (>40 U/l) in the first sample, 15 (11 %) of which exceeded 100 U/l (range 101—280). The results for the 107 patients with a complete series of three ALT determinations three months apart were as follows: 55 (51 %) had at least one abnormal ALT value, and 34 (32 %) had persistently raised values of ALT in all three samples.

The Associations of Anti-HCV with Other Parameters

Raised ALT and anti-HCV positivity were statistically significantly associated ($P<0.001$). The association between anti-HBs or anti-HBc and abnormal ALT was not significant (Table 3). The association between abnormal ALT and anti-HCV was significant ($P=0.0014$), also in the subgroup of patients without hepatitis B markers. Seventy-three per cent of the patients with abnormal ALT had antibodies against HCV, the prevalence of these antibodies being 36 % among those with normal ALT values. On the other hand, 46 % of patients who were anti-HCV positive had normal ALT values.

The mean absorbance readings of anti-HCV did not differ significantly in anti-HCV positive patients with normal or abnormal ALT values (6.0 or 6.5 times the cut-off level of the assay).

Table 2. The age, type and severity of the bleeding disorder, ALT level and hepatitis B serology in 137 Finnish patients in relation to the presence or absence of antibodies to the hepatitis C virus.

Characteristic	Anti-HCV positive (n = 68)	Anti-HCV negative (n = 69)
Mean age (range)	33.4 (7—66)	28.3 (1—65)
Haemophilia A	53	46
Severe	44	24
Moderate or mild	9	22
Haemophilia B	11	17
Severe	3	2
Mild	8	15
Other coag.disorders	4	6
Mean ALT (U/l) (SD)	62.8 (54.7)	31.6 (36.7)
Anti-HBc pos.	42	21
Anti-HBs pos.	41	28

Table 3. The association of abnormal alanine aminotransferase values with antibodies against hepatitis C and B viruses among patients with bleeding disorders.

	ALT norm. (pos/all)	ALT >40 U/l (pos/all)	Significance
Anti-HCV pos	31/86	37/51	$P=0.00008$
Anti-HBs pos	41/86	28/51	NS ($P=0.52$)
Anti-HBc pos	35/86	28/51	NS ($P=0.15$)

The prevalence of anti-HCV among the 107 patients who were tested for ALT three times in 1988 was 31 % (16 out of 52) in those with three normal ALT values, 62 % (13 out of 21) in patients with intermittently abnormal ALT values, and 74 % (25 out of 34) among those with persistently abnormal ones ($P<0.001$).

The association between anti-HCV and anti-HBc as shown in Table 2 was significant ($P<0.001$). The association between anti-HCV and anti-HBs was weaker, but still statistically significant ($P=0.03$). In contrast, the type of the coagulation disorder or the age of the patient were not significantly associated with anti-HCV positivity.

Viral Markers, ALT and Severity of Haemophilia

Patients with severe haemophilia had hepatitis C antibodies significantly more often than those with milder forms ($P<0.001$) (Table 2). Also, the hepatitis B markers anti-HBc or anti-HBs were significantly more common in severe haemophilia A or B than in milder forms (55/73) versus 23/54, ($P=0.001$). The presence of an abnormal ALT value (>40 U/l) did not have a significant association with the severity of haemophilia (31/73 in severe and 16/54 in milder forms, $P=0.20$).

The Type and Amount of Clotting Factor Used

Patients were grouped according to the use of clotting factors (Table 4). The three types of clotting factors did not differ significantly in association with raised ALT values. Users of large-pool factor VIII concentrate (AHF-20) were found to have hepatitis B antibodies slightly more often ($P=0.02$) than those who had used only small-pool cryoprecipitate. Anti-HCV positivity was significantly more common in AHF-20 than cryoprecipitate users ($P<0.01$). Anti-HCV seropositivity was slightly less common in F IX than AHF-20 users ($P<0.05$). The difference in anti-HCV positivity between users of cryoprecipitate or AHF-20 was caused by the subgroup which had used less than 80 000 units of cryoprecipitate.

Anti-HCV Follow-Up

Anti-HCV could be studied in 72 complete series of three samples; 34 were constantly positive, and 36 stayed negative. One patient seroconverted to anti-HCV positivity in the third sample, which also showed a raised concentration of ALT. One initially weakly positive patient was negative in the two last samples.

Discussion

We found a 50 % prevalence of anti-HCV and a significant association of this antibody with raised concentrations of ALT among Finnish patients with bleeding disorders. The prevalence of anti-HCV was highest (74 %) among those with persistently raised ALT values. Patients with severe haemophilia A or B had significantly higher frequencies of both anti-HCV and HBV markers than those with milder forms. (Most Finnish haemophiliac patients have not been vaccinated against HBV). The small-pool cryoprecipitate was associated with a significantly lower anti-HCV prevalence than the F VIII and F IX concentrates of large pool sizes.

The samples for liver tests were gathered as part of a voluntary anti-HIV-round, which may have influenced the composition of the material. Because of the low (0.95 %) prevalence of anti-HIV among Finnish haemophiliacs and the use of virus inactivated preparations the motivation to send a sample seems to have decreased. All the 137 patients in our study group were anti-HIV negative. It is possible that patients who already knew that they had had some kind of liver pathology were more willing to participate in the study. If this was so it would have caused an overrepresentation of abnormal results.

The 50 % prevalence of anti-HCV was lower than in results recently reported for Australian, Spanish, French, British, American, German and Italian haemophiliacs with an overall anti-HCV prevalence of 61 %—82 % (4—10). HCV antibodies sometimes seem to disappear (6), and the above percentages may underestimate the number of patients with a previous HCV contact.

The anti-HCV status of the patients was stable during the six months' observation period. Only two of the patients with a complete three sample series (2.8 %) showed any changes: one lost the low anti-HCV positivity and another seroconverted to positivity with an apparent subclinical acute hepatitis.

Positive anti-HCV ELISA results among haemophiliacs treated with clotting factors have been checked with the new recombinant based immunoblot assay (RIBA, Chiron, Emeryville, California) and shown to be very specific. This means that false positive results were only a minor source of error among these patients (15). We also had the opportunity to test 19 of our samples from anti-HCV ELISA positive haemophiliac patients with RIBA, and they all reacted to the assay, 89 % of them reacting to both its antigens (data not shown).

Table 4. Prevalence of elevated ALT, hepatitis C and hepatitis B antibodies in relation to the clotting factor replacement therapy.

Therapy	Elevated ALT		AHBs or AHBc positive		Anti-HCV positive	
Cryoprecipitate only	18/51	(35 %)	25/51	(49 %)	19/51	(37 %)
<80 000 units	6/25	(24 %)	9/25	(36 %)	5/25	(20 %)
>80 000 units	12/26	(46 %)	16/26	(62 %)	14/26	(54 %)
F VIII concentrate	19/52	(37 %)	38/52	(74 %)	35/52	(67 %)
F IX concentrate	13/29	(45 %)	17/29	(59 %)	12/29	(41 %)
Total	50/132	(38 %)	80/132	(61 %)	66/132	(50 %)

Almost half (46 %) of the patients with anti-HCV had normal ALT values; on the other hand, 31 % of patients with three subsequent normal ALT values had anti-HCV. One explanation for this coexistence of anti-HCV and normal ALT values, a finding confirmed by other authors (10, 16), might be that the antibodies represent only an immune response after past infection, perhaps boosted by repeated antigen challenges. Another possibility is that it corresponds to a quiescent phase of chronic non-A, non-B hepatitis, where the transaminase values may be intermittently normal for months (17). The possible detection of HCV genome within hepatocytes by the polymerase chain reaction (PCR) (18) would be of interest in these patients.

Persistent ALT abnormality can be caused by other factors besides chronic hepatitis, for example, obesity or alcohol abuse. Data about the patients' body mass indices were not available. Only six patients with abnormal ALT values had a higher AST than ALT value, and none had an AST/ALT ratio above 2. The mean GGT of these patients was only slightly above normal: 56.5 U/l (the upper normal value for men being 50 U/l). These biochemical data are consistent with the main cause of liver damage in our patients being non-alcoholic (19).

Finland is self-sufficient in source plasma from voluntary, non-remunerated blood donors. The routine screening includes HBsAg, anti-HIV and cardiolipin, but not surrogate testing for non-A, non-B hepatitis. The prevalence of HBsAg positivity among new blood donors in Finland has been as low as 0.05 % in the period 1985—1988, which accords with the epidemiology of hepatitis B in other Nordic countries (20—22). The prevalence of HCV antibodies among 79 392 Finnish blood donors from February to June 1990 was 0.50 %, a figure similar to values reported from other countries in central and northern Europe (23—25).

Small pool (two or eight donors) lyophilized cryoprecipitate was almost exclusively used in the treatment of haemophilia A and von Willebrand's disease until the second half of 1984, and the intermediate pool concentrate (AHF-20) has been in major use after heat treatment (68°C/72 h, dry heat) only since autumn 1985. No patients have been treated solely with the heat treated concentrate, however, as in Finland the practice for treating patients with haemophilia A is to use small-pool cryoprecipitate in all under seven years old. A factor IX concentrate has been in use since 1969, and its use after heat treatment (68°C/72 h, dry heat) began in 1986.

We conclude that the significant association of raised ALT and anti-HCV points to the possibility of the HCV agent being a major cause of chronic hepatitis among haemophilia patients. Even so, the absence of HCV antibodies in 26 % of the patients with persistently abnormal ALT leaves open the possibilities of heterogeneity in antibody response to various HCV epitopes and also the existence of still more blood borne non-A, non-B hepatitis viruses.

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