Hepatitis C in Hemophilia

Hepatitis C bij Hemofilie

Dirk Posthouwer

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Hepatitis C bij Hemofilie
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1

Introduction

Hemophilia and hepatitis C

Hemophilia is an X-linked bleeding disorder caused by a partial or complete lack of clotting activity factor VIII (hemophilia A) or factor IX (hemophilia B).[1] The prevalence of hemophilia A (85%) is higher than that of hemophilia B (15%). Patients can be classified according to clotting factor activity in three categories: mild, moderate, and severe hemophilia. Patients with mild hemophilia suffer from bleedings mainly after surgery or major trauma. In contrast, patients with severe hemophilia experience joint and muscle bleedings spontaneously or after minor trauma. Since the 1960s hemophilia patients have received intravenous factor VIII and IX replacement therapy prepared from plasma.[2;3]

However, in the 1970s it appeared that patients with hemophilia treated with clotting factor products developed jaundice with biochemical evidence of hepatitis.[4] In the absence of hepatitis A and B, this disease was called non-A, non-B hepatitis.[5;6] After the identification of the hepatitis C virus (HCV) in 1989, it was found that this agent was responsible for the large majority of non-A, non-B hepatitis.[7]

HCV is an enveloped RNA virus that belongs to the family of flaviviruses. The virus appears primarily to infect hepatocytes, although B-cell lymphocytes may also be targeted.[8] Persistent infection appears to be due to lack of adequate T-cell mediated immune response.[9] The high viral replication rate and the frequent mutations result in a HCV population of different variants (quasispecies) which enable the virus to evade host immune mechanisms and leads to chronic infection.[10]

Studies in patients with hemophilia revealed that virtually all patients who were treated with large-pool clotting products before 1990 were infected with HCV.[11-14] In the early 1990s, methods were developed to adequately inactivate HCV and subsequently donor screening for HCV was introduced, resulting in HCV safe clotting products.[15;16]

Natural history of hepatitis C infection

Once infected with HCV, approximately 10-20% of patients clear HCV spontaneously, but the remainder develop chronic hepatitis C.[14;17-19] Most patients with chronic hepatitis C are asymptomatic or suffer from non-specific symptoms including fatigue, malaise, myalgia, arthralgia or pain in the upper right abdomen.[8] However, several studies have shown impaired health-related quality of life (HRQoL) among patients with HCV infection compared with healthy, non-institutionalized members of the general population.[20;21] Data on the effect of HCV infection on HRQoL in patients with hemophilia are lacking.

10% of patients with chronic hepatitis C developed cirrhosis and an additional 10% end-stage liver disease like liver failure or hepatocellular carcinoma after twenty years of infection.[22-25] Faster progression of liver disease is associated with HIV coinfection, alco-

hol abuse, older age at infection, and male gender.[17;22;26] However, the risk of end-stage liver disease after more than 20 years of infection remains poorly defined.

The hemophilic population represents a unique model to study the clinical course of hepatitis C. The date of infection can be determined accurately in these patients, since nearly all patients were infected at the time of their first transfusion.[17;18] Furthermore, these patients are frequently seen in hemophilia treatment centers, resulting in a reliable, long-term follow-up.

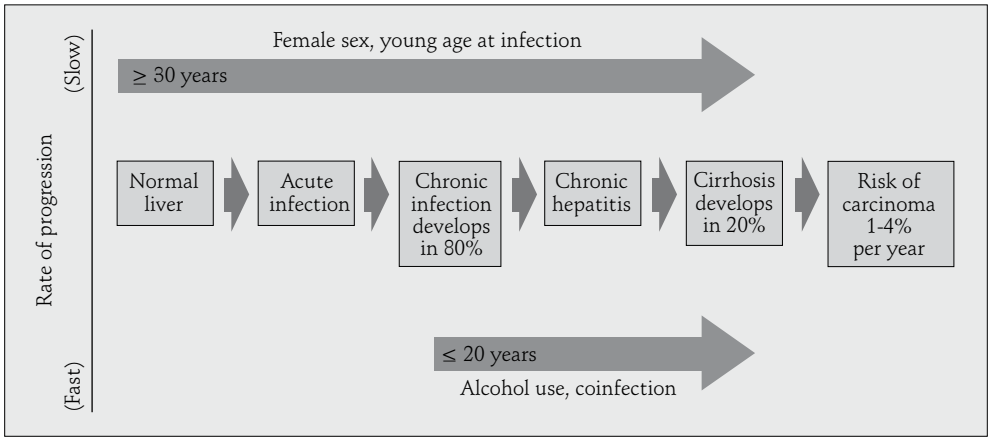

Figure: natural history of HCV infection. (Lauer, NEJM, 2001)[8]

Treatment of hepatitis C – general population

Interferon monotherapy became available in 1987, resulting in normalization of aminotransferases or eradication of HCV in 10-20% of patients treated.[27] The addition of ribavirin to IFN in 1995 markedly improved the virological response; 30-40% of patients became HCV RNA negative after 24-48 weeks of therapy.[28;29] Finally, the introduction of pegylated IFN (PegIFN) in the year 2000 in combination with ribavirin resulted in further improved HCV eradication up to 50-60% of patients.[30;31]

In patients coinfected with HIV the reported efficacy of IFN is lower with responses ranging from 16% for IFN monotherapy to 25-40% for combination therapy with (Peg-) IFN and ribavirin.[32;33]

Treatment of hepatitis C – hemophilic population

Conflicting data exist about the efficacy of IFN-based therapy in the hemophilic population. Previous reports suggested that patients with hemophilia might respond worse to antiviral therapy.[34;35] Although only few trials with IFN have been conducted in patients with hemophilia, results for IFN monotherapy and IFN combined with ribavirin appeared to be similar to those in the general population.[36-38] Until now, there have only been preliminary reports of the efficacy of PegIFN and ribavirin in the hemophilic population.

Aims and outlines of this thesis

The aim of this thesis was to assess the impact of hepatitis C in patients with hemophilia. In order to address this topic, the following questions were formulated:

- What is the prevalence of hepatitis C and the use of antiviral therapies among patients with hemophilia in the Netherlands in the beginning of the 21st century?
- What is the effect of HCV infection on health-related quality of life?
- Can liver fibrosis and cirrhosis be accurately assessed by using non-invasive diagnostics?
- What is the natural history of hepatitis C and what is the risk for developing end-stage liver disease after 30 years of infection?
- What is the effect of IFN-based therapies?

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2

Hepatitis C infection among Dutch hemophilia patients: a nationwide cross-sectional study of prevalence and antiviral treatment

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Summary

Hepatitis C is a major co-morbidity among patients with hemophilia who received inadequately or non-virus inactivated clotting factor concentrates before 1992. The objectives of this study were to investigate the prevalence of hepatitis C and the use of antiviral therapies during the last decade among patients with hemophilia in the Netherlands.

We performed a cross-sectional study and a questionnaire was sent to all 1519 patients known with hemophilia in the Netherlands between 2001 and 2002. The study population for the present study consisted of 771 patients who had received clotting factor products before 1992 of whom 638 reported their hepatitis C status.

In total 441 of the 638 (68%) patients ever had a positive test for hepatitis C virus (HCV); 344 patients (54%) had a current infection, and 97 (15%) had cleared the virus. Among 344 patients currently HCV infected, 111 (32%) had received treatment for hepatitis C, while 34% (33/97) of patients with an infection in the past had been treated for hepatitis C.

In 2002 the prevalence of hepatitis C among patients with hemophilia who received clotting factor products before 1992 was 54%. The majority of patients with a current HCV infection had not been treated with antiviral therapy.

Introduction

Hemophilia is an X-linked bleeding disorder caused by a partial or complete lack of clotting factor activity: factor VIII in hemophilia A and factor IX in hemophilia B. Since the 1960s hemophilia patients have received intravenous factor VIII and IX replacement therapy.[1] In the following years it became apparent that viruses like Human Immunodeficiency Virus (HIV) and hepatitis C virus (HCV), formerly known as non-A non-B hepatitis, were transmitted due to transfusion of infected plasma products.[2;3] Patients treated with large pool products were infected with HCV in 98%, whereas patients treated with cryoprecipitate were infected in 66% of the cases.[4] In the early 1990s, methods were developed to adequately inactivate HCV and subsequently donor screening for HCV was introduced, resulting in HCV safe clotting products.[4-6]

Once infected, about 10-20% of the patients are able to clear the virus spontaneously, while the others develop a chronic carrier state.[7-9] Untreated HCV infection may progress to liver fibrosis, cirrhosis, or hepatocellular carcinoma.[10;11] Liver disease caused by HCV is now recognized as an important cause of morbidity in hemophilia patients.[12] Treatment for non-A non-B hepatitis became available in 1986.[13;14] Today pegylated interferon (PegIFN) in combination with ribavirin is the most effective therapy for hepatitis C.[15] Success of therapy is mainly dependent on genotype and viral load.[16] Antiviral drugs cause side effects, like anemia, neutropenia, depression and flu-like symptoms in the majority of the patients.[17-19]

Little or no information is available on the current prevalence of hepatitis C and antiviral treatment history among patients who have received inadequately or non-virus inactivated clotting factor concentrates before 1992. We therefore investigated the prevalence of hepatitis C infection and assessed the use of antiviral therapy among patients with hemophilia in the Netherlands.

Methods

Setting

Data for the present study were collected within the last survey of a series initiated by Veltkamp in 1972.[20] Since then nationwide surveys were repeated in 1978, 1985, 1992 and in 2001.[21-24] These studies aimed to assess the medical and social consequences of hemophilia in the Netherlands. In 2001, postal questionnaires were sent to all 1519 patients known with hemophilia in the Netherlands, who were either registered at the Netherlands Hemophilia Patients Society, at the hemophilia treatment centers, or known from previous surveys. In this last survey items on hepatitis C were added for the first time.

Data

The study population consisted of patients who were treated with clotting factor products before 1992 and who reported their hepatitis C status. These patients were potentially at risk for HCV infection because they were treated with non-virus inactivated or inadequately inactivated clotting factor concentrates. Severity of hemophilia was defined by the percentage of factor VIII or factor IX clotting activity: severe hemophilia less than 1%, moderate hemophilia 1-5%, and mild hemophilia 5-40% clotting factor activity. Reported type and severity of hemophilia were verified with information from the treatment centers. In addition, data on hemophilia type and severity of non-responders were obtained from treatment centers or from the previous questionnaire performed in 1992. Hemophilia type and severity of 346 non-responders were similar to those in the study population. Items on hepatitis C and HIV were obtained from the questionnaire. Information on the hepatitis B status was not collected.

To assess the validity of the self-reported items on hepatitis C, a random sample of 92 patients (14%) was taken from the two largest participating centers verifying their reported hepatitis C status with information from their treating hematologist.

Data analysis

Infection with HCV was defined as three possible status: never infected with HCV, HCV infection cleared and chronic hepatitis C. *Never infected with HCV* was defined as negative for both HCV antibodies and HCV-RNA in serum. A *cleared HCV infection* or *infection in the past* was defined as positive for HCV antibodies but negative for HCV-RNA. *Chronic hepatitis C* was defined as positive for both HCV antibodies and HCV-RNA. In addition, *ever infected with HCV* was defined as positive for HCV antibodies, regardless of the HCV RNA result.

To study risk of infection according to period of treatment, a sub-analysis was performed comparing infection rates between patients first treated before 1985 with patients first treated between 1985 and 1992. Patients with incomplete treatment history were excluded for this sub-analysis.

HCV status according to type and severity of hemophilia were compared by using the Chi-Square test. Means with 95% confidence intervals (C.I.) of age according to severity of hemophilia and HCV infection status were calculated.

Results

A flow chart of the selection of patients for this study is shown in Figure 1. The response to the questionnaire was 1066 of 1519 (70%). General characteristics of the participants are shown in Table 1.

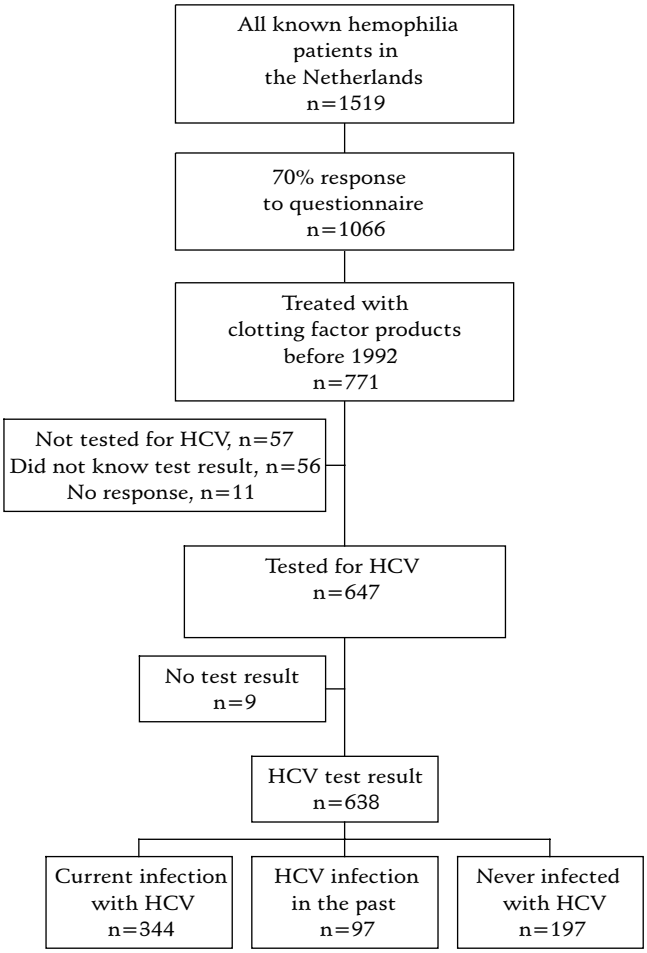

Figure 1. **Flowchart of selection of study population**

Table 1. **Patient characteristics**

Total number of patients	638
Age in years	41 (10-87)
Hemophilia type • A • B	 557 (87%) 81 (13%)
Severity of Hemophilia • Mild • Moderate • Severe	 211 (33%) 112 (18%) 315 (49%)
Patients treated before 1985* • HIV positive	523 (82%) 28 (5%)
Patients treated before 1992† • Anti HCV positive • HCV RNA positive	638 441 (68% of tested patients) 344 (54% of tested patients)

Note: Information of patients treated before 1992 with a reported HCV test result. Values are medians (range) or numbers (percentage)
* At risk for HIV infection due to not adequately or non-virus inactivated clotting factor products
† At risk for HCV infection due to not adequately or non-virus inactivated clotting factor products

Hepatitis C in patients treated with clotting products before 1992

A total of 771 patients were at risk for HCV infection (i.e. treated before 1992); 599 were already treated with clotting products before 1985, 136 were treated exclusively between 1985 and 1992, whereas 36 patients reported to have been treated before 1992 but not whether they were also exposed to clotting products before 1985. 638 of these 771 patients reported their HCV status. Among the 133 patients at risk without a HCV test result, 68% had mild hemophilia.

In the verification sample, 92% (85/92) reported their hepatitis C status correctly; 96% of patients with an HCV infection and 88% of patients with a cleared infection or those who where never infected.

Among 638 patients treated with clotting factor products before 1992 and tested for HCV, 441 (68%) ever had an anti-HCV positive test. 344 (54%) reported to be currently infected with HCV, 97 (15%) reported an infection in the past and 197 patients (31%) had never been infected. No infections with HCV occurred in patients who were treated after 1992 only.

HCV infection was related to type of hemophilia; patients with hemophilia B had been infected more often than those with type A (84% vs. 67%, $p < 0.01$). Among patients at risk for HCV transmission, patients with severe hemophilia had the highest prevalence

of hepatitis C (severe 65%, moderate 53%, mild 37%, $p < 0.001$).

The mean age of patients differed according to severity of hemophilia and HCV status; patients with severe hemophilia, who were never infected, were younger (mean age 23 years, 95% C.I. 19-28) than both patients with severe hemophilia who cleared HCV (37 years, 95% C.I. 33-41), and those currently infected (43 years, 95% C.I. 41-45).

Infection rate of HCV according to treatment period

Although HCV inactivating steps were applied since 1985, risk of HCV infection was not completely eliminated. 523 of 599 patients treated before 1985 and 95 of 136 patients treated 1985-1992 reported their HCV status. Among patients treated before 1985, 62% reported to be currently infected, while 17% cleared HCV. In contrast, the proportion of patients with chronic HCV infection was only 18%, with 7% clearing HCV and 75% never infected in those first treated between 1985 and 1992.

HIV infection

The prevalence of HIV infection among patients treated before 1985 and reporting their HIV status was 5% (28/523).

Treatment of hepatitis C

Among the 344 patients with a current HCV infection, 68% (233) had not been treated with antiviral drugs. The main reasons for refraining from therapy were shrinking from side effects (46%), normal liver function tests (45%), and expected low efficacy (35%). Other reported reasons were: physician not convinced of benefit of treatment (19%), treatment not discussed by physician (18%) and lack of time among patients (9%). Over the last decade, the proportion of patients having been treated, is increasing (Figure 2).

Treatment for HCV was completed among 128 patients and successful treatment was reported in 26% (33/128). Sixteen patients were currently on combination therapy of IFN and ribavirin. Among patients who finished therapy, 57 patients were treated with IFN monotherapy, 51 patients with the combination of IFN and ribavirin, while 13 patients were first treated with monotherapy and later retreated with combination therapy. Seven patients did not remember their treatment regimen.

Patients reported side effects of antiviral therapy in 84% (121/144). Fatigue (78%), flu-like symptoms (73%), and depressive symptoms (46%) were most frequently reported. In 15% of treated patients therapy was discontinued because of side effects.

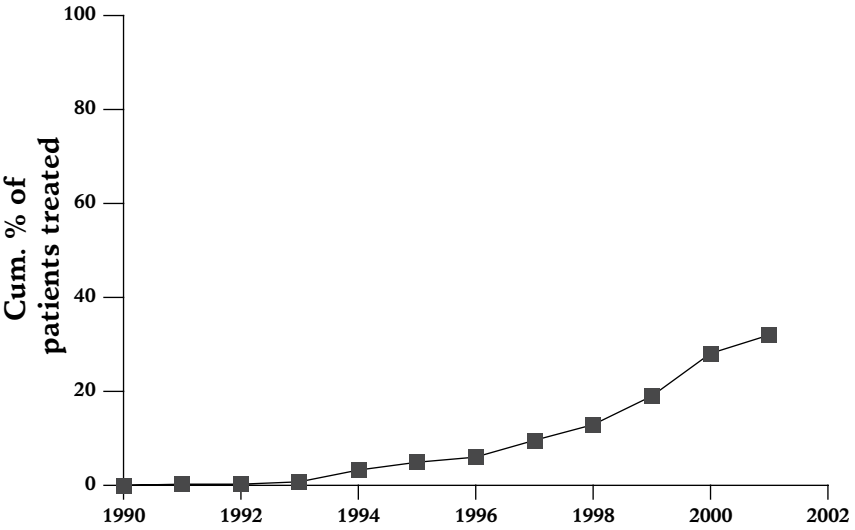

Figure 2. Cumulative percentage of all HCV infected patients with hemophilia treated with antiviral therapy during the last decade. Considering a spontaneous clearance of 15%, the maximum cumulative percentage would be 85%.

Discussion

We report on a nationwide survey on the current prevalence of hepatitis C in hemophilia patients. Of 771 patients at risk for HCV infection, 638 reported their hepatitis C status. 54% of tested patients reported to be currently infected with HCV, of whom 32% had been treated with antiviral therapy.

We performed a cross-sectional study to assess the prevalence of hepatitis C infection among patients with hemophilia in the Netherlands and to examine the use of antiviral treatment. To appreciate our findings some limitations need to be discussed. First, the response rate to the questionnaire was 70%, and selection bias cannot be ruled out. Non-responders to the questionnaire may have been less severely affected, therefore failing to see the need for a survey in this population. This may have led to an overestimation of the prevalence of hepatitis C. However, percentages of type and severity were similar in responders and non-responders, rendering bias less likely.

Secondly, self-reported data may be unreliable. We therefore performed a validation study, and found that these self-reported data were highly reliable, confirming previous observations that, most patients with hemophilia are well informed about their disease and its complications.[21]

In this study, 68% of all tested patients potentially exposed to insufficiently viral inactivated clotting factor products, had ever been infected with HCV and 54% of them reported a current HCV infection. The prevalence of hepatitis C in this population is similar to that reported by others.[4;9;25] As expected, the prevalence was highest among patients with severe hemophilia due to a higher number of exposures to clotting products than patients with mild or moderate disease. Hemophilia B was associated with a higher HCV infection rate (84% vs. 67%) due to exclusive treatment with large pool plasma products, whereas patients with hemophilia A were in many cases exclusively treated with small pool cryoprecipitate.[26] Confirming data in a Dutch study on 316 patients, reported HCV infection rates of 66% and 98% in patients exclusively treated with small pool cryoprecipitate and patients treated with large pool products, respectively.[4] In addition, the proportion of patients with severe hemophilia was higher among patients with hemophilia B than in those with hemophilia A (58% vs. 48%), with concomitant higher exposure rates to potentially unsafe clotting factor products.

In our study, we found that the risk of HCV transmission was lower among younger patients. This may be explained by the lower number of exposures and the introduction of dry heat treatment (up to 68°C) in 1985. Although completely effective for HIV, this method of viral inactivation did not eliminate HCV infection risk, but resulted in a reduction of HCV load only.[27] This is also shown in our study, in which patients exclusively treated with clotting products between 1985 and 1992, had a lower risk of HCV infection than patients treated before 1985. Although this risk was decreased, HCV transmission was not eliminated. Finally, donor screening, pasteurization, steam heat treatment and chemical viral inactivation through the combination of solvent and detergent methods were introduced on a large scale, eliminating transmission of HCV completely in 1992.[6;28;29]

Although there has been a trend towards starting treatment of HCV infection, so far only 32% of the HCV infected patients reported use of antiviral therapy, with a success rate of 26%. The main reasons for refraining from antiviral therapy were expected low efficacy of therapy, normal liver function tests and expected side effects. The argument of low expected effectivity loses its strength as treatment with PegIFN and ribavirin results in a sustained response in 50 to 90% in treatment naive patients dependent on viral genotype.[18] It has been suggested that refraining from therapy in case of normal liver function tests may be appropriate in patients with genotype 1 and 4 with normal histology at liver biopsy.[30] But this is inappropriate in patients with HCV genotype 2, 3 and 5, of whom 80-90% will achieve a sustained response.

Fatigue, flu-like symptoms and depression were the most frequently reported adverse events of antiviral therapy; this is in accordance with other reports.[17] Depression has been a common indication for dose reduction or even discontinuation of therapy.[17;31]

Discontinuation of therapy due to adverse effects was reported in 15% in this study and was similar to that reported by others.[18;19;32;33] The reported reasons for refraining from antiviral therapy indicate that there are still uncertainties about long-term complications of hepatitis C and effectivity of antiviral therapy. Therefore, patients need to be fully informed about HCV infection, its consequences, possibilities of treatment, and its effectivity.

In summary, this study shows that hepatitis C is still a major comorbidity in the Dutch population of hemophilia patients and only a minority of patients with an HCV infection has been treated.

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Hepatitis C and health-related quality of life among patients with hemophilia

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Summary

Hepatitis C has a negative effect on health-related quality of life (HRQoL). It is not clear whether hepatitis C affects HRQoL of patients with hemophilia. The objective of this study was to assess the effect of hepatitis C virus (HCV) infection on HRQoL in patients with hemophilia.

A cross-sectional study was performed among all registered hemophilia patients in the Netherlands. HRQoL was determined by using the self-administering RAND-36 questionnaire. Patients were eligible for the study if they completed the RAND-36, had been treated with clotting factor products before 1992, and had reported their hepatitis C status. Data on severity of hemophilia were obtained from the hemophilia treatment centers. The validity of the self-reported data on hepatitis C status was verified in a random sample of 92 (15%) patients; 92% reported their hepatitis C status correctly.

Fifty-five percent (333/602) of the study population had a current HCV infection. All eight domains of the RAND-36 were lower in patients with a current HCV infection when compared with patients who had never been infected with HCV. After adjustment for age, severity of hemophilia, HIV, employment status, and joint limitations, hepatitis C infection was associated with a decrease of HRQoL on the domains of general health (difference 6.9 [95% confidence interval (C.I.) 2.7 to 11.2]) and vitality (3.8 [95% C.I. 0.1 to 7.7]).

Hemophilia patients infected with HCV scored lower on the HRQoL domains of general health and vitality than hemophilia patients who were never infected with HCV.

Introduction

Hemophilia is an X-linked bleeding disorder caused by a partial or complete lack of clotting factor activity. Health-related quality of life (HRQoL) of these patients is lower than in the general population and is mainly dependent on severity of hemophilia, age, orthopedic status and comorbidities.[1-4] Hepatitis C is a major comorbidity among patients with hemophilia who received non-virus inactivated or insufficiently inactivated large-pool clotting factor concentrates or cryoprecipitate.[5;6] Hepatitis C infection itself has also been shown to be associated with a decrease in HRQoL, which may be explained by chronic liver disease and associated factors present in many infected individuals such as intravenous drug use and low socio-economic status.[7-10] Reports about the effect of hepatitis C virus (HCV) infection on HRQoL in patients with hemophilia are scarce. However, this information may be important with regard to the policy on initiating antiviral therapy. The majority of reports on HRQoL among patients with hepatitis C have focused on patients selected for enrollment into treatment trials, in which patients with hemophilia are usually excluded.[11;12]

The aim of this study was to determine the effect of HCV infection on HRQoL among patients with hemophilia.

Methods

Setting

Data for the present study were collected within the last survey of a series initiated by Veltkamp in 1972.[13] Since then nationwide surveys were performed in 1978, 1985, 1992 and in 2001.[14-16] These surveys aimed to assess the medical and social consequences of hemophilia in the Netherlands. In 2001, postal questionnaires were sent to all 1519 patients known with hemophilia in the Netherlands, who were either registered at the Netherlands Hemophilia Patients Society, at the hemophilia treatment centers, or known from previous surveys. In this last survey items on hepatitis C were added for the first time. The study was approved by the medical ethics committee of the Leiden University Medical Center.

Patients

The overall response to the questionnaire was 70% (1066/1519). Of those responding to the questionnaire, 771 patients had been treated with clotting factor products before 1992 and were at risk for HCV infection. Of those at risk, 638 patients reported their HCV

test result and 602 completed the RAND-36 questionnaire. The study population consisted of these 602 patients, treated with clotting products before 1992, with a reported HCV test result and a completed RAND-36 questionnaire. Hemophilia type and severity of patients not responding to this questionnaire (1519 - 1066 = 453) were similar to those of the responding population (1066). Reasons for not participating in this study were unknown. Sociodemographic and clinical data of patients at risk for HCV, who did not complete the RAND-36 questionnaire (638 - 602 = 36) did not differ from the study population.

Definitions

Hepatitis C status was defined as: never infected with HCV, infection in the past, or current infection. To assess the validity of the self-reported data on hepatitis C, a random sample of the two largest participating centers of 92 patients (15%) was taken and these data were verified with information from the treating hematologist.

Questionnaire

The questionnaire contained questions on type and severity of hemophilia, use of clotting product, health issues, complications and infections, education, and profession. The self-reported type and severity of hemophilia were verified with information from the treatment centers.

HRQoL was assessed by using the Dutch version of the RAND-36 questionnaire.[17-19] This self-administering questionnaire contains 36 items assessing 8 domains of HRQoL: physical functioning, social functioning, role physical, role emotional, mental health, vitality, bodily pain and general health. Each HRQoL domain is given a score ranging from 0 to 100, with higher scores indicative of better quality of life. In addition, the physical and mental health component summaries were calculated using standard algorithms.[20] Table 1 explains the meaning of the different domains.

Joint status was assessed in terms of functional limitation of 16 joints; per joint, scores ranged from 0 (no limitation), 1 (some limitation without daily problems), 2 (some limitation with daily problems), to a maximum of 3 (severe limitation with complete loss of function). The total 'joint limitation score' was calculated by adding up all joint scores resulting in a range from 0 to 48.

The employment and educational status was determined by asking patients about their work or school.

Table 1. Explanation of the domains of the RAND-36.

Domain	Explanation
Physical functioning	Limitations in daily activities (e.g. walking, dressing)
Social functioning	Limitations in social activities (e.g. meeting friends)
Role physical	Difficulties with work or daily activities due to physical health problems
Role emotional	Difficulties with work or daily activities due to emotional problems
Mental health	Presence of depressive feelings or nervousness
Vitality	Loss of energy or presence of fatigue
Bodily pain	Presence of pain and its limitations due to pain
General health	Subjective evaluation of general health status

Data analysis

Descriptive statistics were calculated using means (95% confidence intervals, C.I.) and medians (range). Baseline characteristics and HRQoL scores of patients with a current HCV infection were compared with patients who were never infected; the T-test for continuous and normally distributed data, the Mann-Whitney U-test for continuous and skewed data and the Chi-Square test for ordinal or nominal data. A p-value <0.05 was considered statistically significant.

To quantify the effects of HCV infection on HRQoL, linear regression models were used with physical and mental summary scores as dependent variables. In order to facilitate the interpretation of the effect of hepatitis C on HRQoL and to put this effect in perspective, we also determined the isolated effects of both age and severity of hemophilia on HRQoL. The effect of age was assessed in patients with mild hemophilia who were never infected with HCV. The effect of disease severity was determined by comparing patients with mild versus severe hemophilia, in patients who were never infected with HCV.

Multivariate linear regression models were used to adjust the association between HCV infection and HRQoL for age, severity of hemophilia, HIV, joint limitation and employment/education status. Dummy variables for hepatitis C infection status and severity of hemophilia were created to differentiate among the groups in the regression analyses; the references were 'never infected with HCV' and 'mild hemophilia', respectively. The regression coefficients represent the change in outcome (i.e. scores on domains of HRQoL), per unit increase of the determinant.

Results

Patients

The study population consisted of 602 patients; 171 (28%) patients had never been infected with HCV, 98 (16%) had cleared HCV, and 333 (55%) patients had a current HCV infection. In the verification sample, 92% (85/92) reported their hepatitis C status correctly; these proportions were 93%, 75% and 100% for patients with a current infection, an infection in the past, and patients who have never been infected, respectively. Table 2 presents patient characteristics according to hepatitis C status. Patients with a current HCV infection were older than those who were never infected (44 years vs. 37 years). Furthermore, the proportions of patients with severe hemophilia, HIV, and total score of joint limitations were higher among patients currently infected with HCV compared with patients who were never infected.

Table 2. Baseline characteristics of the study population according to HCV infection status.

	Never infected (n=171)	Infection in the past (n=98)	Current infection (n=333)	p-value*
Age (years)	37 (11-87)	36 (13-87)	44 (13-83)	<0.001
Severe hemophilia	24%	55%	60%	<0.001
HIV positive	1%	2%	7%	<0.005
Joint limitation score (0-48)	2 (0-48)	4 (0-32)	7 (0-35)	<0.001
Employed or student	81%	80%	70%	<0.01

Values are medians (range) or percentages

* Differences in baseline characteristics; patients who were never infected compared with patients with a current HCV infection.

Health-related quality of life

Patients with a current HCV infection had lower HRQoL scores on all domains and the physical component summary of the RAND-36 questionnaire compared to those who were never infected (Figure 1). Physical function, role physical and general health appeared to be mostly affected by HCV infection. The mental component summary was similar in all groups.

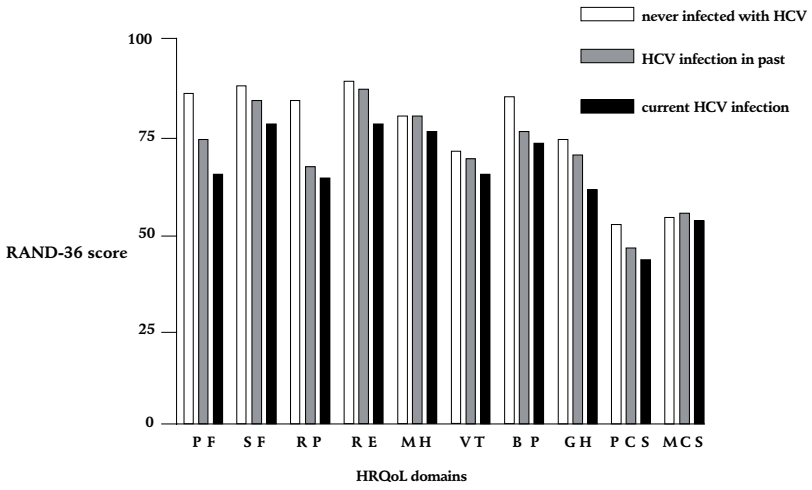

Figure 1. **Crude HRQoL according to HCV infection status.**
Patients who were never infected had higher scores ($p<0.05$) on all domains and the physical component summary score (PCS) compared with patients with a current HCV infection; the mental component summary score (MCS) was similar in all groups.
PF = physical functioning, SF = social functioning, RP = role physical, RE = role emotional, MH = mental health, VT = vitality, BP = bodily pain, GH = general health.

In order to study potential confounders of the association between hepatitis C and HRQoL, we performed univariate analysis of several established determinants of HRQoL on the physical and mental component summaries of the RAND-36 questionnaire (Table 3). Current HCV infection, increasing age, severe hemophilia, HIV, increasing joint limitation and unemployment were associated with lower scores on the physical component summary score. A lower score on the mental component summary was associated with increasing age, increasing joint limitation and unemployment. However, the effects of these determinants on the mental component summary were less pronounced than the effects on the physical component summary score.

A current HCV infection was associated with lower scores on all domains but the mental component summary score in the univariate analysis (Table 4). After adjustment for age, severity of hemophilia, HIV status, joint limitations and employment/educational status, current HCV infection was only associated with a statistically significant decrease of the scores for general health (-6.9, 95% C.I. -11.2 to -2.7) and vitality (-3.8, 95% C.I. -7.7 to -0.1).

Table 3. Crude effects of patient characteristics on physical and mental component summary scores of the RAND-36 (univariate regression).

	Physical component summary	Mental component summary
Current HCV	-8.4 (-10.5 to -6.3) †	-0.9 (-2.7 to 1.0)
Age (per 10 years)	-2.7 (-3.3 to -2.2) †	-0.8 (-1.3 to -0.3) ‡
Severe hemophilia	-8.0 (-10.0 to -5.9) †	1.2 (-0.6 to 3.1)
Joint limitation (per point)	-1.1 (-1.2 to -1.0) †	-0.2 (-0.3 to -0.1) †
HIV infection	-6.4 (-11.1 to -1.7) ‡	2.4 (-1.5 to 6.3)
Employed/student*	11.3 (9.3 to 13.3) †	4.7 (2.8 to 6.5) †

Note. Values are regression coefficients (95% confidence interval).
* patients were classified as 'employed' when they worked full- or part-time; the young patients were classified as student as they attended school
† p-value < 0.001
‡ p-value < 0.05
Interpretation: the presence of a current HCV infection resulted in a decrease of 8.4 points of the PCS.

Table 4. Crude and adjusted effects of current HCV infection on all domains of the RAND-36

HRQoL domain	β current HCV (95% CI) unadjusted	β current HCV (95% CI) adjusted*
Physical functioning	-22.1 (-27.5 to -16.8) †	-1.6 (-5.4 to 2.1)
Social functioning	-9.6 (-14.1 to -5.1) †	-3.2 (-7.7 to 1.2)
Role-physical	-19.5 (-27.0 to -12.0) †	-4.9 (-12.3 to 2.6)
Role-emotional	-11.1 (-17.6 to -4.7) ‡	-3.5 (-10.3 to 3.3)
Mental health	-4.0 (-7.2 to -0.8) ‡	-2.7 (-6.1 to 0.8)
Vitality	-6.5 (-10.1 to -2.8) ‡	-3.8 (-7.7 to -0.1) ‡
Bodily pain	-12.7 (-17.2 to -8.3) †	-3.5 (-7.8 to 0.9)
General health	-13.5 (-17.6 to -9.4) †	-6.9 (-11.2 to -2.7) ‡
Physical component score	-8.4 (-10.5 to -6.3) †	-1.6 (-3.4 to 0.1)
Mental component score	-0.9 (-2.7 to 1.0)	-1.5 (-3.6 to 0.6)

Values are regression coefficients (95% confidence interval)
* adjusted for age, severity of hemophilia, joint limitation, HIV and employment status
† p-value < 0.001
‡ p-value < 0.05
Interpretation: the crude score on general health in patients with HCV infection was 13.5 points lower compared with patients never infected with HCV. After adjustment the score for general health was 6.9 points lower in patients infected with HCV compared to patients never infected with HCV.

In order to facilitate the interpretation of the effect of hepatitis C on the HRQoL domains of general health and vitality by putting this effect into perspective, we studied the isolated effects of age and severity of hemophilia on these domains (Table 5). For every 10 years the score of vitality decreased by 1.2 points (95% C.I. -1.1 to 3.5) and the score of general health by 1.7 points (95% C.I. -0.7 to 4.2) among patients with mild hemophilia without hepatitis C. We found no effect of disease severity on general health and vitality: scores were similar in patients with severe and mild hemophilia without hepatitis C.

Table 5. Effects of age and severe hemophilia on the domains of vitality and general health of the RAND-36 (univariate regression).

	Vitality	General health
Age (per 10 years)	-1.2 (-3.5 to 1.1)	-1.7 (-4.2 to 0.7)
Severe hemophilia	-0.1 (-7.1 to 6.9)	0.6 (-6.6 to 7.8)

Values are regression coefficients (95% confidence interval). Regression coefficients for age were determined in patients with mild hemophilia and who have never been infected with HCV. Regression coefficients for severe hemophilia were determined in patients who have never been infected with HCV and with mild hemophilia as reference variable.

Discussion

In this nationwide study among 602 hemophilia patients, patients with a current HCV infection scored lower than patients who were never infected on all eight domains and the physical component summary of the RAND-36 questionnaire. However, after adjustment for joint limitations, HIV status, age, employment/educational status, and severity of hemophilia, only the scores on the domains of general health and vitality of patients with HCV infection were affected.

To appreciate our findings some limitations of this study need to be discussed. Self-reported data may be unreliable. We therefore performed a validation study to check the accuracy of reported hepatitis C status, and found that these self-reported data were reliable. This confirms previous observations that most patients with hemophilia are well informed about their disease and its complications.[14]

Several studies have shown impaired HRQoL among patients with HCV infection compared with healthy, non-institutionalized members of the general population.[7-10;21;22] The effect of having HCV infection in the non-hemophilia population has been shown to result in a lower score on HRQoL in the range from 10 to 30 points and the domains of

role physical, general health, vitality and role emotional were affected most frequently. Hemophilia itself is also associated with a lower HRQoL compared with the general population.[1;4] Increasing age, severe hemophilia, orthopedic status and HIV are reported to be predictors of a decreased HRQoL in patients with hemophilia.[1-4] The effect of hepatitis C on HRQoL in patients with hemophilia is unclear. One study reported a decreased HRQoL in hemophilia patients caused by hepatitis.[2] However, HRQoL was determined by the health utility index and hepatitis B and C were taken as one determinant in the analyses. This might be inappropriate as Foster et al. showed that hepatitis B and C have different effects on HRQoL.[8] After adjustment for confounding factors, we found that current HCV infection was associated with lower scores on the domains of general health and vitality only. These effects were relatively small but significantly stronger than the isolated effects of age and hemophilia severity. This indicates an important effect of hepatitis C on specific domains of HRQoL (vitality and general health) in patients with hemophilia

Hepatitis C may reduce HRQoL through several mechanisms. Commonly reported symptoms of fatigue and tiredness may be partly responsible for this decrease. This is supported by two studies among HCV infected intravenous drug users showing lower scores of general health and vitality in patients aware of their hepatitis C status.[23;24] It was suggested that a diagnosis of hepatitis C raised concern and fear about current and future health status, resulting in a lower HRQoL. Fear about present and future health status is expected to predominantly affect the domain of general health. Indeed, in our study in which almost all patients were aware of their hepatitis C status, the domain of general health was affected most by the presence of HCV infection.

The majority of patients with hepatitis C in this study population have not been treated.[25] However, our findings demonstrate that specific domains of HRQoL are impaired among patients with hemophilia and hepatitis C. These results support the initiation of antiviral treatment in this population, as successful treatment for hepatitis C has been proven to improve HRQoL and to be cost-effective as well.[11]

In conclusion, hemophilia patients infected with HCV had a lower HRQoL than hemophilia patients who were not infected with HCV. Current HCV infection was associated with lower scores on the domains of general health and vitality.

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4

Significant liver damage in patients with inherited bleeding disorders and chronic hepatitis C: non-invasive assessment of liver fibrosis using transient elastography

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Summary

Many patients with bleeding disorders have been infected with hepatitis C virus (HCV), mainly genotype 1. Liver biopsies are generally not performed in these patients. We assessed liver fibrosis non-invasively using liver stiffness measurement (LSM, Fibroscan®) in 121 patients with bleeding disorders and chronic hepatitis C after 34 (range 14-40) years of infection. In order to assess the validity of LSM in our hands, a separate group of 63 non-hemophilic patients infected with HCV were evaluated with both LSM and biopsy. Liver elasticity was highly correlated to histological (METAVIR score) fibrosis stage ($R = 0.73$, $p<0.001$), and positive and negative predictive value for differentiating F0/F1 from $F\geq2$ were 81% and 78%, respectively. The area under receiver-operator curve was 0.87. Based on LSM, 60% (95% C.I. 51-69) of patients with bleeding disorders and chronic hepatitis C had $F\geq2$: 25% (95% C.I. 19-33) had F2, 18% (95% C.I. 11-25) F3, and 17% (95% C.I. 11-23) F4. Prevalence of cirrhosis based on laboratory and ultrasonographic findings was only 7% (95% C.I. 4-10). Independent risk factors for increase of LSM were older age at infection, higher BMI, presence of viral coinfection, and male gender. Of all eligible patients, 25% of cases with LSM $F\geq2$ but only 2% of cases with LSM $F<2$ started antiviral therapy after LSM. In conclusion, LSM detects a considerable number of unexpected cases with severe fibrosis or cirrhosis in patients with bleeding disorders and long-standing hepatitis C, with significant impact on patient management.

Introduction

Prior to 1990, many patients with bleeding disorders were infected with hepatitis C virus (HCV) due to inadequate viral inactivation of clotting factor products.[1] Indeed, hepatitis C is an important comorbidity and major cause of death in hemophilia patients.[2;3] Hepatitis C progresses slowly but may result in end-stage liver disease in 10-20% of patients after two decades of infection.[4-6] In the vast majority of reports on the natural history of hepatitis C among patients with bleeding disorders, clinical criteria of end-stage liver disease like liver failure, hepatocellular carcinoma, and liver-related mortality have been used as endpoints.[6-8] However, the true incidence of significant liver disease may be underestimated in absence of a liver biopsy.

Currently, state-of-the-art treatment for hepatitis C with Pegylated interferon (PegIFN) and ribavirin is effective in 50% of patients with HCV genotype 1 and 4, and in 80-90% with genotype 2 and 3.[9;10] The objective of treatment is to eradicate HCV and to stop progression to severe liver disease. Although patients with bleeding disorders generally have been offered treatment, most of them have declined therapy because of expected side effects, relatively low efficacy (generally HCV genotype 1), and absence of symptoms of hepatitis C.[11] To determine whether therapy is indicated, individual assessment of fibrosis is necessary, especially in patients with genotype 1. Today, the gold standard for assessing liver damage is liver biopsy. Although some authors report that biopsies can be safely performed in patients with bleeding disorders, there is a risk of fatal bleeding and the costs of correcting clotting factor levels are high.[12] Several small studies have advocated the use of transjugular biopsies in patients with bleeding disorders.[13-15] Although this procedure appears to be safe, biopsies are often small with underestimation of fibrosis, and potentially life-threatening bleeding complications have been described.[16]

Ideally, the decision whether or not to treat patients with bleeding disorders and hepatitis C depends on the patients age, motivation, general health, HCV genotype, and histology or a reliable histology-surrogate.[17] Recently a new device based on liver stiffness measurement (LSM) (by transient elastography) has been developed to assess liver damage non-invasively: Fibroscan®.[18] LSM results correlate strongly with biopsy findings, in particular in patients with hepatitis C.[19;20]

In the present study we assess liver fibrosis and cirrhosis by Fibroscan® in a well-defined cohort of patients with bleeding disorders and long-standing hepatitis C, and determine the impact of LSM results on patient management.

Methods

In this single center study (Van Creveldkliniek) all patients with chronic hepatitis C were invited for liver stiffness measurement (LSM). Onset of infection was estimated as time of first treatment with inadequately or non-virus inactivated clotting factor products and has been shown very reliable in other studies.[4;8] All patients had positive serum HCV RNA tests on at least two occasions. Patients who were currently on treatment for hepatitis C were excluded. LSM was performed between June and September 2005. The study protocol was in accordance with the Helsinki Declaration and was approved by our institutional review board. Patients were enrolled after providing informed consent, and were informed of the results immediately after the procedure. Blood parameters and ultrasonographic examination of the liver and spleen were obtained within six months of the LSM. Alcohol use was assessed at the time of LSM and expressed as units per week during the last two years.

Transient Elastography

One physician (R.d.K., unaware of the clinical status of the patient) performed all LSMs using a Fibrocan® (Echosens, Paris, France). The Fibrocan® induces an elastic shear wave that propagates through the liver. The velocity of the shear wave is measured, assessing the elasticity of the liver. The stiffer the liver, the faster the shear wave propagates. Results are expressed in kilopascals (kPa).

Measurements were performed in the right lobe of the liver through the intercostal space. Patients were lying with their right arm in maximal abduction. Before an LSM, the liver was examined ultrasonographically for ascites, and vascular structures or abnormalities that might hamper the LSM. The measurement depth was from 25 to 65 mm. The median value of ten successful measurements was regarded as representative of the liver elasticity. Only LSM results obtained with 10 successful measurements and a success rate (defined as ratio of successful measurements over the total measurements) of at least 40% were considered for evaluation.[19]

Severity of fibrosis was defined according to Castera et al.: no or minimal fibrosis (F0-F1) < 7.1 kilopascals (kPa), moderate fibrosis (F2) 7.1 – 9.4 kPa, severe fibrosis (F3) 9.5 – 12.4 kPa, and cirrhosis (F4) ≥ 12.5 kPa.[20]

Validation of LSM results

In order to validate LSM results, a separate group of 63 patients without bleeding disorders infected with HCV were evaluated with both LSM and biopsy. An independent pathologist without knowledge of patients' LSM results evaluated liver biopsy specimens for fibrosis according to the METAVIR scoring system.[21]

The median age was 44 years (range 21-70) and all patients were HCV mono-infected. The median length of biopsy was 33 millimeter (range 13-45). According to biopsy, 4 patients had F0 stage of fibrosis, 30 F1, 15 F2, and 8 F3, and 6 F4. Liver elasticity was positively correlated to histological fibrosis stage (Spearman's correlation coefficient 0.73, $p<0.001$). The sensitivity, specificity, positive and negative predictive values for differentiating F<2 from F≥ 2 were around 80% with the area under receiver-operator curve (ROC) of 0.87 (Table 1). For differentiating F<3 from F≥ 3 the sensitivity and positive predictive value were around 70%, and the specificity and negative predictive value were about 90% with an area under ROC of 0.89.

Table 1. Diagnostic accuracy of LSM cut-off values according to METAVIR fibrosis score in a separate group of non-hemophilic patients (n=63) with chronic hepatitis C tested with both biopsy and LSM

	F≥2*	F≥3
Cut-off (kPa)	7.1	9.5
Sensitivity	72%	71%
Specificity	85%	90%
Positive predictive value	81%	67%
Negative predictive value	78%	92%
Area under ROC	0.87	0.89

* F≥ 2 means F0-1 vs F2-4.
Abbreviations: LSM, liver stiffness measurement; ROC, receiver-operating curve.

Ultrasound examination and laboratory tests

We defined clinical cirrhosis as presence of at least 1 major criterion or 3 minor criteria. Major criteria were: obvious cirrhosis on ultrasound recorded by radiologist, and esophageal varices. Minor criteria were: low albumin (<35g/L), low platelet count ($<150*10^9$/L), and splenomegaly, hepatofugal flow, or dilated portal vein on ultrasound.

Data analysis

Data were skewed and therefore expressed as medians (range), or proportions. Correlation between LSM and biopsy was expressed as Spearman's correlation coefficient. A p-value <0.05 was considered statistically significant.

In the separate group of non-hemophilic patients, diagnostic accuracy of LSM for F≥2 (i.e. F0-1 vs F2-4), and F≥3 (i.e. F0-2 vs F3-4) was expressed in sensitivity, specificity, positive predictive value, negative predictive value, and area under the ROC.

In order to identify risk factors for high Fibroscan® scores, univariate and multivariate linear regression analyses were performed. Coefficients are expressed with 95% confidence intervals (C.I.). First, determinants were examined in univariate analysis. All determinants yielding coefficients significant at the 0.2 level, were included in the subsequent multivariate analysis. In the multivariate analysis we used the stepwise regression procedure.

Results

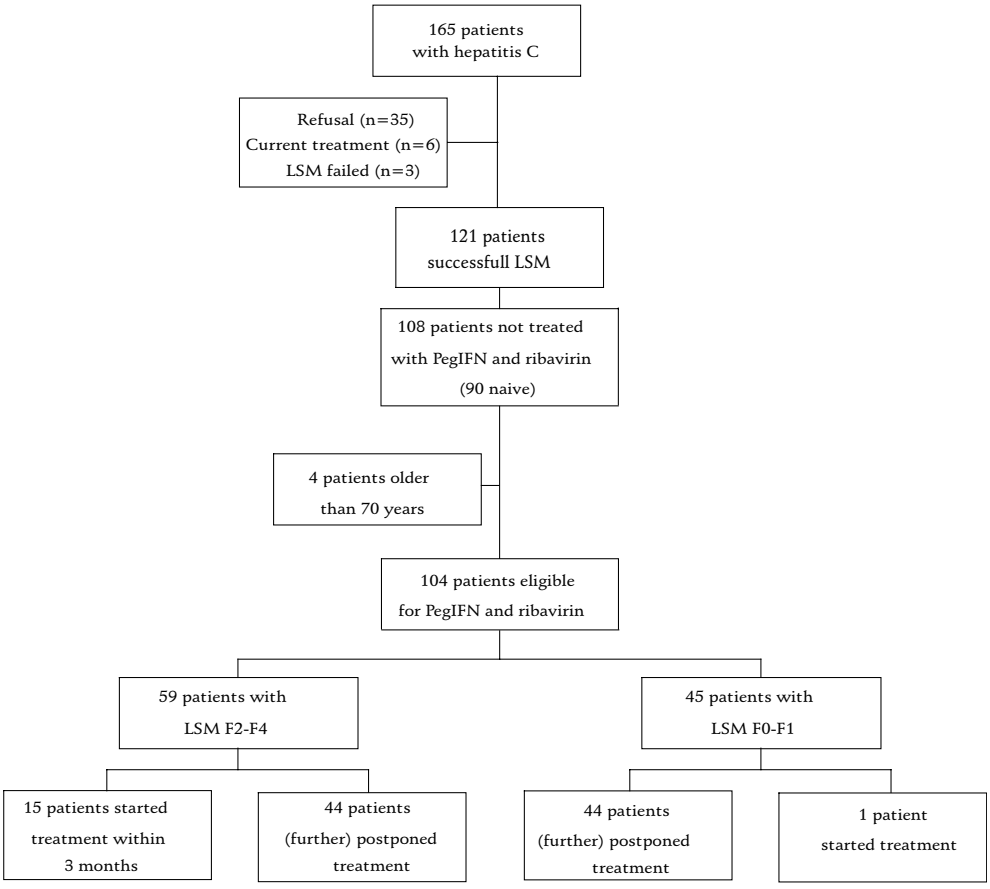

Figure 1. Flowchart of patient selection and clinical consequences of LSM testing. Abbreviations: LSM, liver stiffness measurement; IFN, interferon.

Patients

124 out of 165 patients with chronic hepatitis C and congenital bleeding disorders were enrolled (Figure 1). Six patients currently treated for HCV were excluded. Age, duration of infection, body mass index (BMI), and laboratory and ultrasonographic results were similar in participants and non-participants (data not shown).

Table 2. **Patient characteristics**

Number	121
Type of bleeding disorder	
• Hemophilia	92%
• Von Willebrand's disease	3%
• Other	5%
Age (years)	42 (16-86)
Male	95%
BMI (kg/m^2)	24 (18-41)
Alcohol use (units/week)	1 (0-80)
Age at infection (years)	6 (<1-47)
Duration of infection (years)	34 (14-40)
HCV genotype	
• 1	87%
• 2	3%
• 3	6%
• 4	4%
Coinfection	
• HIV	9%
• HBV	2%
ALT (normal 10-50)	57 (21-195)
AST (15-45)	47 (22-153)
γ-GT (15-45)	52 (16-1257)
α-fetoprotein (<15 μg/L)	4 (1-53)
Albumin (g/L) (34-50)	42 (27-47)
Platelet count (10^9/L) (150-450)	222 (35-590)

Values are medians (range) or proportions
Abbreviations: BMI, body mass index; HBV, hepatitis B virus

In 3 of 124 patients enrolled, LSM could not be obtained because of obesity. Eventually, 121 patients with chronic hepatitis C were successfully examined by LSM. Patient characteristics are summarized in Table 2. The median age of the patients was 42 years (range 16 - 86) and the large majority had HCV genotype 1. HIV coinfection was present in 11 and hepatitis B virus (HBV) coinfection in 3 patients. Thirty-one of 121 (26%) HCV RNA positive patients had a history of unsuccessful antiviral treatment (6 IFN monotherapy, 12 IFN and ribavirin, 13 PegIFN and ribavirin).

Liver stiffness measurement

The LSM was well tolerated, took about 10 minutes and had no complications. Overall, LSM results ranged from 3.4 to 45.0 kPa (median 7.7). 48 patients (40%, 95% C.I. 33-47) with chronic hepatitis C showed no or minimal fibrosis (F0-F1). Moderate fibrosis (F2) was present in 31 (25%, 95% C.I. 19-33), severe fibrosis (F3) in 22 (18%, 95% C.I. 11-25), and cirrhosis (F4) in 20 (17%, 95% C.I. 11-23) patients.

Determinants associated with increase of Fibroscan® score

Determinants associated with increase in Fibroscan® score at the 0.2 level in the univariate analysis were: increased BMI, older age, older age at infection, longer duration of infection, presence of viral co-infection, and male gender (Table 3). Multivariate analysis identified older age at infection, higher BMI, male gender, and coinfection with HIV or HBV as independent risk factors for increase in Fibroscan® result, with age at infection as the strongest risk factor. Duration of infection appeared to be associated with higher LSM results, however this was not statistically significant in the multivariate analysis (regression coefficient per 10 years was 1.2, p=0.18).

Laboratory and ultrasonographic results in patients with cirrhosis

In total, 20 patients were diagnosed by LSM as having cirrhosis. Of these twenty patients, 7 (35%) would also have been clinically diagnosed with cirrhosis (i.e. diagnosis based on the presence of 1 major or three minor criteria: see methods). Of the remaining 13 patients with cirrhosis based on LSM, 3 patients had only low platelet counts, and 4 patients had only splenomegaly. Laboratory tests and ultrasound of the remaining 6 patients revealed no signs of cirrhosis. One patient with a LSM diagnosis of severe fibrosis (9.6 kPa, F3) had clinically evident cirrhosis (bleeding esophageal varices).

Clinical consequences

Of the 121 patients with chronic hepatitis C, 108 patients had not been treated with the current state-of-the-art therapy PegIFN and ribavirin (Figure 1). Of these 108, four patients were older than 70 years (relative contra-indication for treatment). Of 104

remaining candidates for treatment with PegIFN and ribavirin, 59 had an LSM result of F≥ 2, and 25% of these (15 of 59) started antiviral therapy within 3 months of LSM. On the contrary, 44 of 45 (98%) patients not (optimally) treated but with a LSM result F<2, decided to further postpone treatment.

For the 45 patients with LSM F< 2, the distribution between naïve, previous IFN monotherapy, and previous IFN and ribavirin therapy was 90%, 5%, and 5% respectively. Among the 59 patients with LSM F≥ 2, 78% was treatment naïve, 7% has been treated with IFN monotherapy, and 15% with IFN and ribavirin (p=NS for distribution F<2 vs F≥ 2).

Table 3a. **Univariate regression analysis of determinants associated with increase in Fibroscan® score**

Determinant	coefficient	95% C.I.	p-value
BMI (kg/m^2, per 10 units)	4.5	0.5 to 8.5	0.03
Age (per 10 years)	1.7	0.9 to 2.5	<0.01
Age at infection (per 10 years)	2.0	0.8 to 3.1	<0.01
Duration of infection (per 10 years)	2.7	1.0 to 4.3	<0.01
Alcohol use (per 10 units per week)	-0.6	-1.6 to 0.4	0.23
Gender (0=female, 1=male)	4.4	-1.2 to 9.9	0.12
Co-infection HIV or HBV (0=no, 1=yes)	2.2	-1.5 to 6.0	0.20
Genotype 1 (0=no, 1=yes)	0.8	-2.9 to 4.5	0.66
HCV load (per 1*10^7 IU/mL)	-0.2	-1.0 to 0.7	0.65

Abbreviations: BMI, body mass index; IFN, interferon

Table 3b. Multivariate regression analysis of determinants associated with increase in Fibroscan® score

Determinant	coefficient	95% C.I.	p-value
BMI (kg/m^2, per 10 units)	3.9	0.2 to 7.6	0.04
Age at infection (per 10 years)	2.8	1.6 to 4.0	<0.01
Gender (0=female, 1=male)	5.8	0.2 to 11.5	0.04
Coinfection HIV or HBV (0=no, 1=yes)	3.9	0.3 to 7.5	0.04

Interpretation: per increase of 10 years of age at infection, the Fibroscan® score increased with 2.8 points, adjusted for BMI, gender, and coinfection

Discussion

We assessed the prevalence of fibrosis and cirrhosis by LSM in 121 patients with bleeding disorders and infected with HCV for 34 years. In most studies on progression of fibrosis, exact date of hepatitis C is not available. In the present study, we were able to accurately estimate the date of infection based on first exposure to unsafe clotting product. Overall, 60% of patients with chronic hepatitis C had a Fibroscan® result corresponding to F≥ 2, which is a compelling reason to start with antiviral treatment. Severe fibrosis (F3) or cirrhosis (F4) were present in 35% of cases.

Until now, diagnosis of severe fibrosis or cirrhosis in hepatitis C infected patients with bleeding disorders is mostly based on clinical signs, laboratory tests, and ultrasonographic findings. Liver biopsies (percutaneous or transjugular) are very rarely performed in these patients because of risk of bleeding complications and high costs of replacement therapy. Recently, a new device (Fibroscan®) has been developed that measures stiffness of the liver, showing high correlation between liver stiffness and stage of fibrosis in biopsies, as well as excellent receiver operating curves.[18-20] LSM is well tolerated because it is painless, rapid, and has no complications. Furthermore, LSM determines liver stiffness of a volume that is 100 times larger than a biopsy, and is therefore more representative of the entire liver. In our hands LSM findings correlated strongly with biopsy findings, and the diagnostic accuracy was high in a separate group of non-hemophilic patients with chronic hepatitis C, in line with previous studies.[19;20]

Few studies in patients with bleeding disorders and chronic hepatitis C have reported the use of biopsies to assess occurrence of cirrhosis. One study from Sheffield, reports a prevalence of 29% of biopsy-proven cirrhosis after 20 years of infection.[22] We found a similar prevalence with LSM although our patients have been infected for a longer period (i.e. more than 30 years). This difference may be caused by different proportions of HIV coinfection (9% in our study, compared to 41% in the Sheffield study). Other studies determining the natural history of HCV infection among patients with bleeding disorders used end-points like cirrhosis, liver failure, hepatocellular carcinoma, and liver-related mortality. Reported incidences of end stage liver disease vary from 8 to 16% after 12 to 25 years of infection.[4;5;7;8] In some of these studies cirrhosis was defined on the basis of laboratory tests, ultrasonographic examination, and clinical signs.[4] Using the same criteria, we could only diagnose cirrhosis in 7% of the study population compared to 35% of patients with the aid of Fibroscan®. Based on these findings, LSM appears to be a very good alternative to clinical criteria and even biopsy in patients with bleeding disorders. Our suggestion of significant under-diagnosis of cirrhosis is in line with a Canadian study in which patients with congenital bleeding disorders and hepatitis C were evaluated for

liver damage by transjugular biopsies and measurements of hepatic venous pressure gradient.[23] The diagnosis of cirrhosis and/or portal hypertension was made in a substantial proportion of individuals (26%), all without hepatic decompensation. Only a minority of cirrhotic patients in that study had ultrasonographic abnormalities or low platelet counts.

In the non-hemophilic population, progression of fibrosis is associated with age at infection, duration of infection, gender, (excess) alcohol intake, viral coinfection, and obesity.[24-26] Studies in patients with congenital bleeding disorders have confirmed these findings.[2;4;6;7;22] In the present study, we also confirmed the association of most of these factors with LSM results, thus adding to the reliability of LSM in patients with chronic hepatitis C. A high consumption of alcohol is a well-known risk factor for progression to severe liver disease.[27] However, in the present study we could not confirm this association. This may be caused by the low frequency of excess alcohol intake in our cohort. In a recent study among non-hemophilic patients with hepatitis C, low or moderate intake was not associated with fibrosis.[28] Only a few heavy drinkers (more than 20 units per week) were present in our study, but they were all younger than 40 years, were not co-infected with HIV, and had a normal BMI. These favorable prognostic factors might have compensated for their excessive alcohol intake.

Although treatment success for hepatitis C has been improved last years, the majority of patients with bleeding disorders have refused therapy.[11] Most patients fear side effects or expect a low efficacy. Others have no clinical symptoms of hepatitis C and therefore fail to see the need for treatment. LSM may be helpful in making treatment decisions by assessing the progression of fibrosis and help to determine the ideal time point for initiating antiviral treatment. On the other hand, antiviral therapy may be postponed in patients who have been infected for decades and who have only mild fibrosis according to LSM, especially in patients with genotype 1. In the present study, 25% of patients never treated with PegIFN and ribavirin, and a LSM result of F≥ 2, started treatment shortly after LSM. In contrast, 44 of 45 patients with low fibrosis score by LSM (F0-1) further postponed therapy.

In conclusion, the prevalence of severe fibrosis and cirrhosis according to LSM is high in patients with bleeding disorders with long-standing hepatitis C. These findings support the need for increased non-invasive screening in patients, which may have significant impact on the management of the disease.

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5

The natural history of childhood acquired hepatitis C infection in patients with inherited bleeding disorders

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Summary

Although many patients with inherited bleeding disorders have been infected with hepatitis C in early childhood, natural history of infection in this patient group remains poorly defined.

212 patients with inherited bleeding disorders born between 1976 and 1992 were evaluated for hepatitis C virus (HCV) infection, spontaneous clearance, and (by non-invasive tests) progressive liver disease.

120 of 212 patients had been exposed to non-HCV inactivated clotting products and 68 of these 120 patients (57%) were anti-HCV positive. Of these patients, 44 (65%) had chronic hepatitis C (HCV RNA positive) and 24 (35%) showed spontaneous clearance (HCV RNA negative). Five patients with hepatitis C were coinfected with hepatitis B virus and/or HIV. Multivariate analysis indicated that hepatitis C infection was independently associated with longer treatment period (odds ratio [O.R.] 1.6, 95% confidence interval [C.I.] 1.3 – 1.9) and exposure to larger number of donors (O.R. 2.1, 95% C.I. 1.1 – 3.9). Spontaneous HCV clearance was associated with a younger age at first exposure to clotting product ($p=0.02$). After a mean infection period of 21 years, evidence of cirrhosis was present in two patients (5%), both of whom were coinfected with HIV.

Spontaneous HCV clearance is associated with young age at infection. Despite frequent childhood acquired hepatitis C infection among patients with inherited bleeding disorders, progression to cirrhosis after 21 years of infection is rare. However, the diagnosis of cirrhosis without biopsy remains challenging in this population and new, non-invasive means have to be developed to accurately identify cirrhotic patients.

Introduction

Prior to the introduction of effective methods to eliminate the hepatitis C virus (HCV) from blood and blood products (viral inactivation since the mid-1980s, and donor screening for HCV since the early 1990s), many patients with inherited bleeding disorders were infected with HCV.[1;2] Males with hemophilia, in particular, were generally infected at a young age.[3;4]

The natural course of HCV infection in these patients is still uncertain. Hepatitis C appears to progress slowly in adult hemophilia patients. Only a minority develop end-stage liver disease, unless coinfected with human immunodeficiency virus (HIV) and/or hepatitis B virus (HBV).[5-7]

In pediatric patients without bleeding disorders, the natural history of hepatitis C appears to be more benign than in adults.[8-12] However, some authors report that a significant proportion of patients infected in childhood progress to severe liver disease.[13;14] Whether comorbidities or mode of viral transmission play a role in this progression is not clear. One study with 7 years of follow-up reported less liver injury in children with hemophilia compared with children infected by blood transfusion or maternal-fetal transmission.[15] It was suggested that abnormal T-cell immunity in children with hemophilia might result in high viral load but minimal liver injury.

In order to determine the natural history of HCV infection, the onset of infection must be identified, and information on its full course and its potential modifiers must be obtained.[16] These criteria may be met in a cohort of patients with inherited bleeding disorders. At our institution, data on exposures to clotting products are recorded and patients are regularly tested for hematological and chemical parameters, as well as viral infections. Furthermore, patients with inherited bleeding disorders are seen on a yearly basis for their bleeding problems, resulting in a reliable follow-up independent of HCV status or liver disease. Finally, they form a homogeneous group with the same route of infection and are almost all of the same gender.

The primary objective of this cohort study was to assess the natural history of childhood acquired HCV infection in patients with inherited bleeding disorders. The secondary objective was to explore transfusion-associated risk factors for HCV infection

Methods

Patients

We studied all 212 patients with inherited bleeding disorders, born between 1976 and 1992, who were registered at the Van Creveldkliniek. This hemophilia treatment center serves approximately 50% of all hemophilia patients in the Netherlands. From 1976 onwards, full data on the number of exposures and type of product were recorded. In the Netherlands, patients were preferentially treated with clotting products produced from Dutch unpaid donors. This could be either small pool cryoprecipitate or large pool concentrates. Since 1992, no new HCV infections have occurred in our patients due to effective methods of eliminating HCV during preparation of clotting products and introduction of donor screening.

Patients were eligible for the study if they were treated at least once with any product that was not hepatitis C virucidally treated before 1992 ('HCV-unsafe' product) during childhood. The follow-up started at the first visit to our clinic and ended either at the last check-up before February 2005, the start of anti-HCV treatment, or death. Patients visited our clinic at least once a year. We collected basic demographic information, diagnosis and type of bleeding disorder, and date of first exposure to HCV-unsafe clotting product. Severity of hemophilia was defined as: severe < 1.0%, moderate 1-5%, or mild 5-40% of normal clotting activity of factor VIII or IX.

The authors received permission from all patients to collect data anonymously. Informed consent was provided according to the declaration of Helsinki.

HCV-assays and definitions of liver disease

In the early 1990s, routine HCV serology was performed in all patients. In patients without HCV-antibodies, HCV serology was repeated yearly. If patients were anti-HCV positive, they were tested for HCV RNA once per two years.

To assess the date of HCV seroconversion, serum samples stored in the period 1976-1992 were tested retrospectively for HCV antibodies. Samples were stored at -30^0 C. The seroconversion date was assumed to be in the middle of the period of the last negative test and the first positive test, with a maximum of two years between test dates. Data on the seroconversion date according to these stringent criteria and on the number of exposures to clotting products were available in 34 of 68 anti-HCV positive patients.

Samples were tested by the Microparticle Enzyme Immunoassay (MEIA) AxSYM HCV version 3.0 (Abbott, Wiesbaden, Germany) and by the INNO–line immunoassay (LIA) HCV AB III Update confirmation assay (Innogenetics, Gent, Belgium) for the presence of antibodies to HCV. Qualitative HCV RNA detection was performed by using the COBAS AMPLICOR HCV Test, version 2.0 (Roche Diagnostics, Branchburg, NJ, USA)

with a sensitivity of 50-60 IU/mL (i.e. 135-160 copies/mL). For genotypic analysis, the reverse hybridization line probe assay (INNO–LiPA HCVII; Innogenetics) was used, which assesses type-specific sequence variation in the 5' UTR. HCV genotype was available for 41 of 44 patients (93%) with chronic hepatitis C.

HCV infection state was defined as: never infected with HCV (no anti-HCV antibodies), cleared HCV infection (positive anti-HCV antibodies but negative HCV RNA test on at least two occasions), or chronic hepatitis C (positive anti-HCV antibodies and persistently positive HCV RNA tests).

We defined clinical cirrhosis as presence of at least 1 major criterion or 3 minor criteria, excluding non-hepatic causes. Major criteria were: obvious cirrhosis on ultrasound recorded by radiologist, and esophageal varices (≥grade 2). Minor criteria were: low albumin (<35g/L), low platelet count ($<150*10^9$/L), and splenomegaly, hepatofugal flow, or dilated portal vein on ultrasound.

Clotting factor product use and number of donors

In the 1980-1990s, the prevalence of antibodies to HCV was 0.1% in Dutch blood donors.[17] To estimate the number of donors to which a patient was exposed, we collected data until the last exposure to HCV-unsafe clotting factor product, at the latest January 1992. 120 patients were exposed to 'HCV-unsafe' clotting factor product and for 115 of these patients (96%), the date of first exposure was recorded. Among 100 of the 120 patients (83%) the record of their clotting product use was complete. The remaining 20 patients were initially treated elsewhere.

Depending on the product used, the number of 'theoretical' donors per vial to which a patient was exposed was 300 to more than 5500 for large pool products and 2 to 32 for small pool products. The theoretical number of donors was known for each product used during follow-up. For each patient, the cumulative number of theoretical donors he was exposed to was calculated. For example, if a patient had four exposures to a large pool product produced from 1100 donors, this resulted in a cumulative number of theoretical donors of 4400.

In order to estimate the intensity of factor replacement therapy we introduced two variables in the regression analyses: dose and frequency. Dose was defined as number of donors per exposure, and frequency as number of donors per year.

In the Netherlands, dry heat treatment (72 hours, 68^0C) of clotting products was introduced in 1985 to eliminate the risk of HIV infection with the ancillary effect that HCV load was decreased.[18] As a result, risk of HCV infection may be lower for patients exclusively treated after 1985. To study the effect of dry heat treatment on HCV infection, we compared patients treated before 1985 versus those exclusively treated after 1985.

Laboratory assays and ultrasound examination

Mean values of the last three available results of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma glutamyl transpeptidase (γ-GT) were taken. Each result was separated by a minimum interval of six-months. The normal range for ALT was 10-50 U/L, 15-45 U/L for AST, and 15-45 for γ-GT. In addition, alkaline phosphatase, albumin, α-fetoprotein, factor V, prothrombin time, antithrombin III and platelet count at the end of follow up were recorded.

To identify progression of liver disease (i.e. presence of splenomegaly, ascites, cirrhosis), Doppler ultrasound examinations of liver, spleen and flow of portal vein were performed every two years. All patients with hepatitis C were at least 16 years old at their last ultrasound examination, except for two patients. Hepatomegaly was defined as the diameter of the right liver lobe (measured at the midclavicular line) being equal to or larger than 16 cm.[19] Splenomegaly was defined as the length being equal to or larger than 14 cm, or length, width and thickness equal to or larger than 12, 8 and 5 cm, respectively.[20;21]

Data analysis

Data were expressed as mean (standard deviation, sd) if normally distributed, as median (range) if skewed, or proportions. Normally distributed data were compared using the T-test. Comparisons of skewed data were made using the Mann-Whitney U test or Kruskal-Wallis test. Proportions were compared using the χ^2-test. A p-value <0.05 was considered statistically significant.

Uni- and multivariate logistic regression analyses were used to identify risk factors for HCV infection. Coefficients are expressed in odds ratios (O.R.) and 95% confidence intervals (C.I.). First, determinants were examined in univariate analysis. All determinants yielding O.R significant at the 0.10 level, were included in the subsequent multivariate analysis. In the multivariate analysis we used the forward stepwise regression procedure.

Results

Characteristics of study population

Of 212 patients with inherited bleeding disorders born between 1976 and 1992, 120 patients proved to be at risk for HCV infection based on exposure to HCV-unsafe clotting product (Figure 1). Of those at risk, 113 patients suffered from hemophilia, six from Von Willebrand's disease and one from factor VII-deficiency. All were male except for two patients with Von Willebrand's disease. The mean age at first HCV-unsafe exposure was 1.8 years (sd 1.7). Age at the end of follow-up ranged from 11 to 28 (mean 21 years, sd 4) and mean follow-up time was 15 years (sd 7).

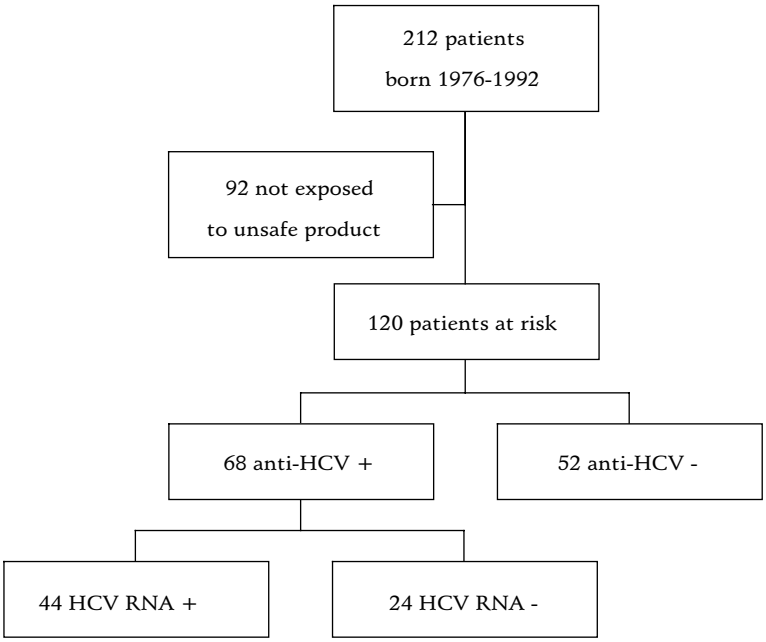

Figure 1. **Flowchart of selection of study population**

At the end of follow-up, two patients had died: one patient with chronic hepatitis C because of pediatric AIDS and the other, who had not been infected, due to a traffic accident. Follow-up was prematurely ended in 13 patients who started antiviral therapy and in five patients who moved to another area and were lost to follow-up.

Among the 120 patients at risk, 68 (57%) were HCV seropositive at the end of follow-up. Of these 68 patients, 44 (65%) were persistently HCV RNA positive (indicating chronic hepatitis C) and 24 patients (35%) HCV RNA negative (indicating spontaneous HCV clearance). All 68 patients ever infected with HCV, remained anti-HCV positive after their first positive test. Fifty-two patients had no evidence of HCV infection.

Three patients with chronic hepatitis C were coinfected with HIV. One patient was coinfected with HBV and another patient was infected with both HIV and HBV. Patient characteristics according to HCV status are given in Table 1.

Clotting products and HCV infection

Compared to those with chronic hepatitis C, patients never infected with HCV more often were those with mild hemophilia and fewer exposure days, were exposed to a lower cumulative number of donors, and were more frequently treated exclusively with small pool products ($p<0.001$, Table 1).

Table 1. **Patient characteristics according to HCV status (n=120)**

	Never infected with HCV	HCV cleared	Chronic hepatitis C
Number of patients	52	24	44
Age at end of follow-up (years)	19.7 (3.5)	21.4 (3.8)	22.5 (3.9)
Hemophilia	94%	92%	96%
• (severe)	(43%)	(82%)	(88%)
Von Willebrand's disease	6%	8%	2%
Other	-	-	2%
Coinfection			
• HBV	0	1	1
• HIV	0	1	4†
Age (years) at first exposure	2.3 (1.9)	1.0 (0.5)	1.7 (1.6)
Total exposure days‡	17 (1-1197)	608 (5-1391)	690 (2-1958)
Cumulative number of donors‡	100	$3*10^4$	$3*10^5$
	(2- $4*10^5$)	(47- $4*10^6$)	(64- $6*10^6$)
Only small pool product	76%	35%	22%

Numbers are means (standard deviation) or proportions unless otherwise stated.
† one patient with chronic hepatitis C was coinfected with HBV and HIV.
‡ median (range)

In Table 2, uni- and multivariate regression analyses for factors associated with HCV infection are given. In the univariate analysis, factors associated with HCV infection were longer treatment period (O.R. 1.6, 95% C.I. 1.3 – 1.9), larger cumulative number of donors (O.R. 2.3, 95% C.I. 1.2 – 4.5) and higher dose (i.e. number of donors/total exposures; O.R. 2.0, 95% C.I. 1.3 – 3.3). Furthermore, patients who had been exposed to clotting factor product before 1985 had a higher risk for HCV infection than patients exclusively treated after 1985 (O.R. 7.7, 95% C.I. 3.3 – 17.9). However, multivariate regression analysis revealed that the variables 'longer treatment period' (O.R. 1.6, 95% C.I. 1.3 – 1.9) and higher 'cumulative number of donors' (O.R. 2.1, 95% C.I. 1.1 – 3.9) were independently associated with risk of HCV infection. Thus, after adjustment for 'treatment period', the O.R. for HCV infection increased by a factor of 2.1 for each additional 100,000 donors.

Table 2. Uni- and multivariate logistic regression of risk factors for HCV infection

	Univariate logistic regression		
	Odds-ratio	95% C.I.	p-value
1st exposure before 1985	7.7	3.3 – 17.9	<0.01
Treatment period (years) *	1.6	1.3 – 1.9	<0.01
Cumulative nr. of donors (per 100,000) †	2.3	1.2 – 4.5	0.01
Dose (per 1,000) ‡	2.0	1.3 – 3.3	<0.01
Frequency (per 100,000) §	1.2	0.9 – 1.5	0.25
	Multivariate logistic regression		
Treatment period (per year)	1.6	1.3 – 1.9	<0.01
Cumulative nr. of donors (per 100,000)	2.1	1.1 – 3.9	0.03

Interpretation: per added unit of exposure to 100.000 donors, patients had a 2.1 times higher risk for HCV infection, adjusted for treatment period.
* period of time during which HCV-unsafe products had been administered
† total number of theoretical donors to which a patient could have been exposed
‡ total number of theoretical donors/total number of exposures
§ number of theoretical donors/ exposure time

Seroconversion and number of exposures

Precise data on both the seroconversion date (according to our stringent criteria) and the number of exposures to clotting products (before seroconversion) were available in 34 of 68 anti-HCV positive patients.

Patient characteristics, data on exposures, and hepatitis C status were similar in patients with and without complete exposure and seroconversion data.

In this subgroup of 34 patients, the median number of exposures prior to seroconversion was 28 (range 1 – 294), and the median cumulative number of donors prior to seroconversion was 1525 (range 8 – $7.2*10^5$). This means that one or two exposures to large pool products could have resulted in HCV infection. The median time between date of first exposure and date of seroconversion was 1.7 years (range 0.1 – 7.5). Figure 2 shows that the proportion of patients infected with HCV correlated strongly with the cumulative number of donors. Fifty percent of patients were infected after being exposed to at least 1500 donors and 90% after exposure to 50000 donors.

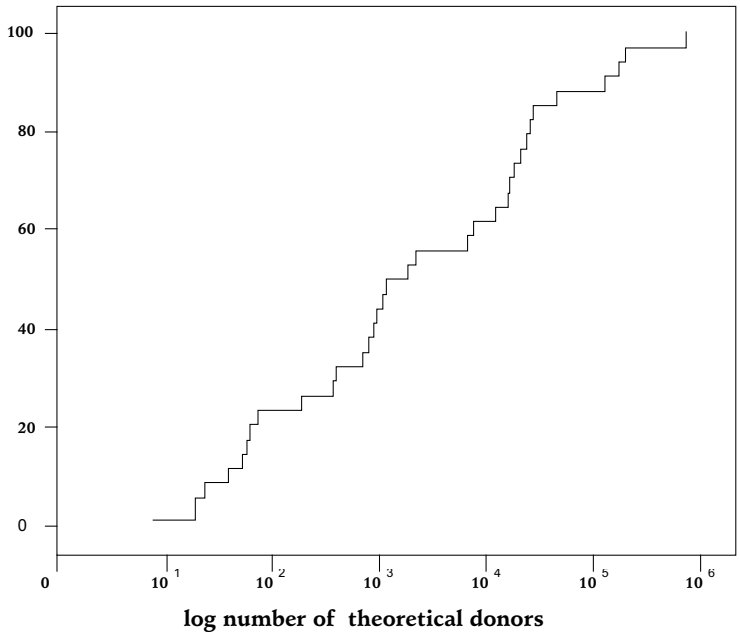

Figure 2. **Cumulative percentage of patients infected with HCV according to the total number of theoretical donors. (n=34)**

Spontaneous clearance of HCV

Twenty-four of 68 (35%) HCV seropositive patients cleared HCV spontaneously. Patients who had cleared HCV spontaneously had been exposed to clotting factor product at a significantly younger age than patients with chronic hepatitis C (mean 1.0 vs. 1.7 years, p= 0.02). The total number of exposure days, theoretical number of donors, and proportion of patients exclusively treated with cryoprecipitate were similar in patients with spontaneous clearance and chronic hepatitis C.

Clinical outcome of chronic hepatitis C

Chronic hepatitis C was present in 44 patients and the mean duration of infection was estimated at 21 years (sd 4). The majority of patients had HCV genotype 1 (63%). The prevalence of the other genotypes was: type 2 (12%), type 3 (23%), and type 4 (2%).

Among these chronically infected patients, 25% had elevated aminotransferases, 9% elevated γ-GT levels and 23% had both. 43% had neither elevated aminotransferases nor elevated γ-GT levels. The mean results of alkaline phosphatase, albumin, alpha-fetoprotein, factor V and AT-3 levels, prothrombin time, and platelet counts were similar in patients with chronic hepatitis C and in those who were not infected.

In total, 5 patients with chronic hepatitis C had been coinfected with HIV and/ or HBV. Two of five coinfected patients had overt ultrasonographic evidence of cirrhosis. One of these patients had been infected with HCV for 25 years and coinfected with HIV and HBV. The other patient had hepatitis C for 13 years, was coinfected with HIV and died of infectious complications of pediatric AIDS at the age of 14 years. Both patients also had low platelet counts, low albumin levels, and splenomegaly. No evidence of cirrhosis was found in the 39 HCV mono-infected patients.

Of the remaining 42 patients without cirrhosis, none had low serum albumin (<35 g/L) and three patients (7%) had low platelets counts ($<150*10^9$/L) after a mean duration of infection of 19 years (sd 5). Abnormalities on ultrasound were found in 20 of 42 (48%) non-cirrhotic patients; 5 patients had an enlarged liver, 8 had splenomegaly, and 7 patients had both hepato- and splenomegaly.

Discussion

Hepatitis C is a major comorbidity and a significant cause of death in patients with hemophilia.[22] Since the early 1980s, physicians involved in hemophilia care have reported the transmission of non-A non-B hepatitis after transfusion of clotting factor concentrate.[1;2] Almost the total population treated with clotting factor concentrates was infected with HCV until the early 1990s, when stringent virucidal treatments were developed and screening of donors was implemented.[3;4] The majority of our patients (65%) exposed to HCV-unsafe clotting product at a young age proved to be infected. As expected, HCV infection was associated with large cumulative numbers of theoretical donors and longer treatment periods. Once infected, 35% of the patients cleared HCV spontaneously, particularly those who had their first exposure at an early age. After a mean period of 21 years' infection, a proportion (5%) of patients with chronic hepatitis C and coinfected with HIV had progressed to cirrhosis.

It has been previously reported that spontaneous clearance of HCV generally occurs within one year of infection, and is associated with viral load, genotype, alcohol use, viral co-infections, gender, and cell mediated immunity.[23-26] In contrast to viral clearance rates of 10 to 20% in adults, spontaneous HCV clearance appears to be 10 to 45% in children.[5;7;8;12;27-29]

In this study we report about the association of spontaneous clearance and early age at infection, which has been suggested previously in hemophilia patients.[7] The present study corroborates the findings of Messick et al. showing that patients with spontaneous clearance were exposed to clotting product at a significant younger age than patients with chronic hepatitis C.[26] Differences in the immune system of children may in part explain

this finding. Young children may have a better immune surveillance and a better control of virus replication than adults. Nevertheless, there has been no definite explanation as to why patients infected at a younger age clear HCV better than patients infected later in life, and future studies are needed to elucidate this observation.

The majority of data regarding the natural history of hepatitis C infection in children report a mild clinical course.[8;12;30;31] However, some studies have reported a progression rate similar to that reported in adults.[13;14] Factors that seem to be associated with the development and progression of liver disease are viral co-infections, alcohol use, older age at infection, mode of infection, and comorbidities. In one study among childhood cancer survivors, liver disease progression was considerable, and it was hypothesized that either immunosuppressive or hepatotoxic effects of chemotherapy could have accelerated the progression of liver disease.[14] However, others reported a benign clinical course in patients cured of childhood cancer (leukemia), suggesting that acquisition of the infection during intrinsic and extrinsic immunosuppression could have inhibited antiviral immune response.[11] This immunosuppression could also explain the mild course of liver disease, assuming that host immune response plays a major role in liver pathology. In our study, 5% of patients with chronic hepatitis C (coinfected with HIV) developed cirrhosis. Furthermore, 48% of our patients with chronic hepatitis C exhibited persistently elevated transaminases and 50% had minor ultrasound abnormalities (enlarged spleen and/or liver) after two decades of infection.

There are potential limitations of our study. First, some patients may have been misclassified as 'never infected', defined as persistent absence of HCV-antibodies. There may be a small risk of loss of antibodies. Previous studies showed that a small part of anti-HCV negative patients had positive earlier (stored) samples.[26;32;33] Nevertheless, we did not observe loss of antibodies in any of anti-HCV positive patients.

Secondly, since liver biopsies were not performed in our patients we diagnosed cirrhosis according to radiological, biochemical, and physical findings. We diagnosed two patients as cirrhotic: both had a rough, granular texture and irregular margins of the liver and splenomegaly on ultra-sound, low platelets and low albumin. However, liver biopsy remains the gold standard for diagnosing cirrhosis and therefore we may have underestimated the proportion of cirrhosis in the remaining patients with chronic hepatitis C. This hypothesis is supported by data from Shin et al., who evaluated patients with congenital bleeding disorders and hepatitis C for liver damage with transjugular biopsies.[34] The diagnosis of cirrhosis and/or portal hypertension was made in a substantial proportion of individuals (26%), all of whom had asymptomatic liver disease.

In hepatitis C patients without bleeding disorders, new non-invasive tests such as the fibrotest and transient elastography were found promising to assess liver fibrosis and cirrhosis.[35-39] Considering the risk of bleeding complications and costs of liver biopsies in

these patients, these tests may be especially useful in patients with bleeding disorders.

In conclusion, our data show high hepatitis C infection rates in patients with bleeding disorders who had been exposed to HCV-unsafe clotting products at a young age. HCV clearance was relatively high and associated with young age at first exposure. Progression to cirrhosis was rare, at least after 21 years of infection. Further data on long-term follow-up are needed to fully assess the sequellae of chronic hepatitis C in patients with bleeding disorders.

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6

Progression to end-stage liver disease in patients with inherited bleeding disorders and hepatitis C: an international, multicenter cohort-study.

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Summary

Prior to 1990, many patients with inherited bleeding disorders were infected with hepatitis C virus (HCV), but the long-term effects of this transfusion-associated infection remain uncertain. The aim of this study was to assess the risk of end-stage liver disease (ESLD) in hemophilia patients with chronic hepatitis C. In a multicenter, international cohort study, all 847 anti-HCV positive patients with inherited bleeding disorders in three centers in the UK and the Netherlands were followed for development of ESLD. Patients were infected between 1961 and 1990, and were followed up to August 2005. Of 847 patients, 160 (19%) spontaneously cleared HCV and 687 (81%) developed chronic hepatitis C. Coinfection with HIV was present in 210 patients. After 35 years of infection the cumulative incidence of ESLD was 11.5% (95% C.I. 8.2-14.8%) in HIV negative patients and 35.1% (95% C.I. 29.2-41.0, $p<0.001$) in HIV co-infected patients. After 20 years of infection, the incidence rate of ESLD for HIV negative patients with chronic hepatitis C was 0.53 per 100 person years, compared to 2.63 for HIV co-infected patients. Independent risk factors of ESLD were: HIV co-infection (hazard ratio 13.8 [95% C.I. 7.5-25.3], older age at infection (hazard ratio 2.3, per 10 years [95% C.I. 2.0-2.8]), alcohol abuse (hazard ratio 4.9 [95% C.I. 2.5-9.6]), and presence of genotype 1 (hazard ratio 2.2 [95% C.I. 1.1-4.2]).

With longer duration of HCV infection, the risk of developing ESLD is emerging in patients with inherited bleeding disorders. Risk factors for rapid progression to ESLD are alcohol abuse, co-infection with HIV, older age at infection, and presence of genotype 1.

Introduction

Prior to 1990, many patients with hereditary bleeding disorders were infected with hepatitis C virus (HCV) through replacement therapy with inadequately or non-virus inactivated clotting factor products during the 1970s and 1980s.[1-4] Once infected, about 80% of patients develop chronic hepatitis C.[5] After twenty years of infection, 10% of patients progress to cirrhosis and an additional 10% to end-stage liver disease (ESLD, i.e. liver failure, hepatocellular carcinoma, liver-related death).[6] HIV coinfection, alcohol abuse, older age at infection, and male gender are the main determinants associated with faster progression of liver disease.[7-11] Whether highly active anti-retroviral therapy (HAART) improves the natural course in HCV/HIV coinfected patients remains controversial.[12-15]

There is growing evidence that progression of fibrosis in patients with chronic hepatitis C may not be linear.[16;17] Patients infected for several decades may be at higher risk for developing ESLD. These findings indicate the need for new studies on patients infected for more than 25 years.

In order to determine the natural history of HCV infection, the onset of infection must be identified, and information on its full course and its potential modifiers must be obtained.[18] These criteria may be met in a cohort of patients with inherited bleeding disorders: data on exposures to clotting products are recorded and patients are regularly tested for viral infections.[19] Furthermore, these patients are seen on a yearly basis for their bleeding problems, resulting in a reliable follow-up independent of HCV status or liver disease. In addition, they form a homogeneous group with the same route of infection and are almost all male. The aim of the present study was to assess ESLD in a cohort of HCV infected patients with inherited bleeding disorders.

Methods

Study design and participants

This international, multicenter cohort study constitutes all HCV antibody positive patients with congenital bleeding disorders from hemophilia treatment centers of Sheffield, UK (Royal Hallamshire Hospital), London, UK (Royal Free Hospital), and Utrecht, the Netherlands (Van Creveldkliniek). Patients were seen at least annually, and blood samples, ultrasounds of liver and spleen, and physical examination were routinely performed. The study was approved by the institutional review boards.

Laboratory methods

HCV and HIV antibody, HCV RNA, and HCV genotype tests were performed in the local hospital laboratories as described previously.[8;9;20] Hepatitis C status was defined as "spontaneous clearance" (positive anti-HCV antibodies but negative HCV RNA test on at least two occasions), or "chronic hepatitis C" (positive anti-HCV antibodies and persistently positive HCV RNA).

Definition of HCV infection, natural history, and ESLD

The date of first exposure to concentrates was assumed to be the date of infection. When this date was unknown, the date of July 1977 (London), January 1972 (Sheffield), or January 1970 (Utrecht) was taken. These different dates correspond to the median date of introduction of clotting factor in the different centers. For patients born after these dates it was assumed to be the date of their first birthday.

The natural history started at the date of HCV infection and ended at the date of ESLD, death, start of antiviral therapy for HCV, or last clinical evaluation whichever came first. Thus, ESLD over HCV infection time was studied in untreated patients. ESLD was defined as the occurrence of liver failure (ascites, bleeding esophageal varices, hepatic encephalopathy), hepatocellular carcinoma, or liver-related death. Alcohol abuse was defined as intake of more than 20 units per week.

Data analysis

In order to estimate the cumulative incidence of ESLD over time since infection, the Kaplan-Meier survival table method was used. The cumulative incidence is the probability of ESLD over follow-up time, taking censoring into account (e.g. proportion of patients with ESLD after 30 years of infection). The analysis was stopped when fewer than 10% of the patients remained under observation. The hazard rates (=incidence rates) for ESLD were calculated as the number of cases divided by the patient-years at risk and expressed in cases per 100 person years (i.e. the probability of ESLD in a certain period, given that the patient is ESLD free at the beginning of that period). Cumulative incidences and hazard rates for ESLD were both calculated for the total cohort of patients with chronic hepatitis C, and after stratification for HIV. In addition, hazard rates were also calculated for the first, second, and third decade of infection.

Cox proportional hazards models were used to assess the effect of several determinants on the risk of ESLD. The model yields a hazard ratio, which is a ratio of the incidence of ESLD with a specific determinant over the incidence without that determinant. The natural history (i.e. untreated infection time) was the survival time variable. Determinants used for the analyses were age at infection, history of alcohol abuse, HCV genotype, and HIV/HAART.

In order to prevent survival bias, HAART was considered as a time-dependent variable, taking the value of 0 prior to the time HAART was given and 1 thereafter. Genotype was not available for 20% of patients. Therefore, we used imputation techniques (missing value analysis, SPSS 12.0, Chicago, Illinois, USA) to predict the missing genotypes using regression models. We performed the analyses on ESLD both without those patients and with imputation of missing genotype. Since results were similar, further analyses were performed with imputed genotype.

Results

Patient characteristics

The present cohort comprises all 847 patients with HCV antibodies evaluated up to August 2005. Patient characteristics are shown in Table 1. The median age at first exposure was 14 years (range <1-77) and the median age at end of follow-up was 43 years (range 11-87). The median follow-up time since infection was 27 years (range 3-42) and total follow-up time since infection was 22,259 person years. Patients suffered predominantly from hemophilia A and B (91%). A history of alcohol abuse was present in 70 patients.

Viral status

Of all 847 patients, 687 (81%) developed chronic hepatitis C. 210 patients (25%) patients were coinfected with HIV. In total, 160 (of 847, 19%) spontaneously cleared HCV. At introduction of HAART in 1996-1997, 116 patients with HIV were still alive and 78 of them (67%) have since received HAART.

Cause of death

Overall, 199 patients (24%) died. 73 patients (37%) died of HIV/AIDS, 55 (28%) of liver disease, and 71 (36%) due to other causes.

ESLD

In total, 71 patients developed ESLD. One patient who cleared HCV spontaneously, developed ESLD after long-term alcohol abuse. The remaining 70 patients with chronic hepatitis C developed ESLD after median 21 years (8-36) of infection. Of these, 50 had two or more features of ESLD. Liver failure was present in 59, hepatocellular carcinoma in 13, and liver-related death in 55 patients. Nine patients with ESLD underwent liver transplantation.

58 patients with chronic hepatitis C who developed ESLD had not been treated with antiviral therapy up to that time (i.e. developed ESLD during natural history). Twelve patients developed ESLD after unsuccessful antiviral therapy.

Table 1. **Patient characteristics**

Patients	847
Age at end of follow-up*	43 (11-87)
Gender (male)	799 (94%)
Diagnosis	
• Hemophilia A	640 (76%)
• Hemophilia B	127 (15%)
• Other	80 (9%)
Total number of deaths	199 (24%)
Cause of death†	
• AIDS	73 (37%)
• Liver-related	55 (28%)
• Other	71 (36%)
Hepatitis C genotype	
•Type 1	361 (53%)
•Type 2 - 5	179 (27%)
• Missing	147 (20%)
Time from 1st exposure to end of follow-up (years)	27 (3-42)
End stage liver disease	71
• Liver failure	59
• Hepatocellular carcinoma	13
• Liver transplantation	9
• Liver related death	55
Coinfection with HIV	210 (25%)
• HIV treatment	161 (of 210 = 77%)
• HAART‡	78 (of 116 = 67%)
History of alcohol abuse	70 (8%)

Note values are numbers (%) or medians (ranges).
* end of follow-up is death or last evaluation.
† proportion of patients died
‡ 116 patients with HIV were alive when HAART became available.

Cumulative incidence of ESLD

After 35 years of infection, the cumulative incidence of ESLD in all patients with chronic hepatitis C was 17.1% (95% C.I. 14.0-20.2%). In contrast, it was 2.1% (95% C.I. 1.0-3.2%) in patients who spontaneous cleared HCV (p<0.001, Figure 1).

HIV was an important determinant of outcome; the cumulative incidence of ESLD increased from 11.5% (95% C.I. 8.2-14.8%) in HIV negative patients, to 35.1% (95% C.I. 29.2-41.0) in HIV coinfected patients. (p<0.001, Figure 2)

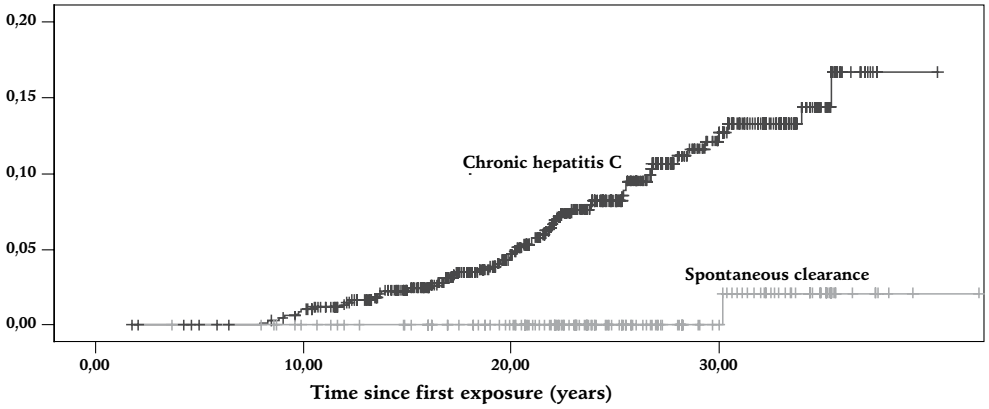

Figure 1. **Cumulative incidences of ESLD in patients with chronic hepatitis C (n=687) and patients who spontaneously cleared HCV (n=160).**

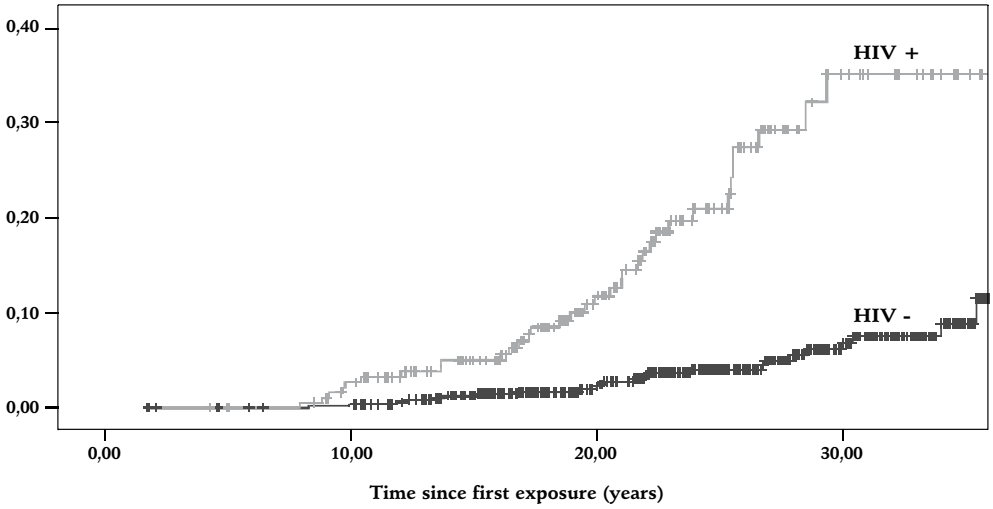

Figure 2. **Cumulative incidences of ESLD in patients with chronic hepatitis C according to their HIV status (190 HIV+, 497 HIV-).**

Hazard rate of ESLD

The risk of developing ESLD increased with duration of infection. In patients with chronic hepatitis C (n=687), the overall hazard rate of ESLD was 0.36 per 100 person years (Table 2). However, when stratified per 10 years of infection it increased from 0.10 in the first 10 years of infection to 0.90 after more than 20 years of infection. For HIV negative patients with chronic hepatitis C (n=497) the hazard rate increased from 0.04 in the first 10 years to 0.53 after more than 20 years. In patients coinfected with HIV (n=190), it increased from 0.27 to 2.63.

Table 2. Hazard rates of ESLD (per 100 person years) for patients with chronic hepatitis C (n=687).

	Duration of infection (years)			
	<10	10-20	>20	Total
Chronic hepatitis C (n=687)				
Hazard rate	7/6820= 0.10	20/5973= 0.33	31/3460= 0.90	58/16253= 0.36
HIV negative (n=497)				
Hazard rate	2/4939= 0.04	7/4504= 0.15	15/2851= 0.53	24/12294= 0.20
HIV positive (n=190)				
Hazard rate	5/1881= 0.27	13/1469= 0.88	16/608= 2.63	34/3958= 0.86

Note. Risk of ESLD related to duration of infection. The risk of ESLD increased with infection time.

Risk of ESLD

Cox's proportional hazards model was used to analyze the effect of age at infection, HCV genotype, HIV and HAART status, and alcohol abuse on the development of ESLD (Table 3). HAART appeared to be associated with reduced risk of ESLD but this was not statistically significant. In the multivariate analysis, ESLD risk was increased with HIV infection (hazard ratio 13.8 [95% C.I. 7.5-25.3]; i.e. independent of other risk factors, patients with HIV had a 13.8 times higher incidence of ESLD compared to HIV negative patients. Older age at infection (hazard ratio 2.3, per 10 years [95% C.I. 2.0-2.8]), presence of genotype 1 (hazard ratio 2.2 [95% C.I. 1.1-4.2]), and alcohol abuse (hazard ratio 4.9 [95% C.I. 2.5-9.6]) were also associated with a higher risk of ESLD.

Table 3. Determinants associated with ESLD in patients with chronic hepatitis C

A: Univariate		
Determinant	Hazard ratio (95% C.I.)	p-value
Age at infection (per 10 years)	1.8 (1.6-2.1)	<0.001
Genotype 1 • Type 2-5 • Type 1	 1.0 1.9 (1.0-3.6)	 0.06
History of alcohol abuse • No • Yes	 1.0 2.5 (1.3-4.7)	 0.01
HIV status • HIV negative • HIV positive	 1.0 5.5 (3.3-9.3)	 <0.001
HAART* • No • Yes	 1.0 0.8 (0.5-1.3)	 0.37

B: Multivariate		
Determinant	Hazard ratio (95% C.I.)	p-value
Age at infection (per 10 years)	2.3 (2.0-2.8)	<0.001
Genotype • Type 2-5 • Type 1	 1.0 2.2 (1.1-4.2)	 0.02
History of alcohol abuse • No • Yes	 1.0 4.9 (2.5-9.6)	 <0.001
HIV status • HIV negative • HIV positive	 1.0 13.8 (7.5-25.3)	 <0.001

* Use of HAART was evaluated in HIV positive patients and considered as a time-dependant variable.

Discussion

This study showed that 17% of hemophilia patients with chronic hepatitis C developed ESLD after 35 years of infection. However, HIV was an important determinant of outcome: cumulative incidence of ESLD ranged from 12% in HIV negative patients to 35%

in patients co-infected with HIV. Duration of HCV infection was also strongly associated with an increased risk of ESLD; hazard rates for all patients with chronic hepatitis C increased from 0.10 in the first decade of infection to 0.90 after more than 20 years of infection. ESLD was independently associated with higher age at infection, HIV co-infection, alcohol abuse, and genotype 1.

The present study has several limitations. First, these findings do not necessarily apply to non-hemophilic patients since almost all patients were male, were repeatedly infected through repeated infusions with clotting factor, and were in general infected at a relatively young age. Secondly, we were not able to assess the incidence of severe fibrosis or compensated cirrhosis as liver biopsies have been performed in only a minority of our cohort-patients. Nevertheless, the present study is strengthened by the large number of patients with long follow-up. Furthermore, date of infection, HCV and HIV status, data on start of HAART, HCV genotype, and history of alcohol abuse were precisely recorded in the large majority of patients. In addition, the time variable used in the analyses was time since infection up to ESLD, death, last evaluation, or start of antiviral therapy (i.e. the natural history without interference of IFN), minimizing the risk of confounding by indication. For the same reason, HAART was introduced as a time-dependent variable. Finally, only solid, clinical endpoints for ESLD were used, leaving little space for misclassification.

In general, two types of classifications have been used in studies on the natural history of HCV infection in patients with inherited bleeding disorders. Some authors define severe liver disease (especially cirrhosis) as presence of certain laboratory, clinical, and/or ultrasonographic findings.[19;21] These authors found a proportion of patients with severe liver disease ranging from 5 to 16% after two decades of infection. Other reports however, described the natural history of hepatitis C using histological or hard clinical outcomes (liver failure, hepatocellular carcinoma, liver-related death).[7-11] In studies from London and Sheffield, histological diagnosis of cirrhosis was made in 30-41% of patients, after 20-25 years of infection.[7;8] The discrepancy in proportions of patients with cirrhosis between non-invasive and histological methods of diagnosis, adds to the hypothesis that the risk of cirrhosis may be underestimated in non-histological studies.

In a large, multicenter cohort in hemophilia patients (MHCS), Goedert reported cumulative incidences of ESLD (hard endpoints) of 15% for HCV/HIV co-infected patients, and of 3% for patients only HCV seropositive after 16 years of follow-up.[11] We found much higher cumulative incidences in our cohort; 35% for those co-infected with HIV and 12% for HCV mono-infected patients. Several factors may account for these differences. The age at end of follow-up in our cohort was much higher (43 vs 20 years) indicating that duration of infection was longer in our study. In addition, age at infection was expected to be lower in the MHCS, resulting in lower risk for ESLD.[8;9] Indeed, in our

study risk of ESLD increased strongly with higher age at infection. Finally, we assessed the risk of ESLD in patients with chronic hepatitis C, i.e. HCV RNA positive patients. In the MHCS paper, incidences of ESLD were determined in HCV seropositive patients. However, the cumulative incidence in the MHCS would probably have been higher when only HCV RNA positive patients were taken into account as about 20% of patients with an HCV infection spontaneously clears HCV (even a higher percentage when infected at a younger age).[9;22]

In the present study, risk of ESLD was associated with older age at infection. Several factors may play a role. There is a reduced availability of anti-oxidizing systems with increasing age which may lead to a more rapid progression to advanced liver disease.[23-25] Furthermore, older patients may have used alcohol for a longer time which may lead to a less favorable course of hepatitis C.[26] Finally, hepatic steatosis may play a role in age-related deterioration of the liver. The amount of fibrosis correlates with body mass index and steatosis.[27] In general, older patients have a higher body mass index and may therefore be more vulnerable for HCV induced liver damage.

Infection with HIV has a major impact on the development of ESLD.[10] Whether HAART could improve this detrimental course remains unclear. Early reports, showed no or even a negative effect of HAART on HCV viremia.[14;28] Others suggested an increased risk of hepatotoxicity in patients using HAART.[29] However, several authors reported a beneficial effect of HAART on fibrosis progression and development of sever fibrosis and cirrhosis.[12;15] Our findings could not corroborate these reports; HAART appeared to slightly reduce the risk of ESLD but this was not statistically significant. The low number of patients on HAART and the relative short follow-up after start of HAART might have contributed to this.

The role of genotype on the clinical course of hepatitis C remains controversial. In the present study, the incidence of ESLD was increased 2-fold with HCV genotype 1. Genotype 1 may be associated with a less robust immune response to HCV and may have a great replication competence, resulting in more severe liver disease (especially in patients with HIV). [9] Several studies have also shown that presence of genotype 1 results in a higher risk of ESLD.[9;30;31] Others suggest that there is no association between genotype and risk of advanced liver disease.[11;25]

Future studies have to be performed to study the effect of IFN based regimens on the development of ESLD. In addition, it would be interesting to study the effect of low and moderate alcohol intake on progression of liver disease. Finally, it is of importance to assess the proportion of hemophilia patients with significant fibrosis or (compensated) cirrhosis by new, non-invasive techniques (e.g. Fibroscian®). Especially in patients with moderate and severe fibrosis further liver damage and ESLD may be prevented using antiviral therapy.[32;33]

In conclusion, after 35 years of infection 12% of HIV negative patients with chronic hepatitis C developed ESLD compared to 35% in patients co-infected with HIV. Risk of ESLD increased with duration of HCV infection and was associated with older age at infection, history of alcohol abuse, genotype 1, and presence of HIV.

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7

Treatment of chronic hepatitis C in patients with hemophilia: a review of the literature

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Summary

Chronic hepatitis C is a major cause of morbidity and mortality in hemophilia patients. Interferon-based antiviral therapy is effective in clearing the hepatitis C virus (HCV) in infected individuals. In contrast to studies in the general population, studies of antiviral therapy in hemophilia patients are limited and often include small numbers of patients. A review of the literature was performed to assess the efficacy of interferon-based therapy for patients with hemophilia chronically infected with HCV.

Studies were identified by electronic searches (Medline, Embase) and hand searches in references of key articles. Data of the included studies were pooled, and responses to therapy were stratified according to treatment regimen, HIV coinfection status, and treatment history. The main outcome was sustained virological response (SVR) defined as absence of HCV RNA both at end of treatment and 24 weeks post-treatment.

35 studies were identified which included 1151 patients. After pooling data of included patients, the SVR in HIV negative, treatment naïve patients was 22% for interferon (IFN) monotherapy, 43% for IFN and ribavirin, and 57 % for pegylated IFN and ribavirin, respectively. Re-treatment with IFN and ribavirin of those who failed to respond to previous IFN monotherapy was successful in 33%. In HCV/HIV coinfected patients, response to IFN monotherapy was 8% and to IFN combined with ribavirin 39%.

Responses to IFN-based therapy in patients with hemophilia have been improved over time and are nowadays approximately 50-60%. These treatment results appear to be similar to those seen in the general population. However, data on hemophilic HCV/HIV coinfected patients and in patients who failed to respond to previous therapy are limited and future studies in these specific patient populations are necessary.

Introduction

The prevalence of hepatitis C is 80 to100% among hemophilia patients who have been treated with clotting factor products before 1990.[1;2] Once infected, about 80% of these patients develop chronic hepatitis C.[2] Approximately 20% of chronically infected patients will develop cirrhosis, liver failure, or hepatocellular carcinoma after 20 years of infection.[3-5] As a result, antiviral treatment is warranted in order to eradicate the hepatitis C virus (HCV) and thus prevent the development of severe liver disease.[6;7]

In the non-hemophilic population, interferon-α (IFN) therapy is associated with a sustained response of 10-20% when used as monotherapy and 30-40% when used in combination with ribavirin.[8;9] When IFN was substituted by pegylated IFN (PegIFN), the response to combination therapy increased to 50-60%.[10;11] Several studies have evaluated the effect of IFN and ribavirin in the re-treatment of patients who failed to respond to IFN monotherapy. HCV RNA was eradicated in 14% of patients who did not respond at all to previous IFN monotherapy and in 49% of patients who had relapsed.[12;13]

In non-hemophilic, HCV/HIV coinfected patients responses to IFN-based therapy were lower than in HCV mono-infected patients. Only 15% of coinfected patients achieved a SVR after IFN monotherapy.[14] Response improved with combination therapy: ribavirin combined with either IFN or PegIFN resulted in a SVR of approximately 20% and 40%, respectively.[15;16]

Although treatment responses appear to be similar in patients with hemophilia, studies are limited and often include small numbers of patients.[17]

We performed a literature review to assess the efficacy of IFNα-based antiviral therapy in patients with hemophilia chronically infected with HCV.

Methods

Literature search

Eligible studies were identified through electronic searches of Medline (1966-January 2006), and Embase (1980-January 2006) using different sets of keywords. The first set consisted of 'hemophilia', 'bleeding disorders', and 'coagulation disorders'; the second set of 'hepatitis C', 'HCV', and 'non-A non-B'; 'the third set of 'interferon', 'IFN', 'ribavirin', and 'antiviral therapy'. In addition, we reviewed the reference lists in key studies and review articles.

Selection

We included both observational studies (pilot studies, non-randomized controlled trials) and randomized controlled trials. Patients could be treatment naïve, relapsers, or non-responders to previous antiviral therapy, irrespective of HIV status. The extracted data of the selected studies included total number of patients, number of patients responding, HIV status, and previous antiviral therapy status. Studies were selected if at least an English abstract was found in which the relevant data could be identified.

Articles about PegIFN and ribavirin in the hemophilic population were lacking. We therefore searched for relevant abstracts in the books of abstracts of the XXVIth International Congress of The World Federation of Hemophilia, the two latest versions of the Annual Meeting of the American Society of Hematology, and the XXth congress of the International Society of Thrombosis and Hemostasis.

Data analysis

The main outcome was sustained virological response (SVR) defined as: absence of HCV RNA at the end of treatment and 24 weeks after completing therapy. One study defined sustained response as normalization of ALT 24 weeks post-treatment.[18] Relapse was defined as: HCV RNA negative at the end of treatment, but positive 24 weeks post-treatment. Non-response was defined as: persistent HCV RNA positive during treatment. For the analyses both relapsers and non-responders were considered as one group (i.e. non-responders). Patients were classified as treatment naïve when the had no history of antiviral therapy.

Data were analyzed by intention to treat principle and presented in two ways. First, studies were stratified according to HIV status, treatment regimen (IFN monotherapy or combination therapy), and history of antiviral therapy (treatment naïve or non-responder to previous therapy) and the responses were expressed as ranges. Secondly, patients of the different studies were pooled together according to treatment regimen, HIV status, and history of antiviral therapy and responses were expressed as SVR with 95% confidence intervals (C.I.).

Results

Literature search

In total, we identified 160 references through electronic and hand searches, including two abstracts of PegIFN and ribavirin (Figure 1). After reading the titles and abstracts, we excluded 115 irrelevant references and 6 duplicates and retrieved 39 references for further assessment. A further four studies were excluded because relevant data could not be

extracted. In Table 1 the selected studies are shown. In total, 35 studies with a total of 1151 patients were selected, including 68 patients who served as controls (i.e. were not treated). Of these 35 studies, seven were randomized controlled trials reporting on 441 patients, including one placebo-controlled trial and one study comparing IFN monotherapy with IFN and ribavirin.[18-24]

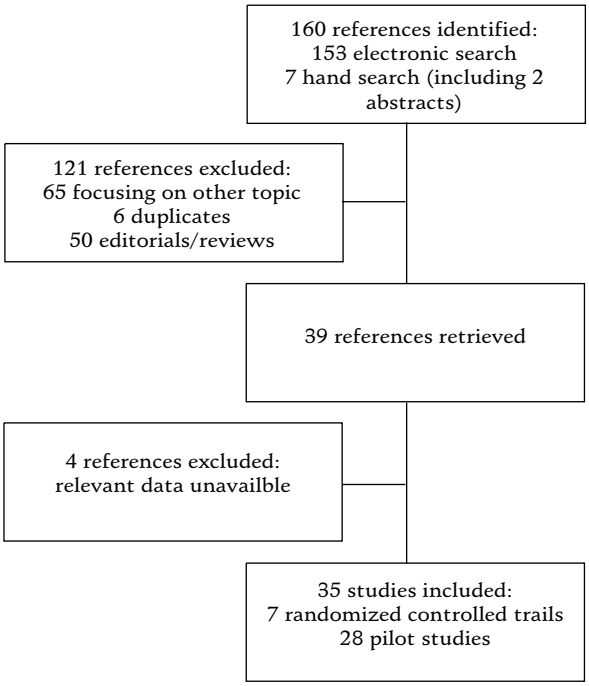

Figure 1. **Flowchart of inclusion of studies.**

25 studies were performed in HIV negative patients, eight studies included both HIV negative and positive patients, and two studies included HIV positive patients only. Median duration of treatment was six months and ranged from three to 36 months. The most commonly evaluated dose of IFN was 3 million units thrice weekly, whereas it was 1.5μg/kg per week for PegIFN. The dosage of ribavirin was 800-1200 mg/day in almost all studies.

Table 1. Response to IFN-based therapy: overview of the selected studies (n=35)

Treatment regimen	Nr. of studies	Nr. of patients	SVR range
HIV negative			
IFN monotherapy			
• Naïve patients[20;22;31-40]	12	269	0-50%
IFN and ribavirin			
• Naïve patients[19;21;41-45]	7	295	29-57%
• Non-responders[26;27] *	2	72	33-36%
• Mixed naïve patients and non-responders[23;46] *	2	72	27-41%
PegIFN and ribavirin			
• Naïve patients[47;48]	2	168	55-59%
Mixed HIV negative and HIV positive			
IFN monotherapy			
• Naïve patients[18;24;49-53]	7	152	0-50%
IFN and ribavirin			
• Mixed naïve patients and non-responders[54] *	1	28	71%
HIV positive			
IFN monotherapy			
• Naïve patients[55]	1	7	0%
IFN and ribavirin			
• Naïve patients[30]	1	20	40%

* Non-responders are both relapsers and non-responders to previous antiviral therapy.

Response to IFN-based therapy in HIV negative patients

In Table 2 pooled SVRs (95% C.I.) are shown. These SVRs were calculated from pooled data of patients extracted from the 35 studies and stratified for coinfection status, treatment regimen, and history of antiviral treatment. For treatment-naïve patients, responses to IFN monotherapy and IFN with ribavirin were 22% and 43%, respectively. Response was higher when patients were treated with PegIFN and ribavirin with 57% of patients showing a sustained response. Re-treatment of patients who relapsed after, or did not respond to IFN monotherapy with IFN and ribavirin resulted in a SVR of 33%.

All 62 patients who were enrolled in these studies but were not treated with IFN-based therapy (whilst serving as controls) failed to clear the virus during the observation period.

Table 2. Pooled SVR (95% C.I.) from data of individual patients according to treatment regimen, coinfection status, and treatment history.

	IFN mono	IFN and ribavirin	PegIFN and ribavirin†	Total
HCV mono-infected, naïve	95/434= 22% (14-30)	136/318= 43% (33-53)	96/168= 57% (53-61)	327/920= 36% (29-43)
HCV mono-infected, non-responders*	-	29/89= 33% (17-49)	-	29/89= 33% (17-49)
HCV/HIV coinfected, naïve	4/51= 8% (2-14)	9/23= 39% (33-45)	-	13/74= 18% (8-28)
Total	99/485= 20% (13-27)	174/430= 40% (32-48)	96/168= 57% (53-61)	369/1083= 34% (28-40)

Pooled data from all studies. Patient data were extracted from the studies and stratified according to HIV status, history of antiviral therapy, and IFN regimen (IFN mono, or combination therapy).
All controls (i.e. 68 untreated patients) remained HCV RNA positive during the observation period.
* Non-responders are both relapsers and non-responders to previous antiviral therapy
† Results based on two abstracts

Response to IFN-based therapy in HIV positive patients

Only 8% (4/51) of HIV positive patients who were treated with IFN monotherapy, achieved a SVR. However, when ribavirin was added to IFN SVR increased to 39%. None of the six controls cleared HCV during the observation period. There were no data of PegIFN and ribavirin for these patients

Discussion

The present review of 35 studies with 1151 hemophilia patients suggests that combination therapy is superior over IFN monotherapy, especially when PegIFN is combined with ribavirin. Reported responses of IFN-based therapy in the hemophilia population are similar to those seen in the general population.

The major limitation of our study is the availability for inclusion of only 7 randomized controlled trials and the low number of patients that were enrolled in these studies. However, the aim of the present review was to provide an overview of the variety of IFN-based therapies used in the hemophilic population, including HIV positive patients. Second, we found only two abstracts of the use of PegIFN and ribavirin in this population, indicating that data on this treatment are still preliminary.

The majority of patients included in the studies were HIV negative and treatment naïve. Responses to IFN-based therapy in these patients were in agreement with those reported in the general population; 10-20% response for IFN monotherapy, 30-40% for IFN combined with ribavirin, and 50-60% for PegIFN and ribavirin.[8;9;11] However, this is in contrast with previous reports which suggested that patients with hemophilia might respond worse to antiviral therapy.[17;25] Patients with hemophilia are often infected with HCV genotype 1, are predominantly male, have long-lasting infection, and often have high levels of viraemia, all of which are factors associated with lower response to therapy.[8;9;19] A possible explanation for the unexpected high response in the hemophilic population might be that patients are more compliant with therapy than the general HCV positive population. Patients with hemophilia are often well informed about their disease and are frequently seen by their physician in a comprehensive care setting which may improve adherence to therapy.

Although IFN-based therapy has been improved over time, there is still a significant number of patients who did not respond to IFN. So far, only two studies have reported the use of IFN and ribavirin in hemophilia patients who failed to eradicate HCV after IFN monotherapy.[26;27] Approximately 35% of these patients achieved a SVR. The use of PegIFN and ribavirin for re-treatment has not yet been reported in the hemophilia population. In non-hemophilic patients, re-treatment with PegIFN and ribavirin resulted in a SVR of 8-21% of patients not responding to IFN monotherapy and IFN with ribavirin, but SVR was 42-55% in patients who relapsed after IFN monotherapy or combination therapy of IFN and ribavirin.[28;29]

The effect of antiviral therapy in HCV/HIV coinfected hemophilia patients remains unclear. We found one study of 20 patients, reporting a SVR of 40% after IFN and ribavirin.[30] Up to now there have been no reports of the use of PegIFN and ribavirin for this specific population.

In summary, IFN-based therapy has been improved over time and treatment with PegIFN and ribavirin appears to be superior with a SVR of 57%. Responses to antiviral therapy in patients with hemophilia appear to be similar to those in the general population. The role of PegIFN and ribavirin in both HCV/HIV coinfected hemophilia patients and in patients who failed to respond to previous therapies remains unclear. However, (re-)treatment may be warranted in these patients as studies in the general population showed that a significant proportion of patients will achieve sustained eradication of HCV.

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8

Antiviral therapy for chronic hepatitis C in patients with inherited bleeding disorders: an international, multicenter cohort study

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Summary

Hepatitis C is a major cause of morbidity in patients with inherited bleeding disorders but little is known about the long-term efficacy of antiviral therapy. The aims of the study were to assess the effect of interferon (IFN)-based therapy on hepatitis C virus (HCV) eradication in treatment-naïve patients, to identify determinants associated with response, and to assess the occurrence of end-stage liver disease (ESLD) after completing antiviral therapy. In a multicenter cohort study all 295 patients who had ever received antiviral therapy were studied. Effect of therapy was expressed as sustained virological response (SVR). The median age at start of antiviral therapy was 36 years (12-68) and median duration of HCV infection was 22 years (range 2-41). Among HIV negative patients (n=235), SVR was 29% (29/101) for IFN monotherapy, 44% (32/72) for IFN combined with ribavirin, and 63% (39/62) for PegIFN with ribavirin. In patients co-infected with HIV (n=60), IFN monotherapy, IFN combined with ribavirin, and PegIFN and ribavirin eradicated HCV in 7/35 (20%), 1/2 (50%), and 19/60 (48%), respectively. SVR increased with genotype 2 an 3 (Odds ratio [O.R.] 11.0 [95% C.I. 5.8-20.5]), and combination therapy (IFN and ribavirin O.R. 3.7 [95% C.I. 1.7-8.4], PegIFN and ribavirin O.R. 4.2 [95% C.I. 1.8-9.5]). Up to 15 years after IFN treatment, none of the patients with a SVR relapsed and none of them developed ESLD. In contrast, median 4 years (<1-15) after antiviral therapy, 12 unsuccessfully treated patients developed ESLD and the cumulative incidence of ESLD after 15 years was 13.0%. IFN-based therapy is effective in patients with hemophilia and has a durable effect. SVR was associated with genotype 2 and 3, and combination therapy. A significant proportion of patients not responding to antiviral therapy developed ESLD.

Introduction

Prior to the introduction of adequate hepatitis C virus (HCV) elimination techniques during the manufacture of clotting factor concentrates, almost 100% of patients with inherited bleeding disorders were infected with HCV.[1;2] Approximately 80% of HCV infected patients develop chronic hepatitis C, which is a major cause of morbidity and mortality in patients with inherited bleeding disorders.[3-5]

In 1987, interferon monotherapy became available, resulting in normalization of aminotransferases and/or eradication of HCV in 10-20% of patients treated.[6-8] Since 1995, the virological response was markedly improved with the addition of ribavirin to IFN; 30-40% of patients became HCV RNA negative after 24-48 weeks of therapy.[7;8] The introduction of pegylated IFN (PegIFN) in the year 2000 in combination with ribavirin resulted in further improved HCV eradication up to 50-60% of patients.[9;10]

The reported efficacy of IFN is lower in patients coinfected with HIV with responses ranging from 16% for IFN monotherapy to 25-40% for combination therapy with (Peg-) IFN and ribavirin.[11;12]

Only a few trials with IFN have been conducted in patients with inherited bleeding disorders and results appeared to be similar to those in the general population.[13-16] However, most of these studies have been limited by a small number of patients treated, a short follow-up, and experience with PegIFN and ribavirin has not yet been reported in peer reviewed publications. Data on response to IFN-based therapies are of importance both for physicians and patients as the majority of hemophilia patients with chronic hepatitis C have received no or sub-optimal treatment.[17] In the present multi-center study the response to initial IFN-based therapy in a large cohort of patients with inherited bleeding disorders was assessed and determinants associated with response were identified. In addition, patients were followed post-treatment in order to assess long-term response and impact on the development of end-stage liver disease (ESLD).

Methods

Study design and participants

All HCV RNA positive patients with inherited bleeding disorders who have ever been treated with IFN monotherapy, IFN and ribavirin, or PegIFN and ribavirin at three large hemophilia treatment centers were included in this analysis. The patients are registered at the hemophilia treatment centers of Sheffield (Royal Hallamshire Hospital, UK), London (Royal Free Hospital, UK), and Utrecht (Van Creveldkliniek, The Netherlands). Patients

were seen at least annually. The study was approved by the institutional review boards of all three hospitals.

Laboratory methods

HIV antibodies, HCV RNA, and HCV genotype testing were performed in the local laboratories as described previously.[4;5;18]

Date of HCV infection

The date of infection was assumed to be the date of first exposure to concentrates. This median date of first exposure varied over the different centers according to the introduction of clotting factor concentrates in the various centers: July 1977 for London, January 1972 for Sheffield, and January 1970 for Utrecht. When the date of first exposure was unknown, the median date was used. For patients born after these dates it was set at the date of their first birthday.

Outcomes

The main outcome was sustained virological response (SVR), defined as absence of HCV RNA in serum at the end of treatment and six months after completing therapy, in treatment-naïve patients. Before HCV RNA testing became available, response to IFN was defined as normalization of aminotransferases (ALT and AST). In the present study, 18 patients were classified as sustained virological responders initially based on normalization of aminotransferases. SVR in all 18 patients was later confirmed by negative HCV RNA test. A relapse was defined as absence of HCV RNA at end of treatment, but positive six months later. Non-responders were defined as HCV RNA positive, both at the end of treatment and six months later. Discontinuation was defined as prematurely ending of treatment, due to adverse events.

ESLD was defined as the occurrence of liver failure (ascites, bleeding esophageal varices, hepatic encephalopathy), hepatocellular carcinoma, or liver-related death.

Data analysis

The analyses were performed for responses to initial IFN-based therapy. In order to identify determinants associated with SVR after antiviral therapy in treatment-naïve patients, multivariate logistic regression was used. Coefficients were expressed in odds ratios (O.R.) and 95% confidence intervals (95% C.I.). All determinants yielding O.R. significant at the 0.20 level in the univariate analysis, were included in the subsequent multivariate analysis. Determinants considered were: gender, age at HCV infection, genotype (1, 4, and 5 vs 2 and 3), duration of infection, age at start first antiviral therapy, history of alcohol abuse, treatment regimen (IFN monotherapy, IFN and ribavirin, and

PegIFN and ribavirin), HIV co-infection, and baseline ALT and AST levels. Alcohol abuse was defined as intake of more than 20 units per week. Genotype was not available for 15% of patients. Therefore, we used imputation techniques (missing value analysis) to predict the missing genotypes using regression models. We performed the analyses both without those patients and with imputation of missing genotype. Since results were similar, further analyses were performed with imputed genotype. HCV load was only available in 60% of patients and was therefore not used in the analyses. In order to estimate the cumulative incidence of ESLD since end of treatment, the Kaplan-Meier survival table method was used.

Results

Patient characteristics

The cohort consisted of 295 patients with chronic hepatitis C, predominantly males with hemophilia (Table 1). The median age at first antiviral therapy was 36 years (range 12-68) and 43 years (19-84) at the end of follow-up. The median duration of infection at the start of initial IFN-based treatment was 22 years (2-41). Coinfection with HIV was present in 60 patients (20%).

Response to initial antiviral therapy

In total, 136 patients have been treated with IFN monotherapy, 74 patients with IFN and ribavirin, and 85 with PegIFN and ribavirin (Table 2). 118 of 295 patients (40%) achieved a SVR after their initial antiviral therapy. In HIV negative patients, response to antiviral therapy ranged from 29% for IFN monotherapy to 63% for PegIFN and ribavirin. In patients coinfected with HIV response was lower at 20% for IFN monotherapy and 48% for PegIFN and ribavirin. All patients who achieved a SVR remained HCV RNA negative after a median follow-up of 5 years (1-15). Discontinuation of therapy due to adverse events was similar for both IFN monotherapy and combination therapies at 10-13%, irrespective of HIV status.

Table 1. Patient characteristics

Patients	295
Diagnose • Hemophilia A • Hemophilia B • Von Willebrand's disease • Other	 224 (76%) 50 (17%) 15 (5%) 6 (2%)
Gender (male)	283 (96%)
Age (years) • At infection • At initial IFN treatment • At end of follow-up*	 13 (<1-56) 36 (12-68) 43 (19-84)
Duration of infection (years)#	22 (2-41)
HCV genotype • 1 • 2 • 3 • 4 • 5 • unknown	 146 (50%) 42 (14%) 58 (20%) 2 (1%) 4 (1%) 43 (15%)
HIV-coinfection	60 (20%)
ALT (U/L) #	82 (16-1091)
AST (U/L) #	48 (12-94)

Note. Values are medians (ranges) or numbers (%).
* End of follow-up is last clinical evaluation or death.
# at start of initial IFN-based treatment

Table 2. Proportion of patients with sustained virological response to initial antiviral therapy

	HIV negative	HIV positive	Total	Follow-up (years)*
IFN	29/101 (29%)	7/35 (20%)	36/136 (26%)	9 (1-15)
IFN and ribavirin	32/72 (44%)	1/2 (50%)	33/74 (45%)	5 (1-8)
PegIFN and ribavirin	39/62 (63%)	11/23 (48%)	50/85 (59%)	1 (1-5)
Total	99/235 (42%)	19/60 (32%)	118/295 (40%)	5 (1-17)

* Median follow-up (range) after end of first treatment

Determinants associated with response to initial antiviral therapy

In the univariate analysis, older age at HCV infection, longer duration of infection, presence of genotype 2 and 3, and combination therapy were associated with a higher SVR (Table 3). In the multivariate analysis determinants independently associated with SVR were: presence of genotype 2 and 3 (O.R. 11.0, 95% C.I. 5.8-20.5), and use of combination therapy (IFN vs IFN and ribavirin: O.R. 3.7 [95% C.I. 1.7-8.4], and IFN vs PegIFN and ribavirin: O.R. 4.2 [95% C.I. 1.8-9.5]). Older age at infection, presence of HIV co-infection, and history of alcohol abuse appeared to be associated with a lower likelihood of SVR, however not statistically significant at p 0.05.

In other words, after adjustment for age at infection, presence of HIV, history of alcohol abuse, duration of infection, and genotype, treatment with IFN and ribavirin resulted in a 3.7 higher chance of SVR than treatment with IFN monotherapy.

Table 3a. Univariate analysis of SVR after initial antiviral therapy.

Determinant	O.R. (95% C.I.)	p-value
Gender		
• male	1.0	
• female	1.1 (0.3-3.4)	0.92
Age at infection (per 10 years)	0.7 (0.6-0.9)	0.01
Duration of infection at start therapy (per 10 years)	2.1 (1.5-3.1)	<0.01
Age at start therapy (per 10 years)	1.0 (0.8-1.2)	0.71
Genotype		
• type 1, 4 and 5	1.0	
• type 2 and 3	7.6 (4.5-12.8)	<0.01
Treatment regimen		
• IFN monotherapy	1.0	
• IFN and ribavirin	2.2 (1.2-4.1)	0.01
• PegIFN and ribavirin	4.0 (2.2-7.1)	<0.01
History of alcohol abuse		
• no	1.0	
• yes	0.5 (0.2-1.3)	0.15
HIV		
• no	1.0	
• yes	0.6 (0.3-1.1)	0.13
ALT* (per 100, U/L)	1.1 (0.9-1.4)	0.43
AST* (per 100, U/L)	0.8 (0.6-1.3)	0.41

*at start of therapy

Table 3b. Multivariate analysis of SVR after initial antiviral therapy.

Determinant	O.R. (95% C.I.)	p-value
Age at infection (per 10 years)	0.8 (0.6-1.0)	0.07
Duration of infection (per 10 years)	1.3 (0.7-2.2)	0.32
Genotype		
• type 1, 4 and 5	1.0	
• type 2 and 3	11.0 (5.8-20.5)	<0.01
Treatment regimen		
• IFN monotherapy	1.0	
• IFN and ribavirin	3.7 (1.7-8.4)	<0.01
• PegIFN and ribavirin	4.2 (1.8-9.5)	<0.01
History of alcohol abuse		
• no	1.0	
• yes	0.3 (0.1-1.1)	0.08
HIV		
• no	1.0	
• yes	0.6 (0.3-1.2)	0.15

*at start of therapy
Interpretation: after adjustment for all other determinants in the multivariate model, treatment with IFN and ribavirin resulted in a 3.7 higher chance of SVR than IFN monotherapy

End-stage liver disease at the end of follow-up

Up to 15 years after completing antiviral therapy, none of the patients with a SVR developed ESLD. However, 12 patients unsuccessfully treated, developed ESLD (10 liver failure, 2 hepatocellular carcinoma) median 4 years (<1-15) after completing treatment and after a median duration of infection of 24 years (18-36). The cumulative incidence of ESLD in these non-responders and relapsers was 13.0% 15 years after initial antiviral therapy and 35 years after infection (Figure 1). Seven of these patients with ESLD were coinfected with HIV.

Re-treatment of previous non-responders and relapsers

177 of 295 patients did not respond to or relapsed after initial IFN-based therapy. Of those, 71 (40%) were re-treated at least once, resulting in a SVR in 28 patients (39%).

Re-treatment of non-responders or relapsers after IFN monotherapy with IFN and ribavirin resulted in a SVR of 35% and with PegIFN and ribavirin in a SVR of 36%. Patients unsuccessfully treated with IFN and ribavirin achieved a SVR of 32% when retreated with PegIFN and ribavirin.

In total, 146 of 295 patients (49%) showed a sustained response after at least one IFN-based therapy.

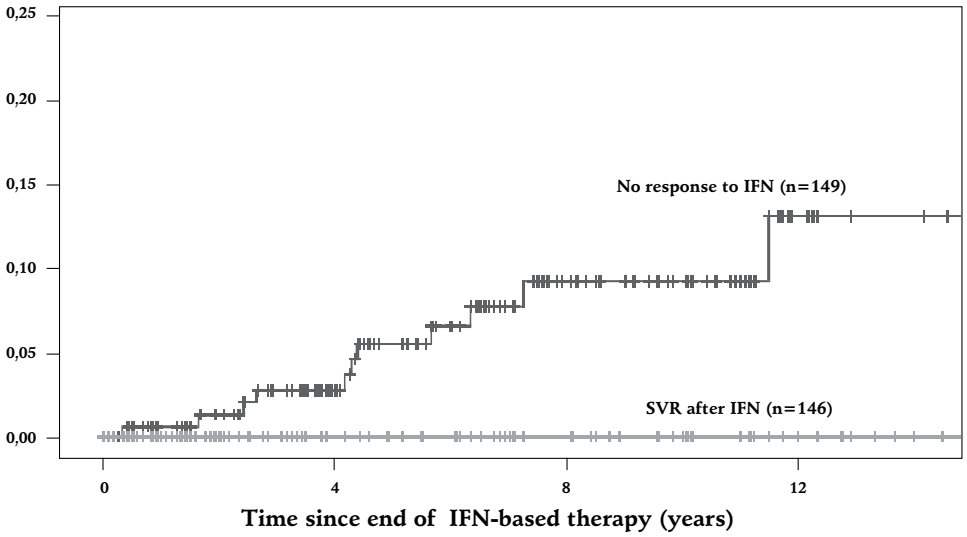

Figure 1. Cumulative incidence of ESLD after antiviral therapy.

Note. 118 patients achieved a SVR after first treatment and an additional 28 patients were successfully re-treated. None of those 146 patients developed ESLD. Of the 149 non-responders, 12 patients developed ESLD: all of them were treated with IFN monotherapy and 3 of them were re-treated with combination therapy.

Discussion

This is the first study describing the long-term responses to three IFN-based therapies in a cohort consisting of almost 300 hemophilic patients with chronic hepatitis C. Sustained response was similar to that seen in the non-hemophilic population and mainly associated with HCV genotype (2 and 3) and combination therapy (IFN/PegIFN and ribavirin).[7;10] None of the sustained responders became HCV RNA positive or developed ESLD up to 15 years after completing initial antiviral therapy. In contrast, 15 years post-treatment 13% of patients unsuccessfully treated had developed ESLD.

To appreciate these findings, some limitations of the study have to be considered. First, direct comparisons between IFN monotherapy and combination therapy have to be interpreted with care. Initially, only patients with elevated aminotransferases were treated with IFN monotherapy. This might have resulted in a selection bias: patients who were

treated with IFN monotherapy might have had more advanced liver disease with possibly lower response to IFN.[19] On the other hand, duration of infection is probably lower in these patients because IFN monotherapy was introduced in the late 1980s and combination therapy several years later.[4;7] However, elevated ALT and AST levels were not associated with sustained response in our study and in the multivariate analysis we included duration of infection and age at infection, rendering bias less likely.

The response to antiviral therapy in HCV-HIV coinfected patients may also have been overestimated. Use of IFN in this population started in the 1990s and at that time, many of the HIV-HCV coinfected patients had died, resulting in a group of 'survivors'.[20] Almost all these patients started HAART with concomitant lower HCV viral load and consequently better response to IFN.[21;22]

Interestingly, we found that an older age at infection appeared to be associated with lower response to antiviral therapy. However, neither age at start of therapy nor duration of infection appeared to be associated with SVR to IFN. The effect of age at infection has been previously described in a small study of twenty hemophilia patients treated with IFN and ribavirin.[23] The mechanisms underlying the reduced response to treatment in patients infected at older age remain elusive, but are probably multifactorial. From studies on the natural history of HCV infection it is known that patients infected at an older age developed ESLD more frequently.[4;5] Possibly, these patients had more severe fibrosis or cirrhosis at start of therapy resulting in less effective treatment compared to those infected at a younger age.

In the present study we found that all patients with a SVR remained HCV RNA negative up to 15 years post-treatment. This corroborates the results of others in the non-hemophilic population and indicates the beneficial and long-lasting effects of successful antiviral therapy.[24;25] Furthermore, ESLD did not occur in patients with SVR, whereas the cumulative incidence was 13% in those unsuccessfully treated 15 years after completing therapy, underscoring the importance of antiviral therapy. However, there are still many hemophilic patients with chronic hepatitis C who have not been treated.[17] Current guidelines recommend treatment in all patients with HCV genotype 2 and 3.[26] In contrast, due to lower SVR and consequently longer duration of treatment, assessment of severity of liver disease is recommended in patients with genotype 1, 4, 5, and 6 before considering treatment. Patients with at least portal fibrosis and septa on liver biopsy ($F \geq 2$) are eligible for treatment. However, biopsies are rarely performed in the hemophilic population because of possible bleeding complications and high costs of clotting factor replacement therapy, resulting in unknown grade of fibrosis in most patients. Recently, a new device (Fibroscan®) has been developed that measures stiffness of the liver, showing high correlation between liver stiffness and stage of fibrosis in biopsies, as well as excellent receiver operating

curves.[27;28] These technique may replace liver biopsy in this population, and offer an alternative way of selecting which patients should be treated with antiviral therapy.

There is no consensus on re-treatment of patients in whom previous IFN-based therapy has failed. However, our data show that re-treatment was successful in 39% of patients. This is in accordance with others, who found that re-treatment of non-hemophiliacs with PegIFN and ribavirin results in a 8-21% SVR in previous non-responders and 42-55% SVR in previous relapsers.[29-31] These data suggest that re-treatment of patients with inherited bleeding disorders is worthwhile due to reasonable chance of response and improved natural history if they eradicate HCV.

Currently, there are no treatment options for those who failed after PegIFN and ribavirin. New drugs are being developed e.g. new IFN molecules, HCV RNA protease and polymerase inhibitors, and immune modulators, but it will take years before they will become available.[32]

In conclusion, IFN-based therapies for chronic hepatitis C are effective in a significant proportion of patients with hemophilia, especially when treated with PegIFN and ribavirin. Treatment responses appear to be similar to those seen in the general population. Improvements in antiviral therapy have led to better virological response over the last decade and have resulted in long term clearance of HCV and prevention of ESLD up to 15 years post-treatment.

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9

Pegylated interferon and ribavirin combination therapy for chronic hepatitis C in patients with inherited bleeding disorders: a single-center experience

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Summary

Chronic hepatitis C is a major comorbidity in patients with inherited bleeding disorders. Although the current state-of-the-art therapy consists of pegylated interferon (PegIFN) and ribavirin, there are no reports of the efficacy of this combination in the hemophilia population. The aim of this study was to assess the response and side-effects of PegIFN and ribavirin in patients with inherited bleeding disorders.

Patients with chronic hepatitis C were treated with PegIFN alpha 2b (1.5μg/kg/week) and ribavirin (800-1200 mg/day) for 24 (genotype 2 and 3) or 48 weeks (genotype 1) and followed for an additional 24 weeks.

In total, 56 patients were enrolled: 31 (55%) had genotype 1, 12 (21%) genotype 2, and 13 (23%) genotype 3. Co-infection with HIV was present in 10 patients (18%) and 7 (13%) had been previously treated with interferon-α (IFN) with or without ribavirin. The overall sustained virological response (SVR) was 55%. In HIV-negative, treatment-naïve patients the SVR was 70%. Successful treatment was associated with genotype 2 and 3, absence of HIV, absence of previous IFN treatment, and decrease of HCV load at week 4 and 12. Although many side-effects occurred, only a minority (11%) discontinued therapy for this reason. Dose reduction of PegIFN was required in 28% and of ribavirin in 35% of patients. Overall, 22% of patients developed a depression and one patients developed a psychosis.

In conclusion, PegIFN and ribavirin is effective in patients with inherited bleeding disorders. Treatment is safe, but severe side-effects may occur and warrant close monitoring during therapy.

Introduction

Many patients with inherited bleeding disorders who received clotting factors before 1990 were infected with hepatitis C virus (HCV).[1-3] Once infected, approximately 80% of patients developed chronic hepatitis C.[4] Nowadays, hepatitis C is a major comorbidity in patients with inherited bleeding disorders and a leading cause of morbidity and mortality.[5;6] In the late 1980s interferon-α (IFN) monotherapy became available resulting in eradication of hepatitis C virus (HCV) in 10 to 30% of patients with hemophilia.[7-9] The addition of ribavirin to IFN resulted in significantly higher sustained response rates of 30-50%.[10-12] Currently, the state-of-the-art treatment for HCV consists of pegylated interferon (PegIFN) and ribavirin.[13;14] This combination yields response rates of 40-50% in non-hemophilic patients with HCV genotype 1 and 4, and 80-90% in genotype 2 and 3. However, response rates of PegIFN and ribavirin in patients with hemophilia have not yet been published.

The aim of this study was to assess the efficacy and safety of combination therapy of PegIFN and ribavirin in patients with congenital bleeding disorders.

Methods

Patients

Between 2002 and 2005, 56 patients with inherited bleeding disorders and chronic hepatitis C were treated with PegIFN and ribavirin at the Van Creveldkliniek, the Netherlands. The study was approved by the institutional review board and all patients gave written informed consent.

Patients were eligible if they suffered from inherited bleeding disorders and chronic hepatitis C (positive anti-HCV antibodies and persistently positive HCV RNA tests). Patients were excluded if they were younger than 18 or older than 70 years of age.

Measurements

Qualitative HCV RNA detection was performed by using the COBAS AMPLICOR HCV Test, version 2.0 (Roche Diagnostics, Branchburg, NJ, USA), and HCV load was assessed by COBAS AMPLICOR HCV MonitorTM Test, version 2.0 (Roche Diagnostics, Branchburg, NJ, USA). Quantitative HCV RNA assessment was performed at start of treatment, after 4 and 12 weeks, at the end of treatment, and 24 weeks thereafter. For genotypic analysis, the reverse hybridization line probe assay (INNO–LiPA HCVII; Innogenetics, Zwijndrecht, Belgium) was used, which assesses type-specific sequence variation in the 5' UTR.

Treatment

Patients were treated with Peginterferon alpha-2b 1.5μg/kg per week (Pegintron ®, Schering-Plough, Maarsen, The Netherlands) and ribavirin 800-1200mg daily (Rebetol ®, Schering-Plough, Maarsen, The Netherlands). Duration of treatment varied across viral genotype: patients with genotype 1 were treated for 1 year. However, if their HCV load was decreased less than 100-fold after twelve weeks of treatment, or HCV RNA was still detectable after 24 weeks, treatment was discontinued. Patients with genotype 2 and 3 were treated for 24 weeks.

Definitions

The primary outcome was sustained virological response (SVR), defined as absence of HCV RNA at the end of treatment and at the end of follow-up (24 weeks post-treatment). Patients were classified as treatment naïve if they had no history of antiviral therapy. Relapse was defined as absence of HCV RNA at the end of treatment but positive HCV RNA in the next 24 weeks. Non-responders were patients who continued to be HCV RNA positive during treatment. For the analyses both relapsers and non-responders were considered as one group (i.e. non-responders). Discontinuation was defined as termination of treatment because of side-effects. Date of infection was estimated to be the date of first exposure to clotting factor concentrate.

Safety assessment

Safety was assessed by laboratory tests (for anemia, neutropenia, and thrombocytopenia) and evaluation of side-effects (flu-like, psychological, dermatological, or weight loss) at week 2, 4, 6, 8, and monthly thereafter. Flu-like symptoms were: fever, malaise, myalgia, and arthralgia). Psychological symptoms were defined as: difficulties in concentrating, irritability, and depressive mood. Dermatological problems (pruritus, rash, eczema), depression, or insomnia were recorded if medication was required (antihistamines, antidepressants, or sleep medication).

Data analysis

Data were expressed as medians (ranges) or numbers (proportions). Comparisons of continuous data were made using the Mann-Whitney U test. Proportions were compared using the χ^2-test. A p-value <0.05 was considered statistically significant.

Results

Patients

In total, 56 patients were treated with PegIFN and ribavirin, starting therapy at a median age of 37 years (range 18-62). Patient characteristics are shown in Table 1. HIV co-infection was present in 10 patients and 7 patients had been previously treated with IFN monotherapy (n=2) or IFN combined with ribavirin (n=5).

Table 1. Patients characteristics (n=56)

Age (years)	37 (18-62)
Male gender	54 (96%)
BMI (kg/m^2)	24 (19-35)
Bleeding disorder • Hemophilia • Other	 53 (95%) 3 (5%)
ALT (U/L)	70 (20-324)
HCV load (IU/mL)	$3*10^6$ ($6*10^3$-$8*10^7$)
HCV Genotype • 1 • 2 • 3	 31 (55%) 12 (21%) 13 (23%
Duration of infection (years)	32 (16-38)
Previously treated for HCV	7 (13%)
HIV co-infected	10 (18%)

Values are medians (ranges) or number (proportions)

Response to treatment

Of 56 patients, 31 (55%) were successfully treated. Eleven patients (20%) did not respond, 8 (14%) relapsed, and 6 patients discontinued therapy because of side-effects. SVRs stratified according to HIV and HCV treatment history are shown in Table 2. SVR was 70% (28/40) in anti-HIV negative, treatment naïve patients. Of the 6 HIV negative patients who did not respond to previous IFN-based therapy, only one patient eradicated HCV. In HIV positive patients SVR was achieved in 2 of 10 patients. SVR was strongly associated with HCV genotype: SVR in genotype 1 was 7/17 (41%) versus 100% (10/10) in genotype 2 and 85% (11/13) in genotype 3 ($p<0.001$ for genotype 1 versus genotype 2 and 3).

Table 2. Sustained virological response after treatment with PegIFN and ribavirin according to HIV status and HCV treatment history.

	Treatment naive	Previously treated	Total
HIV negative	28/40 (70%)	1/6 (17%)	29/46 (63%)
HIV positive	2/9 (22%)	0/1 (0%)	2/10 (20%)
Total	30/49 (61%)	1/7 (14%)	31/56 (55%)

Figures are numbers (proportions)

Treatment failure was associated with genotype 1, having a history of non-response to previous IFN-based therapy (with or without ribavirin), and presence of HIV. Other characteristics of sustained responders versus non-responders are shown in Table 3a.

Baseline levels of hepatitis C viral load were similar in patients with and without sustained response. However, viral load after 4 and 12 weeks were strongly associated with a SVR (Table 3b). 16 of 31 (52%) sustained responders were HCV RNA negative at week 4 compared to 0 of 25 (0%) non-responders. After 12 weeks of treatment, 30 of 31 (97%) sustained responders were HCV RNA negative compared with 9 of 25 (36%) non-responders.

Table 3a. Patient characteristics according to treatment response.

	Sustained response	No sustained response	p-value
Patients	31	25	-
Age (years)	37 (18-58)	38 (20-62)	NS
BMI (kg/m^2)	24 (20-35)	23 (19-30)	NS
Age at infection (years)	4 (<1-27)	4 (<1-26)	NS
Duration infection (years)	32 (16-37)	31 (17-38)	NS
Treatment naive	97%	76%	0.02
Genotype 1	26%	92%	<0.001
ALT (U/L)	60 (20-324)	74 (26-286)	NS
Anti-HIV positive	6%	32%	0.01

Values are medians (ranges) or proportions.
NS = not statistically significant

Table 3b. **HCV load during antiviral therapy according to treatment response.**

	Sustained response	No sustained response	p-value
Viral load (IU/mL) T=0 weeks	$2*10^6$ ($6*10^3$-$8*10^7$)	$3*10^6$ ($6*10^4$-$4*10^7$)	NS
Viral load (IU/mL) T=4 weeks	0† (0-$4*10^5$)	$5*10^5$ ($2*10^3$-$1*10^6$)	<0.001
Viral load (IU/mL) T=12 weeks	0† (0-$1*10^4$)	$2*10^4$ (0-$2*10^7$)	<0.001

Values are medians (ranges)
NS = not statistically significant
† below detection limit of 50 IU/mL

Safety

Side effects were common: psychological problems (irritability, concentration problems) occurred in 83% of patients, and flu-like symptoms were present in 69% of patients (Table 4). Depression (requiring antidepressant drugs) occurred in 22% of patients and one patient developed a psychosis after 3 months of therapy. Weight loss and hematological abnormalities (anemia, neutropenia, and thrombocytopenia) were the only reasons for dose adjustment. Dose reduction of PegIFN or ribavirin was necessary in 15 (28%) and 19 patients (35%), respectively. In total, 6 patients (11%) discontinued therapy due to severe side-effects.

Table 4. **Side effects of PegIFN and ribavirin**

Flu-like	69%
Pruritus /rash*	32%
Concentration problems/irritability	83%
Depression*	22%
Insomnia*	29%
Weight loss ≥ 5 kg	61%
Anemia	63%
Neutropenia	69%
Thrombocytopenia	41%

* requiring medication

Side effects requiring dose reduction or discontinuation	
Dose reduction PegIFN	
• Neutropenia	9%
• Thrombocytopenia	2%
• Weight loss	17%
Dose reduction ribavirin	
• Anemia	26%
• Weight loss	9%
Discontinuation of therapy	
• Flu-like symptoms	7%
• Thrombocytopenia	2%
• Psychosis	2%

Discussion

The present study is the first report to evaluate the response to PegIFN and ribavirin in patients with inherited bleeding disorders. The overall response rate was 55%, and 70% in HIV-negative, treatment-naïve patients. Sustained response was associated with genotype 2 and 3, absence of HIV co-infection, and no previous treatment with IFN. In addition, rapid clearance of HCV at week 4 and 12 was also strongly associated with a SVR. Side-effects were common, but discontinuation of therapy was required in only 11% of patients.

Unfortunately, the number of patients who were co-infected with HIV or had a history of non-response to treatment was too small to draw conclusions about treatment response in these subgroups. Further studies have to be performed, to assess the value of PegIFN and ribavirin in these specific patients.

Previous studies in patients with inherited bleeding disorders have shown the improved response when combining ribavirin to IFN.[10-12] Later on, IFN was substituted by PegIFN resulting in higher SVRs in the non-hemophilic population.[13;14] However, there are only two preliminary reports (abstracts) about the efficacy of PegIFN and ribavirin in patients with bleeding disorders.[15;16] Further studies of efficacy of antiviral therapy in these patients are of importance as patients in the hemophilic population have some distinct features which may lead to different treatment responses. Hemophilia patients are almost exclusively male, most of them have been infected at a young age, have been infected many times, and were all infected before 1990 having a duration of infection of at least 16 years.[17;18] In our study, the response rate in HIV negative, treatment-naïve patients was high (70%), and ranged from 40% in genotype 1 to 90% in genotype 2 and 3.

This is in agreement with data from the general population: 40-50% for genotype 1, and 80-90% for genotype 2 and 3.[13;14]

In the present study, a viral response after 4 and 12 weeks of treatment was associated with a sustained response which is in agreement with previous studies.[19-22] Initially, viral load reduction of at least 100-fold after 12 weeks of treatment appeared to be highly predictive.[19;20] 70-80% of patients who achieved this so-called early virological response achieved a SVR. Patients who failed to achieve this early response did not clear HCV even if therapy was continued for an additional 9 months. More recently, the value of a virological response after 4 weeks of treatment (rapid virological response) was evaluated.[21;22] It was suggested that a treatment of 12-16 week (instead of 24 weeks) was sufficient in patients with genotype 2 and 3 with a rapid virological response at week 4. The virological response at week 4 has not yet been implemented in the guidelines regarding HCV treatment, but early confirmation of viral reduction following initiation of antiviral therapy for chronic hepatitis C is worthwhile. It is a good way to motivate adherence during the first months of therapy and may result in shorter duration of therapy. In addition, patients who fail to achieve a sufficient response after 12 weeks will not clear HCV and therapy can be confidently discontinued in those patients.

Although most patients in the present study completed therapy, side-effects were common and the frequencies of dose reductions or discontinuation of antiviral therapy were similar to other studies in the hemophilic population.[10;23;24] The high adherence to therapy in our population may be caused by several factors. First, most patients were well informed about hepatitis C and its therapy and were motivated to start. In addition, in our center patients were treated in a comprehensive care setting and patients were regularly seen by physician, nurse, and social worker, especially during the first weeks of therapy. Side-effects were closely monitored and treated if necessary. Special attention was paid to depressive symptoms and although 22% of patients developed a depression requiring medication, all of these patients completed therapy.

In conclusion, PegIFN combined with ribavirin is effective in HIV negative patients with bleeding disorders: 40% of patients with genotype 1 and 90% of patients with genotype 2 or 3 achieved a SVR. Although many side-effects occurred, only a minority had to discontinue therapy. Larger studies have to be performed to corroborate these findings, and attention should be paid to re-treatment with PegIFN and ribavirin of patients who failed to respond to previous IFN therapy (with or without ribavirin).

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Discussion

Hepatitis C – a worldwide problem

More than 170 million people worldwide suffer from chronic hepatitis C. Its prevalence is 2% in industrialized countries.[1] Approximately 20% of patients with a chronic infection will develop cirrhosis after 20 years of infection, with an annual risk of developing hepatocellular carcinoma of 1 to 4% in those with cirrhosis.[2;3] Chronic hepatitis C related liver disease is now the leading indication for liver transplantation in both Europe and North America.[4]

Although many studies have been performed on hepatitis C virus (HCV) infection, uncertainties remain about the long-term course of this infection. In order to determine the natural history of HCV infection, the onset of infection must be identified, and information on its full course and its potential modifiers must be obtained.[5]

Hepatitis C and hemophilia

The hemophilic population represents a unique model to study the clinical course of hepatitis C. The date of infection can be determined accurately in these patients, since nearly all patients were infected at the time of their first transfusion.[6] Furthermore, these patients are frequently seen in hemophilia treatment centers, resulting in a reliable, long-term follow-up.[7]

In our study with a follow-up of 35 years, we found that the risk for end-stage liver disease (liver failure, hepatocellular carcinoma, liver-related death) was 12% for HIV negative, and 35% for HIV coinfected patients. We expect the problem of chronic hepatitis C in hemophilia patients to increase in the next decades for two reasons. First, most patients have already been infected for two or three decades, and are currently middle-aged with a life-expectancy that will theoretically be 'sufficient' to develop end-stage liver disease. Moreover, there are indications that progression of liver damage might be exponential, i.e. show accelerated progression with longer duration of infection.[8] Therefore, two issues are essential for adequate care in this population: use of antiviral therapy, and careful follow-up with reliable diagnostics for progression of liver fibrosis.

Antiviral therapy – guidelines for the general population

Currently, antiviral therapy consists of pegylated interferon combined with ribavirin and is recommended to all patients infected with HCV genotype 2 and 3, resulting in a sustained response of 80-90% after six months of treatment.[3]

In contrast, treatment of patients with genotype 1, 4, 5, and 6 takes one year and is successful in only 40-50% of patients. Therefore, therapy is only warranted in patients who have significant liver damage, i.e. at least moderate fibrosis on liver biopsy.

Antiviral therapy – issues in hemophilia

Although a significant proportion of hemophilia patients is at risk for long-term complications of hepatitis, many patients still have not been treated. In the Dutch hemophilia population, approximately 30-40% were not treated mainly due to fear of side effects, lack of clinical symptoms, and expected low efficacy of treatment. However, health-related quality of life was reduced in hemophilia patients with chronic hepatitis C. Thus providing an additional argument in favor of treatment, as health-related quality of life will improve after successful treatment for hepatitis C.[9]

Antiviral therapy in hemophilia – non-invasive assessment of liver fibrosis

Most patients with hemophilia have been infected with the more treatment-resistant HCV genotype 1.[10] However, liver biopsies are rarely performed in these patients because of risk of bleeding, and high costs of clotting factor replacement. Nevertheless, a reliable estimation of liver damage is still needed in order to assess the need for antiviral therapy.

In order to assess liver fibrosis in the hemophilic population and identify patients eligible for antiviral therapy, we were the first to use a new, non-invasive device: the Fibroscan®.[11] With this new technique we found an unexpected high proportion of patients with severe fibrosis or cirrhosis of approximately 35%. Many patients had significant liver damage that would not have been detected with the conventional methods like laboratory tests and ultrasonography. Based on the results of the Fibroscan® examination, approximately 25% of patients started antiviral therapy within three months. The majority of these patients had postponed antiviral therapy for years despite therapy had frequently been offered. These results indicate that the Fibroscan® may play an important role in persuading patients with significant fibrosis to start therapy. Furthermore, this technique may be used to follow patients in order to assess progression of fibrosis and determine prognosis. For this purpose, we recommend regular measurements every 3 to 5 years, in line with recommendations for the frequency of repeat liver biopsy.[12]

Antiviral therapy in hemophilia – response

Conflicting data exist about the efficacy of IFN-based therapy in the hemophilic population. For many years, it was thought that patients with hemophilia would respond worse to IFN-based therapy than patients in the general population.[7;13] Patients with hemophilia are often infected with HCV genotype 1, are predominantly male, have long-lasting infection, and have often high levels of viraemia. However, in our review of IFN-based therapies in the hemophilic population responses were similar to that seen in the general population. This was also corroborated in our pilot study of patients who were

treated with Pegylated IFN combined with ribavirin. The sustained virological response for HIV negative and treatment naïve patients was 70%. This high response may be due to the high compliance to therapy in this population. Patients with hemophilia are frequently seen in a comprehensive care setting and are often well informed about their disease. Possibly, these patients are also better informed about antiviral therapy and prepared for its adverse effects, adding to the adherence of therapy.

Antiviral therapy in hemophilia – long-term effects

The direct responses to antiviral therapy are precisely defined, but what are the long-term effects of this therapy? In a cohort study of approximately 300 patients, we found that all patients who achieved a sustained response after IFN-based therapy remained HCV RNA negative up to 15 years after treatment. Furthermore, none of the successfully treated patients developed liver failure or hepatocellular carcinoma. This was in contrast with patients who failed to eradicate HCV: the risk for developing end-stage liver disease was approximately 13%. In conclusion, these findings indicate that successful therapy postpones or may even prevent long-term complications of chronic hepatitis C during the first 10-15 years after therapy. However, longer follow-up is required to assess whether this favorable effect is sustained over the next decades.

Antiviral therapy in hemophilia – re-treatment and future therapies

In the coming years more attention should be paid to patients who did not respond to antiviral therapy. However, encouraging data about re-treatment exist. A few studies evaluated the effect of re-treatment with pegylated interferon and ribavirin in patients who did not respond to conventional IFN with or without ribavirin. Response was mainly determined by the previous response to IFN: re-treatment of patients who relapsed to previous therapy resulted in a sustained response of 50%.[14-16] In contrast, patients who did not respond at all to previous therapy, achieved a sustained response of only 15%.[15-17] Therefore, the development of new antiviral therapies has to be continued which is in particular important for patients who did not respond to PegIFN and ribavirin. Recently developed drugs like new IFN molecules, HCV RNA protease and polymerase inhibitors, and immune modulators are promising but it will take years before they will become available.[18] Probably, a cocktail of these new agents will be applied in the population infected with hepatitis C and will hopefully result in either a sustained eradication of HCV or in sufficient suppression of the virus replication.

Conclusion

Over the last years, the prognosis of chronic hepatitis C has been improved due to better response to antiviral therapy. However, a significant proportion of patients will still suffer from long-term complications of the disease. Careful follow-up and development of new antiviral drugs remain a challenge for the coming years.

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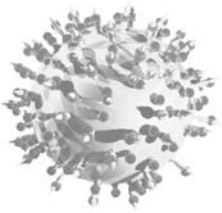
Summary



This thesis aims to describe the clinical consequences of hepatitis C and the role of antiviral therapy in patients with hemophilia.

In **chapter 2** the prevalence of hepatitis C and the use of antiviral therapy is described. A nationwide cross-sectional study was performed and a questionnaire was sent to all patients known with hemophilia in the Netherlands. The study population consisted of 638 patients who had received clotting factor products before 1992 and who reported their hepatitis C status. Of these patients, 54% had a current infection with hepatitis C virus (HCV). 32% of infected patients was treated with antiviral therapy. Main reasons for refraining from therapy were shrinking from side effects, normal liver function tests, and expected low efficacy of therapy.

The effect of hepatitis C on health-related quality of life (HRQoL) in patients with hemophilia is assessed in **chapter 3**. A nationwide cross-sectional study was performed and a questionnaire was sent to all patients known with hemophilia in the Netherlands. HRQol was assessed using the RAND-36 questionnaire. The study population consisted of 602 patients who had received clotting factor products before 1992, had reported their hepatitis C status, and had completed the RAND-36. All eight domains of the RAND-36 were lower in patients with a current HCV infection when compared with patients who had never been infected with HCV. After adjustment for age, severity of hemophilia, HIV, employment status, and joint limitations, hepatitis C infection was associated with a decrease of HRQoL on the domains of 'general health' and 'vitality'.

In **chapter 4** liver fibrosis is non-invasively assessed using liver stiffness measurement (LSM, Fibroscan®). In order to assess the validity of LSM in our hands, a separate group of 63 non-hemophilic patients infected with HCV were evaluated with both LSM and biopsy. Liver elasticity was highly correlated to histological (METAVIR) fibrosis stage (correlation coefficient = 0.73, $p<0.001$). The study population consisted of 121 patients with bleeding disorders and chronic hepatitis C. Based on LSM, 40% had no or mild fibrosis, 25% moderate fibrosis, 18% severe fibrosis, and 17% cirrhosis. The majority of patients with cirrhosis based on LSM would not have been diagnosed with laboratory tests and ultrasonography. Of all eligible patients with at least moderate fibrosis, 25% started antiviral therapy within three months after LSM.

Many patients with inherited bleeding disorders have been infected with hepatitis C in early childhood. In **chapter 5** the natural history of childhood acquired HCV infection is described. In addition, transfusion-associated risk factors for HCV infection are explored.

120 patients with inherited bleeding disorders, born between 1976 and 1990, and treated with non-HCV inactivated clotting products were selected. Of these, 57% (68 patients) had been infected with HCV. Once infected, 65% developed chronic hepatitis C and 35% cleared HCV spontaneously. HCV infection was associated with longer treatment period and with exposure to a larger number of donors (i.e. predominantly exposure to clotting factor concentrates produced from a large number of donors). Spontaneous clearance of HCV appeared to be associated with a young age at infection. After a mean infection period of 21 years, evidence of cirrhosis was present in 5% (two patients) of patients with chronic hepatitis C.

In **chapter 6** the risk of end-stage liver disease (ESLD) is assessed in hemophilia patients chronically infected with HCV. ESLD was defined as liverfailure, hepatocellular carcinoma, or liver-related death. In a multi-center, international cohort study, all 847 anti-HCV positive patients from three hemophilia treatment centers (London, Sheffield, Utrecht) were selected. 19% of patients spontaneously cleared HCV and 81% developed chronic hepatitis C. After 35 years of infection the cumulative incidence of ESLD was 12% in HIV negative patients and 35% in HIV co-infected patients. Independent risk factors for ESLD were: HIV co-infection, older age at infection, alcohol abuse, and presence of HCV genotype 1.

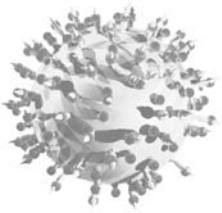
Chapter 7 reviews the literature regarding the antiviral therapy in hemophilia patients with chronic hepatitis C. In total, 35 studies with 1151 patients were identified. Data of the included studies were pooled, and responses to therapy were stratified according to treatment regimen, HIV co-infection status, and treatment history. The main outcome was sustained virological response (SVR): absence of HCV RNA in plasma six months post-treatment. In HIV negative, treatment naïve patients the SVR was 22% for interferon (IFN) monotherapy, 43% for IFN and ribavirin, and 57% for pegylated (Peg) IFN and ribavirin. In HIV co-infected patients, the response to IFN monotherapy was 8%, and 39% to IFN and ribavirin. These results appear to be similar to those seen in the general population.

In **chapter 8** the response to antiviral therapy is described, and determinants associated with this response are identified. In addition, the long-term response and occurrence of ESLD after completing therapy are assessed. In a multi-center cohort study all 295 hemophilia patients with chronic hepatitis C who had ever received antiviral therapy were selected. 235 patients were infected with HCV and 60 with HCV and HIV. In HIV negative patients, the SVR was 29% for IFN monotherapy, 44% for IFN with

ribavirin, and 63% for PegIFN with ribavirin. In HIV co-infected patients, IFN monotherapy was effective in 20%, and (Peg-)IFN with ribavirine in 48%. SVR increased with HCV genotype 2 and 3, and with the use of combination therapy (PegIFN or IFN with ribavirin). Up to 15 years after IFN treatment, none of the patients with a SVR relapsed and none of them developed ESLD. In contrast, the cumulative incidence of ESLD in unsuccessfully treated patients was 13% 15 years post-treatment.

In **chapter 9** the response and side effects of PegIFN with ribavirin are described. 56 patients with hemophilia and chronic hepatitis C were treated. The SVR in HIV negative, treatment naïve patients was 70%. Successful treatment was associated with HCV genotype 2 and 3, absence of HIV, and decrease of HCV load at week 4 and 12. 11% of patients discontinued therapy due to side effects. 22% of patients developed a depression and one patient developed a psychosis.

Samenvatting



Hemofilie is een erfelijke ziekte die gekenmerkt wordt door het ontbreken van stollingsfactor VIII (hemofilie A) of IX (hemofilie B). Patiënten met ernstige hemofilie ervaren spontane bloedingen in spieren en gewrichten, terwijl patiënten met milde hemofilie meestal pas bloedingen krijgen na kiesextracties, operaties of trauma. Hemofilie kan worden behandeld door toediening van stollingsfactorconcentraat. Deze therapie bestaat sinds de jaren zestig van de vorige eeuw. In de jaren die volgden bleek dat het toedienen van stollingsfactoren niet zonder risico was. Door het toedienen van besmette plasma producten werden vele patiënten geïnfecteerd met virussen zoals HIV en hepatitis C. Ter voorkoming van deze infecties worden bloeddonoren gescreend op de aanwezigheid van virale infecties en zijn er methoden ontwikkeld om de stollingsproducten virusvrij te maken. Deze ontwikkelingen hebben in Nederland er toe geleid dat HIV sinds 1985 en hepatitis C sinds 1992 niet meer door plasmaproducten worden verspreid.

Eenmaal besmet met het hepatitis C virus (HCV) ontwikkelt 80% van de patiënten een chronische hepatitis C. Deze chronische leverontsteking kan na jaren leiden tot ernstige leverschade (levercirrhose en leverfalen), leverkanker .

In **hoofdstuk 2** wordt de prevalentie van hepatitis C en het gebruik van antivirale medicatie beschreven. Gegevens voor deze studie werden verkregen uit een landelijke enquête gehouden onder alle Nederlandse hemofiliepatiënten (HiN-5). De studiepopulatie bestond uit 638 patiënten die voor 1992 behandeld waren met stollingsproducten. Van deze patiënten had 54% een chronische hepatitis C, en 32% was behandeld met antivirale therapie. De belangrijkste redenen om zich niet te laten behandelen waren: angst voor bijwerkingen, het ontbreken van laboratorium afwijkingen wijzend op leverschade en een verwachte lage kans op succes van de behandeling.

De invloed van hepatitis C op de kwaliteit van leven bij patiënten met hemofilie wordt onderzocht in **hoofdstuk 3**. Wederom werd gebruik gemaakt van gegevens verkregen uit de landelijke enquête gehouden onder alle Nederlandse hemofiliepatiënten (HiN-5). Kwaliteit van leven werd bepaald door een vragenlijst, de RAND-36. Voor deze studie werden 602 patiënten geïncludeerd; al deze patiënten waren voor 1992 behandeld met stollingsproducten en hadden de RAND-36 volledig ingevuld. Patiënten met hepatitis C scoorden lager op de RAND-36 dan patiënten zonder hepatitis C. Naast hepatitis C bleken leeftijd, ernst van de hemofilie, HIV, gewrichtsbeperking en werkeloosheid geassocieerd te zijn met kwaliteit van leven. Nadat gecorrigeerd was voor deze factoren, bleek hepatitis C geassocieerd te zijn met een lagere score op specifieke domeinen van kwaliteit van leven, namelijk 'vitaliteit' en 'algemene gezondheid'.

In **hoofdstuk 4** wordt de mate van leverfibrose bepaald in hemofilie patiënten met chronische hepatitis C. Dit gebeurde niet-invasief met behulp van de Fibroscan®. Met dit apparaat wordt de elasticiteit van de lever bepaald. De validiteit van het onderzoek met de Fibroscan® werd eerst onderzocht in een groep van 63 patiënten met hepatitis C maar zonder hemofilie. In deze subanalyse bleek dat de resultaten van de Fibroscan® sterk correleerden met die van de huidige 'gouden standaard', namelijk de leverbiopsie: correlatiecoëfficiënt 0.73, $p<0.001$).

In de studiepopulatie bestaande uit 121 patiënten met hemofilie en chronische hepatitis C bleek na onderzoek met de Fibroscan® dat 40% van de patiënten geen tot milde fibrose, 25% matige fibrose, 18% ernstige fibrose en 17% cirrhose had. Het merendeel van de patiënten met cirrhose (65%) zou niet als zodanig gediagnosticeerd zijn op basis van alleen laboratorium uitslagen en bevindingen bij echo. Van alle patiënten met een indicatie voor antivirale therapie (i.e. minstens matige fibrose) startte 25% binnen 3 maanden na het onderzoek.

Een groot deel van de hemofilie patiënten is geïnfecteerd met hepatitis C op jonge leeftijd. In **hoofdstuk 5** worden risicofactoren voor het krijgen van hepatitis C alsook het natuurlijk beloop van deze infectie bepaald. De studiepopulatie bestond uit 120 patiënten, allen geboren in de periode 1976-1992 en behandeld met HCV-onveilige stollingsproducten. Van deze 120 patiënten, raakte 57% (68 patiënten) geïnfecteerd met hepatitis C. Eenmaal geïnfecteerd ontwikkelde 65% chronische hepatitis C en 35% klaarde het virus spontaan. Risico op infectie met hepatitis C was geassocieerd met een langere behandelperiode met onveilig stollingsproduct en blootstelling aan een hoog aantal donoren (i.e. vooral blootstelling aan producten gemaakt uit plasma van vele donoren). Spontane klaring van HCV was geassocieerd met een jonge leeftijd ten tijde van besmetting. Uiteindelijk ontwikkelde 5% van de patiënten met chronische hepatitis C een levercirrhose na een infectieduur van 21 jaar.

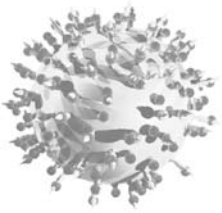
In **hoofdstuk 6** wordt het risico op het ontwikkelen van ernstige leverziekte bepaald. Ernstige leverziekte werd gedefinieerd als leverfalen, hepatocellulair carcinoom of dood door leverziekte. Voor deze studie werden alle patiënten met antilichamen tegen hepatitis C geïncludeerd uit drie hemofilie centra (Sheffield, London, Utrecht). In totaal werden 847 patiënten geselecteerd; 19% klaarde HCV spontaan en 81% ontwikkelde chronische hepatitis C. Na 35 jaar HCV infectie, was het risico op ernstige leverziekte 12% in HIV negatieve en 35% in patiënten met een HIV co-infectie. Onafhankelijke risicofactoren voor ernstige leverziekte waren: co-infectie met HIV, oudere leeftijd ten tijde van besmetting, alcohol misbruik en het besmet zijn met HCV genotype 1.

Hoofdstuk 7 beschrijft de tot nu toe gepubliceerde literatuur over antivirale therapie in hemofilie patiënten met chronische hepatitis C. In totaal werden 35 studies met 1151 patiënten geselecteerd. De data van de verschillende studies werden samengevoegd en gestratificeerd volgens type behandeling en HIV status. Succes van therapie werd weergegeven als sustained virological response (SVR): geen HCV-RNA meer aantoonbaar in het bloed een half jaar na het staken van therapie. In HIV negatieve patiënten die nog nooit eerder behandeld waren was de SVR 22% voor interferon monotherapie, 43% voor de combinatie van interferon met ribavirine en 57% voor Peg-interferon met ribavirine. In patiënten met een HIV co-infectie resulteerde interferon monotherapie in een SVR van 8% en in een SVR van 39% voor de combinatie van interferon met ribavirine. Deze resultaten geven weer dat de behandeling van hepatitis C bij hemofilie patiënten even effectief lijkt te zijn als in de algemene populatie.

In **hoofdstuk 8** wordt gekeken naar het succes van antivirale therapie en de factoren die dit beïnvloeden. Vervolgens wordt er gekeken naar de duurzaamheid van dit effect en het optreden van ernstige leverziekte na het beëindigen van de therapie. In een multicenter cohort studie werden alle patiënten geselecteerd die behandeld waren met antivirale therapie. In totaal werden er 295 patiënten geselecteerd; 235 met chronische hepatitis C en 60 met chronische hepatitis C en HIV. In HIV negatieve patiënten was de SVR voor interferon monotherapie 29%, 44% voor de combinatie van interferon met ribavirine en 63% voor Peg-interferon met ribavirine. In patiënten met een HIV co-infectie was de SVR voor interferon monotherapie 20% en voor de combinatie van (Peg-)interferon met ribavirine 48%. De kans op succes was groter bij patiënten met genotype 2 en 3 en bij patiënten die behandeld waren met combinatietherapie van (Peg-) interferon en ribavirine. Alle patiënten met een SVR bleven HCV negatief gedurende een maximale follow-up van 15 jaar en niemand ontwikkelde ernstige leverziekte. Dit in tegenstelling tot patiënten die zonder succes behandeld werden: 15 jaar na het staken van de therapie was het risico 13% voor het ontwikkelen van ernstige leverziekte.

Tenslotte worden in **hoofdstuk 9** het effect en de bijwerkingen beschreven van Peg-interferon in combinatie met ribavirine. In totaal werden 56 patiënten met deze therapie behandeld. De SVR voor patiënten zonder HIV die nog niet eerder behandeld waren was 70%. Een succesvolle behandeling was geassocieerd met HCV genotype 2 en 3, afwezigheid van HIV en een daling van de HCV load op week 4 en week 12. Bij 11% van de patiënten werd de therapie gestaakt vanwege bijwerkingen. 22% van de patiënten ontwikkelde een depressie en één patiënt een psychose.

Dankwoord



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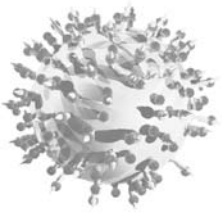
Curriculum Vitae



De auteur van dit proefschrift werd op 3 december 1976 geboren te Ermelo. Hij volgde het middelbaar onderwijs in Amersfoort (gymnasium aan het Van Lodestein College) en behaalde het eindexamen in 1995. Aansluitend begon hij aan de studie Farmacie aan de Universiteit Utrecht (propedeuse behaald in 1997). In 1996 begon hij met de studie Geneeskunde aan de Universiteit Utrecht, welke in 2002 werd afgerond. Vanaf januari 2003 tot februari 2006 werkte hij als arts-onderzoeker in de Van Creveldkliniek in het Universitair Medisch Centrum Utrecht. Tijdens deze periode werd ook de opleiding tot klinisch epidemioloog aan het Netherlands Institute for Health Sciences (NIHES) te Rotterdam succesvol afgerond. Sinds maart 2006 is hij werkzaam als arts-assistent op de afdeling Interne Geneeskunde van de Gelre Ziekenhuizen te Apeldoorn.

De auteur is gehuwd met Marlies Posthouwer-Veldhuijzen en samen hebben zij een zoon (Jasper) en een dochter (Judith).