The natural history of chronic hepatitis C in a cohort of HIV-negative Italian patients with hereditary bleeding disorders

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This study looked at 102 anti–hepatitis C virus (HCV)–positive, hepatitis B virus (HBV)–negative, and HIV-negative patients (median age, 45.1 years; range, 15-71) affected by hereditary bleeding disorders who have been infected with HCV for 15 to 34 years (median, 25.1). All these patients were infected before the mid 1980s because of non–virally inactivated pooled blood products. Fourteen patients (13.7%) were HCV-RNA negative with no signs of liver disease and were considered to have cleared the virus. Eighty-eight patients (86.3%) were HCV-RNA positive. The HCV genotype distribution was 1a in 20.5%, 1b in 36.4%, 2 in 17.0%, 3 in 15.9%, 4 in 3.4%, and mixed in 6.8% of cases. Twenty-four patients (23.5%) had serum cryoglobulins, symptomatic in 4 cases, and associated with liver disease and with genotype 1. Among the 88 HCV-RNA–positive patients, 15 (17.0%) had normal alanine aminotransferase levels and abdominal ultrasound, 61 (69.3%) had nonprogressive chronic hepatitis, and 12 (13.7%) had severe liver disease (6 [6.9%] liver cirrhosis, 4 [4.5%] hepatic decompensation, and 2 [2.3%] hepatocellular carcinoma) after a follow-up period of 25 years. There were 3 (3.4%) liver-related deaths. HCV genotype 1, patient's age at evaluation, duration of infection, and severity of congenital bleeding disorder were associated with more advanced liver disease. The results confirm the slow progression of HCV infection in HIV-negative hemophiliacs. (Blood. 2001;98: 1836-1841)

Introduction

Hepatitis C virus (HCV) infection is a major problem for patients affected by hereditary bleeding disorders treated with factor concentrates during the 1970s.[1-4] In fact, because the concentrates were non–virus inactivated and were prepared from a large pool of plasma, virtually all patients treated with these products were infected with HCV at the time of the first infusion.[5-8] Because the onset of the infection can be reasonably estimated (first treatment with non–virus-inactivated blood products), these patients represent a unique model for studying the natural history of HCV infection and associated complications.[9-21] The course of hepatitis can be accurately assessed in these patients because they are seen regularly at hemophilia centers with laboratory, clinical, and instrumental tests.

Studies of the natural history of HCV infection in persons with hemophilia are limited.[21-25] Furthermore, most of the few studies published so far refer to hemophiliacs coinfected with HIV,[26-30] which is a well-known risk factor for a more rapid progression of liver disease and could be a confounding factor in evaluating the natural history of HCV infection.

In this retrospective study, we report the natural history of HCV infection and the progression of chronic HCV-related liver disease in a cohort of 102 HIV and hepatitis B virus (HBV) seronegative hemophiliacs followed at 3 hemophilia centers in northern Italy and exposed to the virus for a period of up to 34 years.

Patients and methods

Patients

Our cohort comprises 102 patients with hereditary bleeding disorders positive for anti-HCV antibodies treated at 3 Italian hemophilia centers (Verona, Trento, and Parma). All the patients were negative for anti-HIV antibodies and hepatitis B surface antigen (HbsAg) and were infected before 1985 due to non–virus-inactivated concentrates manufactured from a large pool of plasma. None of them had a history of drug addiction or alcohol abuse or had received antiviral treatment for hepatitis C before evaluation. The median age of the anti-HCV antibody–positive patients was 45.1 years (range, 15-71); the ratio of males to females was 6.3 (88 men and 14 women). Thirty-three patients (32.4%) were affected by mild hemophilia A (factor VIII $>$ 4 U/dL), 9 (8.8%) by moderate hemophilia A (factor VIII 1-4 U/dL), and 32 (31.3%) by severe hemophilia A (factor VIII $<$ 1 U/dL); 4 (3.9%) patients were affected by mild hemophilia B (factor IX $>$ 4 U/dL) and 2 (2.0%) by severe hemophilia B (factor IX $<$ 1 U/dL); 20 (19.6%) patients were affected by von Willebrand disease; and 2 (2.0%) female patients were carriers of hemophilia A.

Study design

Every 6 months the patients underwent clinical examination, with particular attention to liver status, laboratory tests (routine liver chemistry analysis, search of serum cryoglobulins, serum markers of HBV and HIV) and abdominal ultrasound examination. We recorded HCV genotype from all 88 HCV-RNA–positive patients and the time of the first infusion of non–virus-inactivated clotting factor concentrates. Chronic hepatitis C was established

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in the presence of abnormal serum alanine aminotransferase (normal range, 8-45 U/L) in monthly determinations for 6 months. We defined severe liver disease when cirrhosis, liver decompensation, or hepatocellular carcinoma was present. Cirrhosis was diagnosed clinically in the presence of laboratory (platelet count $<$ 100 000/mL [normal range, 150-400 $\times$ 10^9/L], serum albumin $<$ 35 g/L [normal range, 35-50 g/L], serum cholinesterase $<$ 4500 U/L [normal range, 4650-14 400 U/L]), endoscopic (presence of esophageal varices), and/or abdominal ultrasound (irregular margins of liver, dilated portal vein, splenomegaly) signs of liver failure. Liver decompensation was defined as the presence of at least 2 of the following: ascites, jaundice, prolonged prothrombin time, and encephalopathy. Hepatocellular carcinoma (HCC) was suspected on the basis of increased serum α-fetoprotein and abdominal ultrasound finding of a focal lesion. The diagnosis was confirmed bioptically. Only the 2 patients with HCC underwent liver biopsy. Because no stored sera were available to define dates of seroconversion for any of the patients, we have estimated the time and the duration of the HCV infection, assuming that the first exposure to non–virally inactivated clotting factor concentrate prepared from pooled donations had transmitted the HCV.

Laboratory assays

Serum cryoglobulins. The presence of serum cryoglobulins was evaluated as follows. After collecting the blood at 37°C, serum was cleared by centrifugation at 2000*g* for 15 minutes, stored in a cryocrit tube at 4°C for 7 days, and examined daily. The formation of the precipitate was confirmed visually. Samples were considered positive for cryocrit values more than 0.5%, after checking the heat resolubility of the cryoprecipitate. Cryoglobulins were identified as immunoglobulin composition by immunoblotting assay and classified as type II when rheumatoid factor was monoclonal or as type III when rheumatoid factor was policlonal.[31]

Serologic anti-HCV assay. For the detection of immunoglobulin G antibodies to HCV we used an indirect enzyme immunoassay (Cobas Core Anti-HCV EIA; Roche Diagnostic Systems, Branchburg, NJ) containing epitopes from the core and the NS3, NS4, and NS5 proteins to improve the clinical sensitivity as well as the clinical specificity of anti-HCV antibody detection. Specimens with absorbance values more than 1.1 times the cutoff value were retested in duplicates, and, if the values were again more than 1.1 times the cutoff value, the specimens were considered reactive for HCV antibodies. In this case the specimens were confirmed by more specific methods as immunoblot assay (Inno-Lipa HCV II; Innogenetics, Zwijndrecht, Belgium).

HCV RNA qualitative testing and HCV genotyping. HCV RNA was reverse transcribed and amplified using a commercially available HCV RNA assay (Cobas Amplicor HCV; Roche Diagnostic Systems), with primers to the highly conserved 5′ noncoding region. Biotin-labeled amplified products were genotyped using a commercially available line probe assay (Inno-Lipa HCV II; Innogenetics). In brief, the labeled amplicons were reversely hybridized to oligonucleotide probes directed against variations found on the 5′ noncoding regions of the HCV genome.[32] The specificity can be obtained with very stringent hybridization condition (50°C $\pm$ −0.5°C). After hybridization, streptavidin labeled with alkaline phosphatase was added to trace the hybrid previously formed, and nitroblue tetrazolium and 5-bromo-4 chloro-3 indoyl-phosphate were used as substrate, resulting in a colorimetric reaction. Positive line is detected only when a perfect match between the probe and the biotinylated amplicons occurs. With this standardized assay the 6 major HCV types and their most common subtypes can be detected simultaneously, and it has been reported to be highly concordant with other tests for the assignment of genotype.[33] The nomenclature system used was the same used by the international scientific community.[34] HbsAg and antibodies to HIV were tested by commercially available immunoassays (Abbott Laboratories, Chicago, IL).

Statistical analysis

For analysis of normally distributed continuous data, we used Student *t* test; for analysis of categorical data, we used the chi-square or Fisher exact tests. We used the one-way analysis of variance to compare the categories of patients.

Results

Fourteen of the 102 patients (13.7%) enrolled were HCV-RNA negative, whereas the remaining 88 (86.3%) were HCV-RNA positive. All the patients were infected before 1985 (date of introduction of sterilized concentrates) because of non–virus-inactivated clotting factor concentrates. The median age at infection was 20.1 years (range, 1-56). The average duration of infection was 25.1 years (range, 15-34 years). The 14 patients (13.7%) who have cleared the virus and 15 (17.0%) of the 88 HCV-RNA–positive patients had no laboratory, clinical, or instrumental signs of liver disease during the regular follow-up controls. Seventy-three (83.0%) of the 88 HCV-RNA–positive patients showed liver disease; 61 of them (69.3%) had chronic hepatitis and 12 (13.7%) had liver cirrhosis. The average time lapse between the infection and the onset of liver cirrhosis was 21.6 years (range, 6-32). Among the 12 patients with liver cirrhosis, 4 patients (4.5%) developed hepatic decompensation after an average period of 4.3 years (range, 2-8) from the onset of liver cirrhosis and 27.5 years (range, 23-32) from the HCV infection, respectively. Two patients (2.3%) with liver cirrhosis developed HCC 4 and 8 years, respectively, after the diagnosis of liver cirrhosis (29 and 32 years from the HCV infection). There were 3 (3.4%) liver-related deaths (2 patients with hepatic decompensation and 1 patient with HCC).

All the 88 HCV-RNA–positive hemophiliacs were tested for HCV genotype. The HCV genotype distribution was 1a in 18 patients (20.5%), 1b in 32 patients (36.4%), 2 in 15 patients (17.0%), 3 in 14 patients (15.9%), 4 in 3 patients (3.4%), and mixed in 6 patients (6.8%; 1a + 1b in 5 cases and 1a + 2 in 1 case). There were no type 5 and 6 infections. Genotype 1 was the most frequent among HCV genotypes because it was detected in 56 of 88 cases (63.6%; 18 cases 1a, 32 cases 1b, 5 cases 1a + 1b, and 1 case 1a + 2). Table 1 shows epidemiologic, clinical, and virologic characteristics of the 102 HCV antibody–positive hemophiliacs. There was no difference in demographic features or severity of hemophilia between the patients who had spontaneously recovered and those who developed persistent infection. Table 2 shows the HCV genotypes according to liver status. Ten of the 56 patients (17.9%) with genotype 1 showed a progression of HCV infection with liver cirrhosis in 4 cases (7.1%), hepatic decompensation in 4 cases (7.1%), and HCC in 2 cases (3.6%). On the contrary, only 2 of the 32 patients (6.2%) with other genotypes had liver cirrhosis ($P < .001$). None of them had hepatic decompensation or HCC. Table 3 correlates the liver status with epidemiologic and clinical features of the 88 HCV-RNA–positive hemophilic patients. Patients with severe liver disease were older and had been exposed for longer to HCV virus than patients with normal serum transaminases or chronic hepatitis ($P = .02$ and .03, respectively). Furthermore, the progression of HCV infection was associated with a more severe congenital bleeding disorder. On the contrary, there was no correlation between the hepatic status and gender.

Twenty-four patients (23.5%) had serum cryoglobulins typed in 16 of 24 cases: type II (mixed cryoglobulinemia) in 14 patients (87.5%) and type III in 2 patients (12.5%). No HCV-RNA–negative patients had circulating cryoglobulins. Cryocrit ranged from 1% to 10%. The 1b HCV genotype that was more frequently present (8 of 24, 33.3%) in cryoglobulinemic patients, followed by 1a (6 of 24, 25.0%), 2 (5 of 24, 20.8%), 3 (4 of 24, 16.7%), and 1a + 1b (1 of 24, 4.2%). The difference between HCV genotype 1 and other genotypes was statistically significant (15 of 24 [62.5%] versus 9 of 24 [37.5%], $P < .001$). Twenty-three of the 24 cryoglobulinemic

Table 1. Epidemiologic, clinical, and virologic features of the 102 HCV antibody–positive hemophiliacs

Features	HCV antibody–positive hemophiliacs (n = 102)
Median age, y (range)	45.1 (15-71)
Median age at infection, y (range)	20.1 (1-56)
Median duration of infection, y (range)	25.1 (15-34)
Sex	
Male	88 (86.3)*
Female	14 (13.7)*
Diagnosis	
Mild/moderate hemophilia	48 (47.1)*
Severe hemophilia	34 (33.3)*
von Willebrand disease	20 (19.6)*
HCV genotype†	
1a	18 (20.5)*
1b	32 (36.4)*
2	15 (17.0)*
3	14 (15.9)*
4	3 (3.4)*
1a + 1b	5 (5.7)*
1a + 2	1 (1.1)*
Liver status	
Normal ALT levels‡	29 (28.4)*
Chronic hepatitis	61 (59.8)*
Liver cirrhosis	6 (5.9)*
Hepatic decompensation	4 (3.9)*
Hepatocellular carcinoma	2 (2.0)*

HCV indicates hepatitis C virus; ALT, alanine aminotransferase.
*Number (percentage).
†HCV genotype was performed on the 88 HCV-RNA–positive patients.
‡Among the patients with normal ALT levels, 14 (13.7%) were HCV-RNA negative and 15 (14.7%) were HCV-RNA positive.

patients (95.8%) had liver disease: 18 (75.0%) had chronic hepatitis, 2 patients (10.0%) had liver cirrhosis, 2 patients (10.0%) had hepatic decompensation, and 1 patient (5.0%) had HCC. Four (16.7%) of the 24 patients with serum cryoglobulins developed cryoglobulinemic syndrome (type II mixed cryoglobulinemia in 3 cases and type III in 1 case); 2 of them had mild purpura and 2 developed systemic vasculitic disease associated in one case with cryoglobulinemic nephropathy. Three of those 4 symptomatic patients had chronic hepatitis and 1 had liver cirrhosis. HCV genotype was 1b in 2 cases, 2 in 1 case, and 3 in 1 case. The average time lapse between the HCV infection and the appearance of cryoglobulins was 14.4 years (range, 7-22). Table 4 compares the characteristics (sex, age at infection, duration of infection, genotype, primary disease, and hepatic status) of the patients with and without serum cryoglobulins. The HCV genotype 1, the age at infection, the duration of infection, and the severity of the congenital bleeding disorders were associated with the presence of serum cryoglobulins.

Discussion

The introduction of lyophilized large donor pool clotting factor concentrates in the early 1970s dramatically changed the hemophilia treatment permitting home therapy for bleeding episodes and a safer management of surgical procedures.[1-4] However, until 1985 when virucidal treatment was introduced, the concentrates were not subjected to viral inactivation during preparation, and they were largely responsible for the transmission of HCV infection in hemophiliacs. In fact, because clotting factor concentrates are prepared from plasma pools obtained from thousands of donors, virtually all patients treated with non–virally inactivated concentrates were infected with HCV at the time of the first infusion.[5,6]

Chronic hepatitis C is an important cause of morbidity and mortality in patients affected by hereditary bleeding disorders treated with factor concentrates during the 1970s, and sometimes it is a more serious problem than the primitive coagulopathy.[2,3,7]

Because the onset of infection can be reasonably estimated (first treatment with non–virus-inactivated blood products), these patients represent a unique model for studying the natural history of HCV infection and associated complications.[22-25] The course of hepatitis can be accurately assessed in these patients because of the long-term follow-up and because they are seen regularly throughout their life at hemophilia centers with laboratory, clinical, and instrumental tests.

In this retrospective study we reported a cohort of 102 HCV antibody–positive hemophiliacs treated at 3 hemophilia centers in northern Italy who had been exposed to the virus for 15 to 34 years. To avoid any possible interference with the natural history of HCV infection, we evaluated only patients that were HBV and HIV seronegative with no history of drug addiction or alcohol abuse and untreated for hepatitis C.

Fourteen percent of these patients were HCV-RNA negative and none of them had alterations of alanine aminotransferase values during the follow-up period; these patients were considered to have cleared the virus. Among the 88 HCV-RNA–positive patients, 17.0% (15 patients) had no signs of liver disease during the follow-up period, 69.3% (61 patients) had a stable liver disease

Table 2. HCV genotypes of the 88 HCV-RNA–positive hemophiliacs and hepatic status

HCV genotype	Normal ALT levels, no. (percentage)	Chronic hepatitis	Severe liver disease			All patients
			Cirrhosis	Hepatic decompensation*	Hepatocellular carcinoma*	
1	6 (6.8)	36 (40.9)	3 (3.4)	4 (4.5)	1 (1.1)	50 (56.9)†
1a	4 (4.5)	12 (13.6)	2 (2.3)	1 (1.1)	—	18 (20.5)
1b	2 (2.3)	24 (27.3)	1 (1.1)	3 (3.4)	1 (1.1)	32 (36.4)
2	3 (3.4)	11 (12.5)	1 (1.1)	—	—	15 (17.0)
3	3 (3.4)	10 (11.4)	1 (1.1)	—	—	14 (15.9)
4	2 (2.3)	1 (1.1)	—	—	—	3 (3.4)
1a + 1b	1 (1.1)	2 (2.3)	1 (1.1)	—	1 (1.1)	5 (5.7)
1a + 2	—	1 (1.1)	—	—	—	1 (1.1)
All patients	15 (17.0)	61 (69.3)	6 (6.8)	4 (4.5)	2 (2.3)	88 (100.0)

HCV indicates hepatitis C virus; ALT, alanine aminotransferase.
*Hepatic decompensation and hepatocellular carcinoma were only observed in patients with established cirrhosis.
† Ten of the 56 patients (17.9%) with genotype 1 had a severe liver disease (4 liver cirrhosis, 4 hepatic decompensation, and 2 hepatocellular carcinoma), whereas only 2 of the 32 patients with other genotypes (6.2%) had liver cirrhosis ($P < .001$).

Table 3. Correlation between liver status and epidemiologic and clinical features of the 88 HCV-RNA–positive hemophilic patients

Features	Normal ALT levels (n = 15)	Chronic hepatitis (n = 61)	Severe liver disease* (n = 12)	P†
Median age, y (range)	43.1 (15-67)	44.6 (25-70)	52.1 (26-68)	.02
Median age at infection, y (range)	22.3 (1-56)	19.6 (1-52)	22.0 (1-42)	NS
Median duration of infection, y (range)	21.1 (15-32)	25.0 (15-50)	29.6 (15-36)	.03
Sex				
Male	13 (86.7)‡	52 (85.2)‡	10 (83.3)‡	NS
Female	2 (13.3)‡	9 (14.7)‡	2 (16.7)‡	
Diagnosis				
Mild/moderate hemophilia	8 (53.3)‡	30 (49.2)‡	3 (25.0)‡	< .01
Severe hemophilia	3 (20.0)‡	21 (34.4)‡	7 (58.3)‡	< .001
von Willebrand disease	4 (26.7)‡	10 (16.4)‡	2 (16.7)‡	NS

HCV indicates hepatitis C virus; ALT, alanine aminotransferase; NS, not significant.
*Severe liver disease = liver cirrhosis, hepatic decompensation, and hepatocellular carcinoma.
†*P* value = severe liver disease versus normal ALT levels and chronic hepatitis.
‡Number (percentage).

(nonprogressive chronic hepatitis), and 13.7% (12 patients) developed liver cirrhosis over a period of 21.6 years from infection. Liver failure and HCC occurred in 4.5% (4 patients) and 2.3% (2 patients) of patients after 27.5 and 30.5 years, respectively, from infection. On the whole, among the 102 HCV antibody-positive hemophiliacs evaluated in this study, only 11.8% had a progressive liver disease after an average follow-up period of 25.1 years (Table 1). Thus, our data are similar to those reported in literature[9,14-18] for non-hemophilic HCV-infected patients and confirm the slow progression of HCV infection in HIV-seronegative hemophiliacs.[24,25]

Genotype 1 was the most frequent among HCV genotypes (Table 1). In fact, it was detected, alone, or associated with other genotypes in 63.6% of cases. HCV genotype distribution in our cohort of patients is similar to that observed generally in the Italian population[35] and in other studies on hemophiliacs from the same Italian regions,[36,37] but it is quite different from data coming from trials on hemophiliacs in other countries.[38-44] Furthermore, as hemophiliacs have been exposed to clotting factor concentrates prepared from thousands of donors, some of them have been infected by multiple HCV genotypes. In our study the prevalence of mixed infection is 7% which is similar to that reported in literature.[36-44]

On analyzing the epidemiologic, clinical, and virologic features of the 88 HCV-RNA–positive patients (Tables 2,3) we observed that a more advanced liver disease was associated with HCV genotype 1, a higher age at evaluation, and a more severe congenital bleeding disorder. As in other studies,[23,26] we also identified the duration of infection as an important variable of progression of liver disease: The longer the exposure to HCV, the more severe the liver disease. These last risk factors are strictly correlated. In fact, patients with more severe hemorrhagic disorders had received non–virally inactivated factor concentrates and had been infected at a younger age than patients with mild bleeding disease and thus had a longer-lasting infection. Similarly, the older

Table 4. Epidemiologic, clinical, and virologic characteristics of HCV-RNA–positive patients with and without serum cryoglobulins

Characteristics value	All patients (n = 88)	Cryoglobulins negative (n = 64)	Cryoglobulins positive (n = 24)	P
Median age, y, (range)	44.1 (15-71)	41.8 (15-67)	47.2 (23-71)	.05
Median age at infection, y (range)	20.3 (1-56)	21.7 (1-56)	15.4 (1-38)	NS
Median duration of infection, y (range)	24.3 (15-34)	21.3 (15-36)	31.4 (15-36)	< .001
Sex				
Male	77 (87.5)‡	58 (90.6)‡	19 (79.2)‡	NS
Female	11 (12.5)‡	7 (10.9)‡	4 (16.7)‡	
Diagnosis				
Mild/moderate hemophilia	43 (48.9)‡	34 (53.1)‡	9 (37.5)‡	< .01
Severe hemophilia	29 (32.9)‡	19 (29.7)‡	10 (41.7)‡	.02
von Willebrand disease	16 (18.2)‡	11 (17.2)‡	5 (20.8)‡	NS
Genotype				
1a	18 (20.5)‡	12 (18.8)‡	6 (25.0)‡	< .001§
1b	32 (36.4)‡	24 (37.5)‡	8 (33.3)‡	
2	15 (17.0)‡	10 (15.6)‡	5 (20.8)‡	
3	14 (15.9)‡	10 (15.6)‡	4 (16.7)‡	
4	3 (3.4)‡	3 (4.7)‡	—	
Mixed*	6 (6.8)‡	5 (7.8)‡	1 (4.2)‡	
Liver status				
Normal ALT levels	15 (17.0)‡	14 (21.9)‡	1 (4.2)‡	< .001
Chronic hepatitis	61 (69.3)‡	43 (67.2)‡	18 (75.0)‡	.02
Severe liver disease†	12 (13.6)‡	7 (10.9)‡	5 (20.8)‡	< .01

HCV indicates hepatitis C virus; NS, not significant; ALT, alanine aminotransferase.
*Three patients with genotype 1a + 1b and one patient with genotype 1a + 2b were cryoglobulin negative; one patient with genotype 1a + 1b had serum cryoglobulins.
†Four patients with liver cirrhosis, 2 patients with hepatic decompensation, and 1 patient with hepatocellular carcinoma were cryoglobulin negative; 2 patients with liver cirrhosis, 2 patients with hepatic decompensation, and 1 patient with hepatocellular carcinoma had serum cryoglobulins.
‡Number (percentage).
§HCV genotype 1 versus other genotypes: 15 of 24 (62.5%) vs. 9 of 24 (37.5%), *P* < .001.

age at the time of evaluation reflects the longer duration of infection. Furthermore, we must consider the immunosuppressive effect of the clotting factor concentrates that could have favored the HCV progression.[45] Patients with severe hemophilia were likely to have been treated more frequently with blood products than patients with mild hemophilia.

Previous studies[22-24] have found an association between the age at infection and the liver disease progression. Patients infected older than age 40 years had the most aggressive liver disease. We did not find this correlation because of the younger median age at infection (20 years) of our cohort of patients.

Among HCV genotypes, type 1 appeared to be associated with the fastest progression of liver disease in our study. In fact, it was detected in 64% of patients with chronic hepatitis and in 75% of patients with severe liver disease. If there are no doubts about the association between the duration of HCV infection and severity of liver disease,[16-18,23,26] more uncertainties exist as to the role of HCV genotype 1 on liver disease progression.[37-44] Our study also confirms for hemophiliacs the observations in non-hemophilic patients[15,18,44] about the greater virulence of genotype 1.

HCV infection is now recognized as a major risk factor for the development of HCC.[46-49] This complication was first described in patients with congenital bleeding disorders in 1991 by Tradati et al.[46] They reported a survey of 11 801 hemophiliacs from 54 centers in the United States and Europe and found 10 cases of HCC, all in patients with cirrhosis, with a prevalence 30 times higher than normally expected. The high prevalence of HCC in hemophiliacs infected with HCV was confirmed by further studies.[47-49] A prospective trial[47] analyzed the risk of developing HCC in a cohort of 385 Italian hemophiliacs of whom 6 developed HCC during a 2-year follow-up. We found a similar prevalence in our Italian hemophiliacs infected with HCV because 2% of them developed HCC. Our study also confirmed the previous findings[48,49] about the presence of liver cirrhosis, the duration of infection, and the genotype 1b as important risk factors for developing HCC. Both patients with HCC had liver cirrhosis and had been infected 30 years previously by HCV genotype 1b.

Many studies have shown a strong association between HCV infection and the presence of serum cryoglobulins.[50-53] Serum cryoglobulins have been detected in 19% to 55% of patients with chronic HCV infection. Such cryoglobulins may be clinically significant and associated with a systemic vasculitic disorder in 12% to 30% of cases.[52,53] Although most of these studies refer to non-hemophiliacs, our results are consistent with them. In our study of 102 HCV antibody–positive hemophiliacs, 24% of the patients developed serum cryoglobulins after an average time of 14 years from infection, with clinical symptoms in 17% of them. This last finding is in contrast with what Santagostino et al[36] reported. They found no clinical signs or symptoms of systemic vasculitis in a cohort of 135 hemophiliacs with chronic HCV infection. In accordance with previous studies,[36,50-53] we identified a positive correlation between genotype 1, the presence of chronic hepatitis or severe liver disease, the duration of hepatitis C, and the risk of producing serum cryoglobulins.

In our study, only the 2 patients with HCC underwent liver biopsy. Whereas liver biopsy is strongly recommended in non-hemophilic HCV-infected patients to assess their liver status, its role in hemophilic patients with HCV liver disease is still controversial.[54-58] Even if many groups have reported that liver biopsy could be done safely in hemophiliacs after coagulation factor replacement,[55,57-59] fatal bleeding following liver biopsy has been reported.[58] Nowadays, the availability of many laboratory (serological, polymerase chain reaction testing, and genotype analysis of HCV) and instrumental (ultrasound and computed tomography) techniques offers the possibility to follow accurately and safely these patients. For this reason we have chosen to manage our HCV-infected hemophiliacs without liver biopsy using clinical history (first time of infusion of nonvirus-inactivated clotting factors and duration of infection), laboratory, and instrumental tests to evaluate their liver status.

Studies of the natural history of HCV infection in persons with hemophilia are limited.[22-25] Furthermore, most of the few studies published[26,27] so far refer to hemophiliacs coinfected with HIV, which is a well-known risk factor for a more rapid progression of liver disease. Thus, in these studies, HIV coinfection could be a confounding factor in evaluating the natural history of HCV infection and could explain the higher incidence of severe liver disease observed. On the contrary, our study shows the slow progression of hepatitis C in HCV-positive and HIV-negative hemophiliacs and confirms that the natural history of HCV infection in these patients is not different from those without congenital bleeding disorders.

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