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Update on chronic hepatitis C in hemophiliacs

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Background and Objectives. Hepatitis C virus (HCV) infection is an important cause of morbidity and mortality in patients affected by hereditary bleeding disorders and treated with non-virus inactivated clotting factor concentrates during the 1970s.

Information sources. In this review, we briefly report the present knowledge about HCV infection in hemophilic patients. The natural course of hepatitis C virus infection in hemophiliacs is described, by analyzing the prevalence of HCV infection, the genotype distribution and the risk factors involved in the progression of chronic hepatitis into severe liver disease such as cirrhosis, liver decompensation and hepatocellular carcinoma.

State of the Art and Perspectives. We focus on the most important advances in the treatment of hepatitis C in hemophiliacs.

Key words: HCV infection, hemophilia, therapy.

Hepatitis C virus (HCV) infection is frequently observed in hemophiliacs who received clotting factor concentrates prior to the mid 1980s.[1-5] In fact, until 1985 when heat 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.[1,2,6] Hepatitis C virus infection in hemophiliacs differs from the infection in non-hemophilic patients, mainly because the non-virus-inactivated clotting factor concentrates were prepared from plasma pools obtained from thousands of donors and in this way many hemophilic patients were co-infected with different HCV genotypes or with human immunodeficiency virus (HIV) and hepatitis B virus (HBV).[7-10] Hemophiliacs represent a unique model for studying the natural history of HCV infection and associated complications since the onset of the infection is known (first treatment with non-virus-inactivated blood products) and the course of hepatitis can be accurately assessed due to their long-term and periodic follow-up at hemophilia centers by laboratory, clinical and instrumental tests.[11-15]

In this review, we briefly describe the main characteristics of HCV infection in hemophilic patients with particular attention to the prevalence of HCV infection, the natural history of this disease and the co-infection with other viruses. Finally, we focus on the most important advances in the treatment of hepatitis C in hemophiliacs.

The prevalence of HCV infection and genotype distribution in hemophiliacs

Virtually all hemophiliacs who received clotting factor concentrates prior to the availability of viral inactivation techniques were infected with hepatitis C virus at the time of the first infusion and most studies[6,15-17] report a high prevalence of HCV-antibody positivity in hemophilia patients treated with concentrates before 1985, ranging from the 83% of an Italian study6 to the 98% of a Dutch study.[17] In a previous study collecting data from 3 Hemophilia Centers of Northern Italy, we reported a 100% anti-HCV positivity in 102 hemophilic patients who had been exposed to large pool non-virally inactivated coagulation factor concentrates.[15]

Since hemophiliacs were infected by clotting factor concentrates manufactured from many thousands of donors, the HCV genotype distribution reflects that of the donor population; moreover different genotypes may have infected the same patient.[18,19] Thus, genotypes in hemophiliacs show a marked ethnic and geographic variation.[2] In fact, whereas types 1, 2 and 3 are more frequent in northern Europe[20,21] and North America,[22] type 4 is the principal genotype in the Middle

East[23] and North Africa and type 5 in South Africa.[2] Table 1 shows the HCV genotype distribution reported from six recent studies.[15,24-28]

The natural history of HCV infection in hemophiliacs

Hepatitis C virus infection is a world major health problem: it is estimated that there are 170 million infected individuals world-wide and that the prevalence of infection is nearly 3%.[29] Although our knowledge about the epidemiology of this infection has been clarified after the discovery of hepatitis C virus in 1989, much uncertainty remains about the long-term course of HCV, mainly because the primary infection is often asymptomatic and remains unrecognized in most patients. Moreover, the chronic phase remains silent for decades, thus preventing a precise definition of the onset of the infection.[30] The study of the natural course of hepatitis C is crucial for defining the pathogenesis,[31-39] the gravity, the complications[40-43] and the prognostic factors[15] associated with this illness and epidemiologically acquired information has important therapeutic implications. The most useful information about the natural history of HCV infection can be obtained from retrospective studies on patients transfused before the discovery of hepatitis C virus[35,38] and on hemophiliacs treated before 1985 with large-pool non-virus inactivated factor concentrates;[10-15] in fact, for these patients the date of infection can be accurately assessed since nearly 100% of them were infected at the time of their first transfusion with blood components or products.[44] Moreover, hemophilic patients represent a unique model to study the natural course of HCV infection because of their accurate and long-term follow-up.[15] Many studies have been recently published on HCV-infected hemophiliacs with the aim of elucidating the time elapsed between the infection and the onset of complications (liver cirrhosis, hepatic failure and hepatocellular carcinoma)[9-15] and the factors influencing disease progression.[45-49]

However, the study of the natural history of HCV in hemophiliacs is limited by two factors. The first limitation is represented by histologic studies of the liver. Whereas liver biopsy is strongly recommended in non-hemophilic HCV-infected patients in order to assess their liver status, its role in hemophilic patients with HCV liver disease is still uncertain.[50-55] Although many groups have reported that a liver biopsy can be safely done in hemophiliacs soon after coagulation factor replacement,[53-55] fatal bleeding following liver biopsy has been reported.[52] We think that the availability of many laboratory (serological, polymerase chain reaction testing and genotype analysis of HCV) and instrumental (ultrasound and computed tomography) techniques together with the clinical history (first time of infusion of non-virus-inactivated clotting factors and duration of infection) offers the potential to follow these patients accurately and safely.[15] Our beliefs were confirmed by Hanley *et al.*,[53] who compared ultrasound and laparoscopic inspection of the liver surface with liver biopsy in 87 hemophiliacs, finding evidence of liver cirrhosis in about 25% of patients, showing a high sensitivity (80%) and specificity (88%) of the former method. The second limitation is that hemophiliacs are frequently co-infected with other viruses, in particular HIV which is a well-known risk factor for a more rapid progression of liver disease (*see below*)[7-10,56-58] and could be a confounding factor in evaluating the liver status. For this reason the study of the natural history of HCV infection should be performed only in HIV-negative hemophiliacs.[14,15]

Studies on the natural history of HCV infection in non-hemophiliacs showed that approximately 20 to 30% of the patients with chronic hepatitis

Table 1. Prevalence of HCV genotype in HCV infected hemophiliacs: data of literature.

Authors	*Number of patients*	*HCV genotype*						
		1	*2*	*3*	*4*	*5*	*mixed*	*not performed*
Franchini et al.[15]	102	57%	17%	16%	3%	–	7%	–
Eyster et al.[24]	32	45%	7%	42%	3%	–	3%	–
Telfer et al.[25]	189	64%	12%	19%	1%	1%	2%	1%
Tagariello et al.[26]	45	56%	19%	11%	–	–	11%	3%
Santagostino et al.[27]	135	74%	6%	10%	2%	–	3%	5%
Preston et al.[28]	96	50%	13%	18%	4%	2%	7%	6%

will develop cirrhosis about 20 years after exposure. For those patients who develop cirrhosis, the 5-year risk of liver decompensation is between 15 and 20% and that of hepatocellular carcinoma is around 10%.[28-38] Studies on hemophiliacs seem to confirm these observations.[11-15] Makris *et al.*[11] reported the liver biopsy results of 63 hemophilic patients and found cirrhosis in 19 (30%) of them. They also followed 138 HCV-positive hemophiliacs and reported hepatic failure in 9 (6.5%) of them after an average duration of infection of 19 years. Telfer *et al.*[12] retrospectively studied 255 HCV seropositive patients with congenital coagulation defects: the risk of liver failure was 10.8% at 20 years after the first treatment with non-virus-inactivated large pool factor concentrate. However, the analysis of the data in these two latter studies could have been influenced by the high percentage (40%) of HCV-positive hemophiliacs co-infected with HIV. The most useful information on the natural history of HCV infection in hemophiliacs came from studies on HCV-positive/HIV-negative patients. Meijer *et al.*[14] reported cirrhosis in 7 (16%) out of their 45 patients with congenital coagulation disorders 19 years after infection. In a study on 88 HCV-RNA-positive/HIV-seronegative hemophiliacs15 we found that 61 (69.3%) had a non-progressive chronic hepatitis and 12 (13.7%) had a severe liver disease (6 [6.9%] liver cirrhosis, 4 [4.5%] hepatic decompensation and 2 [2.3%] hepatocellular carcinoma) with 3 (3.4%) liver-related deaths after a 25-year follow-up. Thus, these two latter studies show the slow progression of hepatitis C in HCV^{+}/HIV^{-} hemophiliacs and confirm that the natural history of HCV infection in these patients is not different from that in patients without congenital bleeding disorders. The very slow course of HCV infection in HIV-negative hemophiliacs was also confirmed by Yee *et al.*[13] in a study of 310 patients (*see below*).

HCV infection is now recognized as a major risk factor for the development of hepatocellular carcinoma (HCC).[47-49] This complication was first described in patients with congenital bleeding disorders in 1991 by Colombo *et al.*,[47] who reported a survey of 11,801 hemophiliacs from 54 centers in the United States and Europe and found 10 cases of HCC, all in patient with cirrhosis, with a prevalence 30 times higher than normally expected. The high prevalence of HCC in HCV-infected hemophiliacs was confirmed by further studies. A prospective study[48] analyzed the risk of developing HCC in a cohort of 385 Italian hemophiliacs: during a 4-year follow-up, six patients developed HCC. Age at infection greater than 45 years, presence of cirrhosis and high serum α-fetoprotein (AFP) baseline levels were associated with an increased risk of cancer.

Retrospective studies on the natural history of HCV infection in hemophiliacs are also important because they allow identification of those factors influencing the disease progression. Age at infection, mode of HCV transmission, alcohol consumption, HCV RNA levels, viral genotype and HIV co-infection are the most important co-factors involved in liver disease progression reported by different authors.[11-15] Several reports have identified the age at infection as an important variable for the rate of progression to severe liver disease in HCV infected hemophiliacs. On multivariate analysis, Makris *et al.*[11] showed that patients with a higher age at infection (> 40 years) and with a longer duration of infection (> 15 years) had an increased risk of developing severe liver disease. The higher age at evaluation was the only independent risk factor for more advanced disease identified in multivariate analysis by Meijer *et al.*[14] in their cohort of HCV-RNA positive and HIV-seronegative hemophiliacs. In addition to the time of the first infusion, another important factor affecting the long-term outcome of HCV infection is the mode of HCV transmission. In fact, two studies[59,60] found more severe liver disease in patients who acquired the infection through blood transfusion, suggesting that the size of the infectious inoculum may influence the disease progression. The viral load could also play an important role in liver disease progression in those hemophiliacs who were exposed to massive doses of non-virus-inactivated concentrates for several years. Relationships between viral genotype and severity of disease have been reported by several authors with contradictory results.[23-29] Many studies[15,23-25] have found that HCV genotype 1 is associated with the presence of more severe liver disease, higher viral loads, a poorer response to therapy and an increased risk of liver-related death, but not all authors agree with those findings.[26] In our study[15] we observed that the HCV genotype 1, a higher age at evaluation, a more severe congenital bleeding disorder and the duration of infection were associated with more advanced liver disease.

Studies in non-hemophilic patients showed that chronic alcoholism accelerates progression of chronic HCV-related liver disease, leading more frequently to liver cirrhosis and hepatocellular carcinoma.[61] Similarly, Yee *et al.*[13] reviewed clinical and treatment records from 310 HCV-infected patients with inherited bleeding disorders and

observed a higher mortality in those who had an increased alcohol consumption.

Co-infection with HIV

HCV infection in HIV+ hemophiliacs is associated with higher HCV-RNA levels and faster progression of liver disease than in HIV-negative patients. Moreover, an anti-HIV combination treatment may be hepatotoxic and worsen hepatic status.[7-10,56,57]

The importance of HIV co-infection as a risk factor for the progression of liver disease in HCV infected hemophiliacs was shown by Eyster *et al.*[9] who found, in a prospective cohort study of 236 hemophiliacs followed for 10 to 20 years, that 9% of the co-infected hemophiliacs and none of the HCV^+/HIV^- patients developed liver failure. In a subsequent report[10] on 223 persons with hemophilia, the same author found significantly higher HCV-RNA levels in HIV-seropositive than in HIV^- seronegative hemophiliacs: over a 15-year period, HCV-RNA levels increased threefold in HCV^+/HIV^- patients and 58-fold in HCV^+/HIV^+ hemophiliacs. These findings suggest that HIV-induced immune deficiency may promote HCV replication with increased liver damage and, finally, more rapid progression of liver disease. Evidence that HIV infection increases HCV viral load and accelerates HCV liver disease was also given by Telfer *et al.*[12] who found, in a retrospective study of 255 HCV^+ hemophiliacs, that the risk of developing hepatic decompensation was 21 times higher in HIV^+ patients than in HIV^- ones. Other studies found an association between HCV genotype and the rate of HIV progression. Sabin *et al.*[7] demonstrated a more rapid progression to both acquired immuno-deficiency syndrome (AIDS) and death in patients with HCV genotype 1 than in those with other genotypes.

Mortality

Long-term follow-up studies of hemophilia populations recognize hepatitis C virus infection as a major cause for morbidity and mortality.[49,56] Darby *et al.*[56] analyzed the mortality from liver disease and liver cancer in a cohort of 4,865 hemophiliacs in the UK. After a follow-up of 8-24 years, they discovered that mortality from liver disease was 16.7 times higher than in the general population and 5.6 times higher for liver cancer. The cumulative risk of death from liver disease or liver cancer was strongly related to age at infection (> 45 years) and HIV co-infection. The highest risk (18.7 times) was seen for HCV-infected patients older than 45 years with HIV infection. A fatal combination of HIV and HCV co-infection was also seen by Yee *et al.*,[13] who found that, after a 25-year follow-up, 26 (8%) out of the 310 HCV^+ hemophiliacs had died from a liver-related death. The liver-related mortality was 3% for HIV negative and 21% for HIV positive patients. A similar mortality rate (3.4%) was observed in our study[15] on HCV^+/HIV^- hemophiliacs.

Treatment

Trials in non-hemophilic patients with chronic HCV-related liver disease demonstrated that the association of ribavirin to monotherapy with interferon-α significantly increases the percentage of sustained biochemical and virological responses (persistence of normal serum alanine aminotransferase [ALT] levels and undetectable serum levels of HCV-RNA 6 months post-treatment) from 20% to 40%.[62-65] These studies also identified two virological factors (i.e. HCV genotype 1 and a viral load higher than 2×10^6 copies/mL) as negative predictors of a response to treatment.[62,64]

Studies on the treatment of HCV-infected hemophiliacs are limited and include a small number of patients.[66-85] As to monotherapy with interferon-α in HCV-infected hemophiliacs, Hanley *et al.*[67] treated 31 patients with 3 MU three times weekly for 6 months and reported sustained responses in 2 of them (6.5%). Similar results were obtained by Peerlink *et al.*[73] in 13 patients with coagulation disorders. A higher rate of responders (38%) was found by Pinilla *et al.*[85] by an intensive protocol using 6 MU three times per week for 1 year but 19% of enrolled patients stopped the treatment due to interferon toxicity. The largest study was the trial conducted by Rumi *et al.*[69] on 102 HIV-negative hemophiliacs randomized to receive interferon-α 3 MU thrice weekly for 1 year versus no treatment. After a 12-month post-treatment follow-up, six out of the 50 treated patients (12%) had a sustained biochemical and RNA response. Finally, a poor clinical outcome has been reported by Hayashi and colleagues,[72] who found that all the 7 HCV^+/HIV^+ hemophiliacs treated with a high dose of interferon-α (9 MU daily for 2 weeks, then 9 MU thrice weekly for 22 weeks) for 6 months failed to achieve a sustained response.

A significant advance in the treatment of hepatitis C in patients with hereditary bleeding disorders was represented by the combination therapy of interferon-α and ribavirin. Shields *et al.*[81] treated 28 patients with interferon-α (3 MU three times per week) and ribavirin (1-1.2 g daily depending on body weight) for 1 year and reported a high rate of virological responses (71%). The same schedule

Table 2. Treatment of chronic hepatitis C in hemophiliacs: literature data.

Authors	*Protocol*	*N.° pat.*	*Sustained responses (%)*
Interferon-α			
Hanley et al.[67]	3 MU three times weekly for 6 months	31	2/31 (6.5)
Peerlink et al.[73]	3 or 6 MU three times weekly for 6 months	13	1/13 (7.7)
Pinilla et al.[85]	6 MU three times weekly for 12 months	26	10726 (38.5)
Rumi et al.[69]	3 MU three times weekly for 12 months	50	6/50 (12.0)
Hayashi et al.[72]	9 MU daily for 2 weeks, then		
	9 MU three times weekly for 22 weeks	7	0/7
Interferon-α plus ribavirin			
Shields et al.[81]	IFN-α 3 MU three times weekly for 12 months		
	ribavirin 1-1.2 g daily for 12 months	28	20/28 (71.4)
Sauleda et al.74	IFN-α 3 MU three times weekly for 12 months		
	ribavirin 0.8-1.2 g daily for 12 months	20	7/20 (35.0)

was used by Sauleda *et al.*[74] in 20 hemophiliacs and a sustained remission was obtained in 7 out of the 20 treated patients (35%). The most important trials on the therapy of chronic hepatitis C in hemophiliacs are reported in Table 2.

The addition of polyethylenglycol to interferon produces a molecule with a longer half-life and duration of therapeutic activity, allowing a more convenient once-weekly administration. Treatment of chronic hepatitis C infection with pegylated interferon results in a rate of sustained virologic response which is approximately twice that achieved with standard interferon.[58] Recently, a randomized trial comparing peginterferon plus ribavirin with interferon plus ribavirin showed a significantly higher sustained response rate in the peginterferon group (54% vs.47%).[86] Ongoing trials in hemophiliacs with chronic hepatitis C are evaluating the efficacy of pegylated interferon plus ribavirin.

Liver transplantation is the only available treatment option for patients with end-stage HCV-related liver disease (decompensated liver cirrhosis and/or hepatocellular carcinoma).[87,88] Moreover, liver transplantation in HCV-infected hemophiliacs cures hemophilia by providing a long-term correction of coagulopathy.[88-92] The first successful orthotopic liver transplantation in a patient with hemophilia was reported by Lewis *et al.* in 1985.[89] A review published in 1998 by Gordon *et al.*[93] reporting the experience on 26 liver transplants in hemophilic patients showed that post-transplant three-year survival was significantly higher in HIV-negative recipients than in HIV-positive ones (83% vs. 23%). Post-operatively, all patients achieved normal clotting factor levels after an average of 24 hours.

Conclusions

HCV infection is a major problem for hemophiliacs treated before 1985 with non-virus-inactivated factor concentrates since nearly 100 % of them were infected at the time of the first infusion. The treatment of concentrates with virucidal methods (dry-heating, pasteurization, vapor heating, solvent-detergent and nanofiltration) and the improvement of screening tests on plasma donations markedly lowered the risk of HCV transmission from clotting factor concentrates. A further, important advance in viral safety was the development, thanks to the progress of DNA technology, of recombinant clotting factors (factor VIII, factor IX and factor VII) during the 1990s.[94]

HCV-infected hemophiliacs are a particularly suitable model for studying the natural history of HCV infection, since the date of infection (first treatment with non-virally inactivated clotting factor concentrates) is known for most of them. Moreover, as they were infected before 1985 (when virus-inactivated concentrates were introduced), the follow-up period is often sufficient for analyzing the long-term outcome of HCV infection.

The literature data show that the course of chronic hepatitis C in HIV-negative hemophiliacs is slow and similar to that of patients without congenital bleeding disorders.[15] The results of treatment with interferon-α of hemophiliacs with HCV-related hepatitis are disappointing, with a post-treatment sustained remission rate of nearly 10%, which is approximately half that achieved in non-hemophilic patients.[67,69] Combination therapy with interferon–α and ribavirin improves the percentage of responses to about 40% which is similar to the results obtained in non-hemophilic patients treated with the same regimen.[74,81] Unfortunately, HIV

co-infection in hemophiliacs worsens both liver disease and response to the anti-HCV treatment.[72]

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