The natural history of hepatitis C virus infection in Italian patients with von Willebrand's disease: a cohort study

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Background and Objectives. Unlike patients with hemophilia, those with von Willebrand's disease (VWD) have a mild to moderate bleeding tendency requiring a lower rate of transfusion: moreover, the use of blood products in most Italian patients with VWD was greatly reduced following the introduction of desmopressin in 1977. The main objective of this study was to compare the prevalence and outcome of hepatitis C virus (HCV) infection in multi-transfused patients with VWD and in those with hemophilia A or B.

Design and Methods. In a large cohort of 356 patients with VWD (41% type 1, 53% type 2 and 6% type 3) and 340 with hemophilia A (85%) or B (15%), all of whom were negative for human immunodeficiency virus (HIV) serum HCV markers, liver function tests and abdominal ultrasound were performed every 6 months for 6 years.

Results. VWD patients were less often transfused with any type of blood products than were hemophiliacs (40% versus 96%) and were, therefore, less frequently infected with HCV (39% versus 82%). HCV infection in patients with VWD occurred at an older mean age (22 versus 7 years), was of shorter duration (20 versus 31 years), and manifested less often with elevated transaminases (58% versus 83%). The risk of infection by HCV genotype 1a was significantly lower in patients with VWD than in hemophiliacs. Despite these differences in the features of HCV infection, the cumulative incidences of advanced liver disease (11% versus 10 %) and hepatocellular carcinoma (2% versus 4%) were very similar in the two groups of patients.

Interpretation and Conclusions. VWD patients had lower prevalences of HCV infection with HCV and genotype 1a infections than did hemophiliacs, reflecting the different source and type of transfused blood products. HCV infection in both groups seems to run a relatively mild course, until now, but the high prevalence of genotypes resistant to curative antiviral therapies is of concern.

Key words: von Willebrand's disease, hemophilia A and B, desmopressin, plasma factor concentrates, hepatitis C virus, end-stage liver disease.

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Prior to the mid 1980s, the risk of patients with inherited bleeding disorders being infected with hepatitis C virus (HCV) after transfusional therapy with coagulation factor concentrates manufactured from large pools of plasma was very high; this risk was influenced by the source and type of blood products received.[1–3] After the adoption of highly effective methods of viral inactivation in 1984-1987, HCV transmission substantially ceased among recipients of factor concentrates.[4–7] An additional step towards safety was made in the early 1990s, when donated blood was screened with HCV assays of greater sensitivity. These assays helped to reduce the risk of HCV transmission by single donor blood products (whole blood, plasma or cryoprecipitate) and to reach the current rates of less than two cases per 100,000 donations.[8–10] Both prospective and cohort studies have clearly shown that the clinical consequences of hepatitis C in patients with inherited bleeding disorders are not trivial, because a number of patients

may ultimately develop end-stage liver disease or cancer.[11,12] The progression of liver disease appears to be accelerated by host factors such as male gender, old age at first infection and environmental factors including alcohol abuse or co-infection with the hepatitis B virus (HBV) and human immunodeficiency virus (HIV).[13–15] In contrast to the many studies of hepatitis C in patients with hemophilia A and B, less attention has been paid to patients with von Willebrand's disease (VWD), who generally have a milder bleeding tendency than hemophiliacs. Patients with VWD tended to be treated more frequently with single donor blood products and were, therefore, less exposed in the past to non-virus-inactivated coagulation factor concentrates.[16] Additional differences between the two groups of patients that may influence the progression of hepatitis C are gender (unlike hemophilia A and B, VWD also affects females) and age of infection (VWD patients usually receive their first replacement therapy later in life). On this

background, we compared the frequency and outcome of hepatitis C in patients with VWD and those with hemophilia who had been prospectively followed up for 6 years.

Design and Methods

Design of the study

This was a cohort study of HCV infection in Italian patients with VWD followed up at a single center: internal controls were patients with hemophilia A and B followed up in the same way. All the patients were aware of the experimental nature of the study and gave their informed consent according to the Declaration of Helsinki. In order to be included in the study, patients had to have a diagnosis of VWD, hemophilia A or hemophilia B and have been born before 1992. This birth limit was chosen because it was the year that recombinant factor VIII and IX products were introduced into the market, an event that has eliminated transmission of HCV to such patients. Other inclusion criteria were available information on serum markers of blood-borne viruses, obtained yearly, in all patients who had received replacement therapy and results of liver function tests in all HCV-infected cases, performed at intervals of 6 months or less, starting from January 1998.

Exclusion criteria were: (i) co-infection with HIV, to avoid a major confounding factor on the progression of HCV infection, known to be present frequently in patients with hemophilia but much less in those with VWD; (ii) co-infection with HBV; (iii) alcohol consumption exceeding 40 g per day; (iv) patients lost to follow up; (v) patients with no information on first exposure to blood products. For the purposes of this study, the following types of blood products were considered: single donor products such as whole blood, packed red cells, plasma and cryoprecipitate; and large pool concentrates containing factor VIII and von Willebrand factor (FVIII/VWF), non-virus-inactivated before 1985 and virus-inactivated thereafter (factor IX concentrates for hemophilia B). A pasteurized FVIII/VWF concentrate was the product most widely employed in the treatment of VWD.

Patients

As of January 1998, 428 VWD patients were registered at the Angelo Bianchi Bonomi Hemophilia Thrombosis Center. Of these, 356 met the inclusion criteria: two were excluded because they were positive for HIV, one because positive for HBsAg, two because of alcohol abuse and 67 because they were born after 1992, lost to follow up, or information on their first exposure to blood products was lacking. For each included patient, detailed information about bleeding history and treatment was available. Of these 356 patients, 73 (20%) had never required any therapy, 141 (40%) had had their bleeding episodes treated exclusively with desmopressin (DDAVP) and 142 (40%) had been exposed at least once to blood products. The classification of VWD was based on the recommendations of the International Society on Thrombosis and Haemostasis.[17] The diagnosis of VWD type was confirmed in all 356 patients during the 6-year observation period according to the criteria of the Italian Association of

Table 1. Transfused blood products and hepatitis C virus infection in patients with von Willebrand's disease and in those with hemophilia A or B and the effects on liver status.
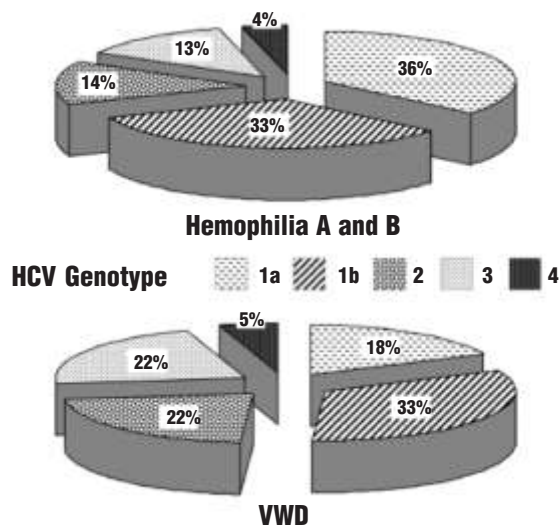
	Willebrand's disease n=356	Hemophilia A or B n=340	p value
Patients exposed at least once to blood products	142/356 (40%)	327/340 (96%)	<0.0001*
Type of blood product received at first treatment			
whole blood or plasma	83/142 (58%)	115/327 (35%)	
cryoprecipitate	31/142 (22%)	44/327 (13%)	
large-pool coagulation factor concentrates	28/142 (20%)	168/327 (52%)	<0.0001*
HCV infection among treated patients			
Serum anti-HCV positive	56/142 (39%)	268/327 (82%)	<0.0001*
Median age at first transfusion (mean – SD)	22.0 (23.3-17.7)	7.0 (11.7-11.2)	<0.0001°
Male/ Female	22/34	265/3	<0.0001*
HCV RNA negative	11/56 (20%)	56/268 (21%)	1.0*
Liver status of HCV RNA positive cases			
Elevated ALT	26/45 (58%)	177/212 (83%)	0.02*
End stage liver disease	5/45 (11%)	22/212 (10%)	0.6 *
Hepatocellular carcinoma	1/45 (2%)	9/212 (4%)	0.1*
Antiviral treatment	4/45 (9%)	58/212 (27%)	< 0.0001*

*Fisher's exact test; °Median test.

Hemophilia Centers[18]: 148 (41%) had type 1 VWD, 188 (53%) had type 2 and 20 (6%) had type 3 VWD. Of 427 hemophiliacs born before 1992 who met the inclusion criteria, 87 (20%) were also co-infected with HIV and were not, therefore, included in this study. Among the 340 patients enrolled, 288 (85%) and 52 (15%) had hemophilia A and B, respectively; 178 (HA/HB=148/30) had severe disease (52%), 41 (HA/HB=32/9) had moderate disease (12%) and 121 (HA/HB=108/13) had mild disease (36%). Of these 340 patients, 327 (96%) had been exposed at least once to blood products, mainly to large pool non-virus-inactivated coagulation factor concentrates before 1985, then concentrates inactivated with various methods. DDAVP and single donor blood products were seldom used in hemophiliacs (Table 1) .

Assessment of HCV

During the 6-year follow-up (April 1998-April 2004) serum markers of blood-borne viruses (hepatitis A, B and C and HIV) were evaluated at yearly intervals in all patients transfused at least once. In all the HCV-infected patients, liver function tests and abdominal ultrasound examination were carried out at 6-month intervals. The HCV genotype from all HCV-RNA positive patients and the time of the first infusion of blood products was also evaluated.[1,2] Chronic hepatitis C was defined by the presence of serum HCV-RNA for more than 6 months. Cirrhosis was diagnosed when the following laboratory criteria were concomitantly found: platelet count lower than 100×10^9/L (normal range $150-400 \times 10^9$/L), serum albu-

Figure 1. Distribution of HCV genotypes in patients with hemophilia A or B (upper panel) and in patients with von Willebrand's disease (VWD, lower panel). The overall distribution of HCV genotype was not significantly different (*p*=0.06) in VWD and hemophilia, but the risk of being infected with HCV genotype 1a (*Table 2*) was significantly lower in VWD (OR=0.39; 95% CI 0.17-0.91).

min lower than 35 g/L (normal range 35-50 g/L) and serum cholinesterase lower than 4,500 U/L (normal range 4,650-11,400 U/L). Additional diagnostic criteria were endoscopic demonstration of esophageal varices or detection of an irregular liver margin, dilated portal vein, and splenomegaly at ultrasonography. Liver decompensation and hepatocellular carcinoma (HCC) were considered to be end-stage liver disease (ESLD). Liver decompensation was defined by the finding of cirrhosis with at least one of the following signs: ascites, jaundice, hemorrhage from ruptured varices, or encephalopathy. HCC was diagnosed according to internationally agreed criteria.[19]

Laboratory methods

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were measured by an automatic method at 37°C (normal value ≤ 37 IU/L). Commercially available enzyme immunoassays were used to determine serum HbsAg, antibodies to hepatitis B core antigen, anti-HCV and anti-HIV. Serum HCV-RNA was detected in-house by nested reverse transcription polymerase chain reaction (RT-PCR) using primers of the 5' non-coding region.[20] The minimum detectable level was approximately 50 international units (IU/mL) using a panel of infected sera calibrated to the WHO International Standard.[21] HCV was typed by a nested RT-PCR using universal biotinylated primer in the 5' non-coding region (Line Probe Assay, Immuno-LIPA HCV2, Immunogenetics; Zwijndrecht, Belgium).

Statistical analysis

The distribution of selected variables was evaluated by simple descriptive statistics. Differences between the distributions of the selected variables were evaluated using Fisher's exact test for categorical data. Wilcoxon's test was carried out for statistical evaluation of differences between continuously distributed variables. The case-only design was used to compare the association between HCV genotype and VWD/hemophilia case groups and multivariate unconditional logistic regression was used to analyze the data. Odds ratios and 95% confidence intervals (CI) were used as indices of relative risk, with the CI computed using the standard errors of the estimated logistic regression coefficients.

Results

The 356 patients with VWD were older than the 340 hemophiliacs (median age 42 versus 38 years, *p*=0.02) and more often female (57 versus 2%, *p*<0.0001). Data on the exposure of patients to either blood products and hence to the risk of HCV infection, together with the effects of HCV infection on the status of the liver are shown in Table 1.

HCV infection and blood transfusion

More hemophiliacs than patients with VWD (96% versus 40%, *p*<0.0001) required treatment at least once with blood products. On the occasion of the first treatment VWD patients were exposed more frequently than hemophiliacs to whole blood or plasma (58% versus 35%) and cryoprecipitate (22% versus 13%), and less frequently to large-pool non-virus-inactivated coagulation factor concentrates (20% versus 52%). Patients with VWD were exposed to blood transfusion at an older age than were hemophiliacs (median 22 versus 7 years, *p*<0.0001). Infection with HCV, as defined by anti-HCV positivity, was less frequent in VWD than in hemophiliacs (39% versus 82%, *p*<0.0001): those infected were less frequently males (39% versus 99%, *p*<0.001). The prevalence of viremic patients (serum RNA positive) was similar in the two groups of anti-HCV positive patients (80% versus 79%). HCV RNA was not detectable in the sera of 11 VWD patients (20%) and 52 hemophiliacs (21%) already in 1998, at the time of study enrollment (Table 1).

HCV genotypes and risk factors for HCV infection

Even though the overall distribution of HCV genotypes was not significantly different (*p*=0.06) between VWD patients and hemophiliacs (Figure 1), the risk of being infected with HCV genotype 1a (Table 2), was significantly lower in patients with VWD (OR=0.39; 95% CI 0.17-0.91). However, the risk of infection with HCV 1a genotype was greater in VWD patients (OR=21.25, CI 2.48-182.2) than in hemophiliacs (OR=1.96, CI 1.05-3.65) treated with large-pool, non-virus-inactivated coagulation factor concentrates than in those treated only with single donor blood products (Table 3). Among the 142 transfused patients with VWD the risk of becoming infected with HCV, evaluated as anti-HCV positivity, was not affected by sex or age but was associated with the degree of clinical severity of VWD, being more frequent in type 3 VWD than in type 1 or 2 (OR 17.16; CI 3.29-89.56). The risk of becoming infected with HCV was also associated with the year of first exposure to blood products, being higher when transfu-

Table 2. Distribution of hepatitis virus in genotypes in patients with VWD and hemophilia A or B.

Genotype	von Willebrand's disease n (%)	Hemophilia A or B n (%)	OR* (95% CI)	
1a	8 (18%)	77 (36%)	0.51 (0.20-1.32)	0.39 (0.17-0.91)
1b 2 3 4	15 (33%) 10 (22%)10 (22%) 2 (5%)	71 (33%) 29 (14%) 28 (13%) 7 (4%)	1# 1.55 (0.60-3.98) 1.59 (0.62-4.08) 2.44 (0.43-13.7)	⎱ ⎰ 1#)
Total	45	212		

Relative risks are presented as odds ratios (OR) and corresponding 95% confidence intervals (95% CI) according to an unconditional logistic regression model based on case-only design; #reference category.

Table 3. Distribution of type of transfused blood products according to inherited bleeding disorder and HCV genotypes (1a versus others genotype).

	von Willebrand's disease			Hemophilia A or B		
	Other genotypes	1a	OR* (95% CI)	Other genotypes	1a	OR* (95% CI)
Blood/plasma/cryoprecipitate	31 (84%)	2 (25%)	1#	75 (56%)	31 (40%)	1#
Large pool concentrates	6(16%) 37	6 (75%) 8	21.25 (2.48-182.2)	60 (44%) 135	46 (60%) 77	1.96 (1.05-3.65)

Relative risks are presented as odds ratios (OR) and corresponding 95% confidence intervals (95% CI); according to an unconditional logistic regression model based on case-only design # reference category.

sion occurred before 1986 (OR 4.29; CI 1.22-15.05) than during the interval 1986-1992 (Table 4). The risk of HCV infection was not significantly different depending on whether the first transfusional treatment was a single donor blood product or large pool coagulation factor concentrates. This was clearly related to the introduction of pasteurized FVIII/VWF concentrate in 1984-1985. In fact, none of the 15/28 (54%) VWD patients exposed exclusively to this pasteurized concentrate was infected whereas all the remaining 13/28 (46%) cases exposed to large-pool, non-virus-inactivated FVIII/VWF concentrates became anti-HCV positive (Table 4).

Outcomes of HCV infection

Several laboratory and clinical parameters describing the liver status of cases with chronic HCV hepatitis in both cohorts of patients are summarized in Table 1. Serum ALT activity was persistently or intermittently high in a significantly lower proportion of chronically infected HCV-RNA positive patients with VWD than in the corresponding hemophiliacs (58 versus 83%, p=0.02). However, the proportions of patients who developed ESLD or HCC were similar: 11% versus 10% and 2% versus 4%, respectively. Fewer patients with VWD were treated with antiviral agents (9% versus 27%).

Discussion

Patients with VWD are a unique model to investigate hepatitis associated with multiple transfusions because they have long been exposed to single donor blood products such as whole blood, plasma or cryoprecipitate but

Table 4. Variables affecting the risk of HCV infection (evaluated as anti-HCV positivity) in the 142 transfused patients with von Willebrand's disease.

Variable	HCV antibodies (negative/positive)	OR* (95% CI)
Sex		
Males	33/22	1#
Females	53/34	1.02 (0.49-2.16)
Phenotype		
Type 1 VWD	26/9	1#
Type 2 VWD	55/34	2.21 (0.89-5.47)
Type 3 VWD	4/13	17.16 (3.29-89.56)
First transfusion (calendar year)		
Before 1986	60/50	4.29 (1.22-15.05)
1986-1992	26/6	1#
Type of transfusion at first exposure		
Blood/plasma/cryoprecipitate	71/43	1#
Large pool concentrates	15/13	0.67 (0.21-2.09)
non-virus-inactivated	0/13	
pasteurized	15/0	

*#reference category; *from a unique multivariate logistic regression model containing all variables presented in the table simultaneously.*

less frequently to large-pool coagulation factor concentrates, which until 1985-1987 carried a very high risk of transmitting hepatitis C. Unlike patients with hemophilia, those with VWD have a mild to moderate bleeding tendency so that their rate of transfusion treatment is usually much lower than in hemophiliacs. In addition, the use of blood products in most Italian patients with VWD was greatly reduced following the introduction of DDAVP in 1977,[22] a synthetic drug that increases endoge-

nous VWF. Not unexpectedly, therefore, the prevalence of HCV infection, as expressed by serum anti-HCV, was lower in this cohort of VWD patients than in hemophiliacs (39% versus 82%), who more often required treatment with some type of blood product (96% versus 40%) and with large-pool, non-virus-inactivated coagulation factor concentrates (51% versus 20%). The fact that large-pool coagulation factor concentrates are derived from plasma donated in geographical areas characterized by a high prevalence of the HCV genotype 1a explains why the hemophiliacs, who more often received these concentrates, in this cohort and in others, had a particularly high prevalence of genotype 1a.[23,24] Conversely, a lower prevalence of genotype 1a was observed in our patients with VWD, with a relative increase of genotypes 2 and 3. The prevalence of genotype 1b, albeit not statistically different in patients with hemophilia and VWD, was higher in the latter, in agreement with the findings in multi-transfused patients, drug-addicts and small hemophilia cohorts from France and Italy.[25-27] A high prevalence (65%) of HCV genotype 1b was also reported in Italian patients with thalassemia who are mainly exposed to red cell components from unpaid donors.[28] Interestingly, the source of blood products from single donors seems to play a role, since also American patients with thalassemia have a prevalence of HCV infection of 35-40%,[29] similar to that found in our Italian VWD patients. In our cohort, genotype 1a was mainly observed in those VWD patients who, like hemophiliacs, were given large-pool, non-virus-inactivated coagulation factor concentrates. In agreement with previous reports [5,30-32] we found that the introduction of pasteurized FVIII/VWF concentrates abolished new HCV infections. From a clinical standpoint, however, infections with HCV genotypes 1a and 1b have similar clinical consequences, as both carry a similar risk of disease progression and are relatively more resistant to interferon therapy than genotypes 2 and 3.[33-35] Despite the different rates of exposure of the two groups of patients to HCV (as expressed by the seroprevalence of anti-HCV) the rate of chronically infected (HCV-RNA seropositive) patients was the same in VWD and hemophilia. Unfortunately in our study we could not prospectively assess HCV clearance in 11 VWD patients and 56 hemophiliacs because they were already HCV RNA seronegative in 1998, at the time of their enrollment in the study: these VWD and hemophilia patients could have cleared HCV a long time before. In fact, approximately one-tenth of all infected patients have been shown to spontaneously clear HCV, as demonstrated by the presence of serum antibody anti-HCV in the face of persistently negative results for serum HCV-RNA.[36] Moreover, viral clearance usually occurs within 1-2 years after the onset of infection in young patients with hemophilia[37] while in non-hemophiliacs with self-limiting hepatitis, the median time for HCV RNA clearance is approximately 12 weeks, with a range of 2-24 months.[38] Our data are in agreement with the recent observations of Zhang et al. who prospectively showed that 20% of HCV-infected hemophiliacs spontaneously clear the virus and also shed light on the importance of non-genetic determinants in spontaneous recovery from HCV infection.[39]

Our study indicates that the clinical consequences of HCV infection were relatively mild in most patients with VWD. Even though as many as 58% of the chronically infected patients (HCV-RNA seropositive) had persistently elevated serum ALT values, only six (13%) of them had clinical signs of ESLD. The prevalence of VWD patients with persistently elevated ALT values compares well with the rates reported in other settings of non-hemophilic HCV-infected patients with chronic hepatitis followed up for longer than 10 years after transfusions.[40,41] We may have underestimated the risk of ESLD because we did not perform liver biopsies. However, the 13% cumulative incidence of ESLD in VWD confirms previous reports in non-hemophilia patients followed for 20 years after blood transfusion.[42,43] Our decision not to perform liver biopsies was dictated by the associated costs and risks in bleeders, as well as by the fact that this strategy is not required for optimal management of hepatitis C infection, as indicated by a recent NIH consensus conference.[44] Even though the lack of a correlation between HCV genotype 1 and hepatitis C severity confirms findings in previous studies in non-hemophilic patients,[33,45] this genotype is unfavorable, because patients infected with genotype 1 respond less often to interferon therapy than those with genotype 2 or 3.[46-48] Due the relatively low number of VWD patients with active HCV infection, only a small number of them underwent treatment with anti-viral agents such as interferon and ribavirin, therefore precluding any analysis of their responsiveness to antiviral therapy.

In conclusion, the prevalences of HCV infection and HCV genotype 1 infection were lower in patients with VWD than in hemophiliacs, reflecting the different source and type of transfused blood products. HCV infection in both groups seems to run a relatively mild course, so far, but the higher prevalence of genotypes resistant to potentially curative antiviral therapies is of concern.

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