Co-morbidity in adult haemophilia patients

Dietje Elisabeth Fransen van de Putte

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Co-morbidity in adult haemophilia patients

Co-morbiditeit bij volwassen hemofiliepatiënten

(met een samenvatting in het Nederlands)

Proefschrift

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Dietje Elisabeth Fransen van de Putte
geboren op 17 april 1980 te Delft

Promotor:	Prof. dr. D.H. Biesma
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	Dr. K. Fischer

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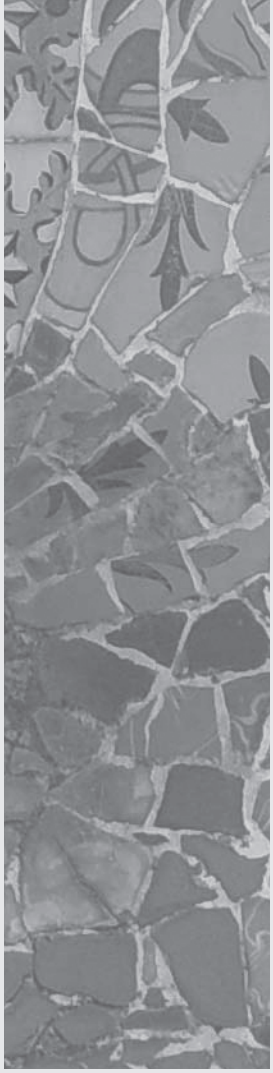
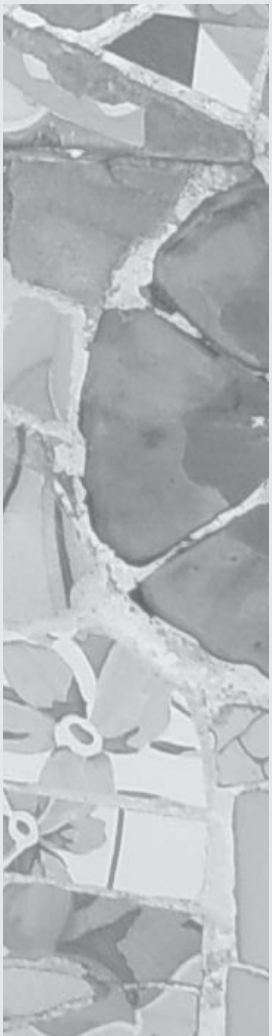
Chapter 1

Introduction





Haemophilia is an X-linked inherited bleeding disorder. Patients with haemophilia A (85% of all haemophilia patients) have a deficiency of clotting factor VIII, while patients with haemophilia B lack clotting factor IX. Haemophilia patients can be classified according to their residual factor VIII or IX activity (Table 1): patients with < 1% factor activity have severe haemophilia, patients with 1-5% activity have moderate disease and patients with 6-40% activity have mild haemophilia. Factor activity levels above 40% are considered normal and do not lead to increased bleeding risks. Patients with severe haemophilia experience spontaneous bleeding, most often in muscles and joints, which can lead to significant morbidity and impairment. Frequent joint bleeding causes joint damage and arthropathy. Moderate and mild haemophilia are usually not associated with spontaneous bleeding, but these patients do have an increased bleeding risk after (minor) trauma and during and after surgery.

Table 1. Classification of haemophilia A and B according to disease severity.

Haemophilia severity	Remaining clotting factor activity	Symptoms
Severe	< 1%	Frequent spontaneous bleeding in muscles and joints; abnormal bleeding after minor injuries, surgery or tooth extractions
Moderate	1-5%	Spontaneous bleeding is rare; abnormal bleeding after minor injuries, surgery or tooth extractions
Mild	6-40%	No spontaneous bleeding; abnormal bleeding after major injuries, surgery or tooth extractions

Before the mid 1960s, treatment of bleeding episodes consisted of (long-term) bed rest, in combination with application of ice and fixation of the affected joint or limb, and occasional blood or plasma transfusions in case of life threatening bleeding. Later, treatment with cryoprecipitated factor VIII derived from small-pool frozen plasma became available for haemophilia A and prothrombin complex concentrates derived from large plasma pools for haemophilia B [1]. In the 1970s, factor VIII clotting factor concentrates could also be produced from large-pool donor plasma. Since 1988, recombinant clotting factor products, which are synthetical agents not produced from human blood, have been available [2]. Clotting factor concentrates can be administered to haemophilia patients either prophylactically (several times per week to prevent bleeding) or on demand (when bleeding occurs).

Older haemophilia patients, who received no or limited treatment for bleeding episodes in their youths, are now experiencing the consequences of lack of treatment in the shape of severe arthropathy, mainly in the ankles, knees and elbows. Surgical joint replacements (of the ankles, knees or elbows) or arthrodeses (of the ankles) are often indicated.

For younger patients, the availability of (prophylactic) treatment has enabled them to live (relatively) normal lives, without significant impairment [3,4].

After the introduction of clotting factor concentrates, life expectancy of haemophilia patients has increased dramatically, from 27 years in 1960 to 67 years in 2001 [5,6]. It is now approaching that of the general male population, especially in patients who are not infected with hepatitis C or HIV [6,7]. Figure 1 shows the age distribution of the 721 haemophilia patients who were treated at the Van Creveldkliniek in 2012. Of these patients, 44% were aged 40 years or older. One hundred patients (14%) were in their sixties or seventies, and eleven (1.5%) were eighty years or older. The number of patients with severe haemophilia aged 40 years or older who are treated at the Van Creveldkliniek has increased from zero in 1975 to 136 in 2012 (Figure 2).

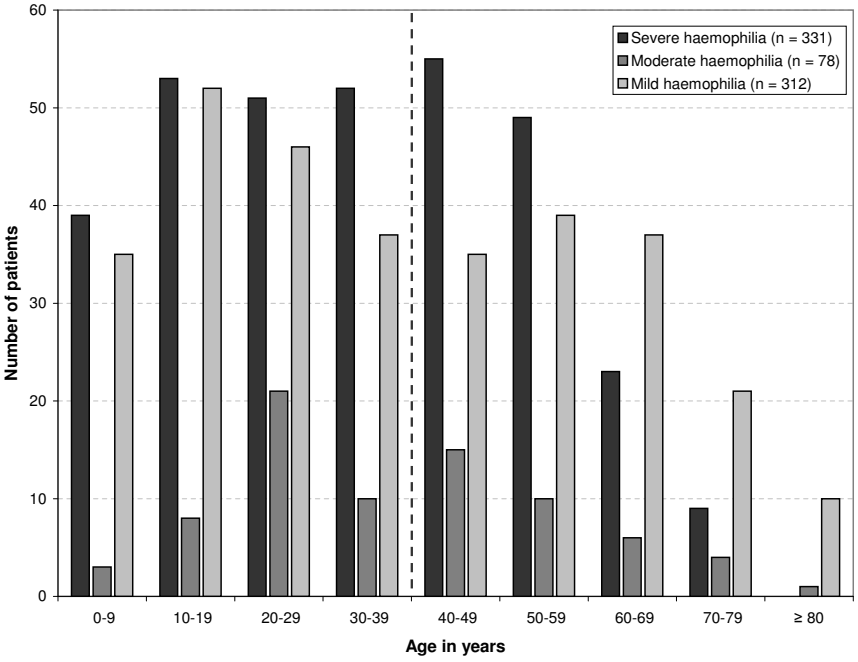


Figure 1. Age distribution of the 721 haemophilia patients who were treated at the Van Creveldkliniek in 2012. The dotted vertical line indicates age 40 years.

Definition of co-morbidity

The term 'co-morbidity' indicates any medical condition existing simultaneously with, but independently of a patient's primary disease [8]. In haemophilia patients, this includes all medical problems which are not directly haemophilia-related.

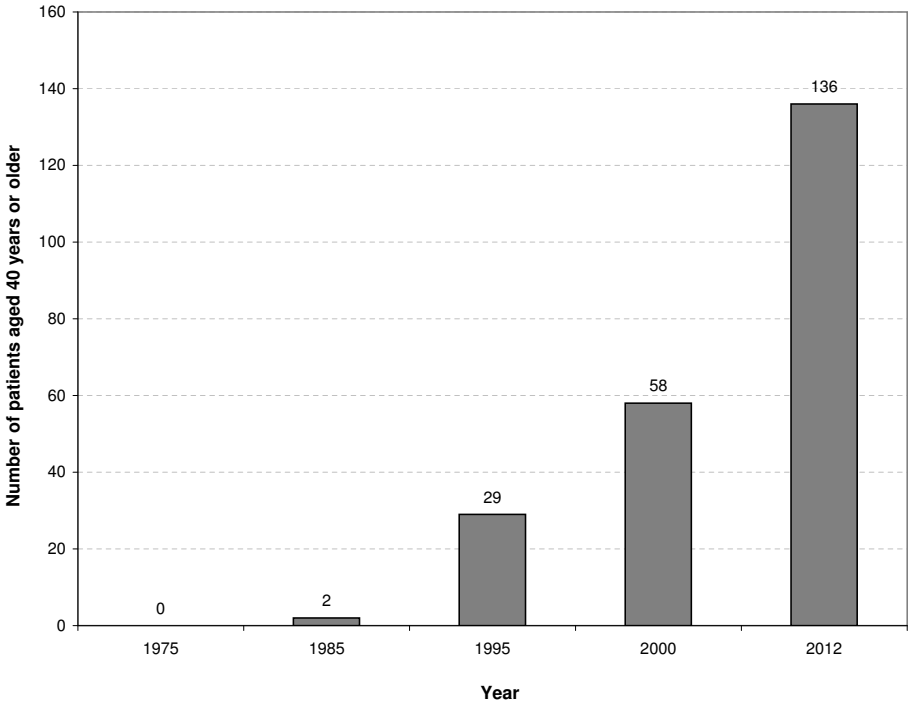


Figure 2. Number of patients with severe haemophilia aged 40 years or older who were treated at the Van Creveldkliniek between 1975 and 2012.

Age-related co-morbidity

As a consequence of their improved life expectancy, haemophilia patients are increasingly experiencing age-related types of co-morbidity, such as cardiovascular disease (CVD) and malignancies. The interest in these types of co-morbidity is increasing. Because, until recently, the number of older haemophilia patients was relatively small, no large studies on co-morbidity in this patient population could be performed, and little was known about the prevalence of age-related problems in haemophilia patients and whether there are any differences between haemophilia patients and non-haemophilic males. A mortality study, with follow-up until 1998, reported increased mortality from bleeding complications, liver disease and Hodgkin disease, but decreased mortality from ischemic heart disease in 6018 HIV negative haemophilia patients from the United Kingdom compared with the general population [7]. A decreased mortality from ischemic heart disease has also been reported in Dutch haemophilia patients [6,9]. Little is known, however, about the occurrence of non-fatal CVD in haemophilia patients, and the association with known cardiovascular disease risk factors. Reports on the prevalence of hypertension in haemophilia patients, for example, are conflicting, with some studies reporting increased prevalences [9-11], while others report no differences with

the general population [12-14]. The prevalence of hypercholesterolemia is reported to be lower in haemophilia patients [9,15], while the prevalences of other CVD risk factors such as smoking and overweight appear to be similar in haemophilia patients and the general population [9,12,14,16].

Virus-related co-morbidity

Unfortunately, in the past, clotting factor products turned out to be contaminated with hepatitis C (HCV) and/or human immunodeficiency virus (HIV). Between 1979 and 1985, depending on the type of clotting factor products used in different countries, 1% to 62% of haemophilia patients were infected with HIV [17-19]. In our own centre, 16% of patients were infected [20]. Before the introduction of highly active antiretroviral treatment (HAART) in 1996, many HIV infected patients died. Moreover, before the introduction of adequate inactivating methods for HCV in 1992, nearly all haemophilia patients who were treated with large-pool clotting factor products were infected with HCV [21-24]. The course of HCV infection has been studied extensively in patients with and without inherited bleeding disorders. About 20% of infected patients spontaneously clear the virus, while 80% develop chronic hepatitis C [22,25-27]. In these patients, the virus can be present in the liver for several decades, without causing any clinical problems. Over time, however, progressive scarring (fibrosis) of the liver tissue occurs, resulting in severe liver damage with impaired liver function (cirrhosis). This, in turn, can lead to complications such as liver failure, portal hypertension, bleeding of esophageal varices, hepatocellular carcinoma and death (Figure 3). After over three decades of HCV infection, 8% of patients with inherited bleeding disorders and chronic HCV, from an international study including patients from Utrecht, Sheffield and London, were reported to have developed end-stage liver disease [28]. Co-infection with HIV, alcohol abuse, older age at infection and the presence of HCV genotype 1 are associated with faster progression of liver disease [27-30].

Antiviral treatment against hepatitis C with interferon became available in 1987 [31]. In 1995 ribavirin was added, and since 2000 a combination of pegylated interferon (PegIFN) and ribavirin is used [32,33]. The aim of antiviral treatment is to eradicate the hepatitis C virus and stop progression of liver damage. Treatment duration is 24 or 48 weeks, depending on HCV genotype and treatment effect. Currently, treatment is successful in about 50% of patients with HCV genotypes 1 or 4, and in 80-90% of patients with genotypes 2 or 3 [32,33]. Unfortunately, antiviral treatment has many side-effects. This, in combination with the relatively low success percentage, especially in HCV genotype 1, which is most prevalent in haemophilia patients, has resulted in many patients being reluctant to start antiviral treatment. To ensure optimal timing of antiviral treatment in HCV infected patients (neither too soon, exposing patients to

unnecessary side-effects, nor too late, increasing the risk of irreversible liver damage), it is important to know the extent and progression rate of liver damage.

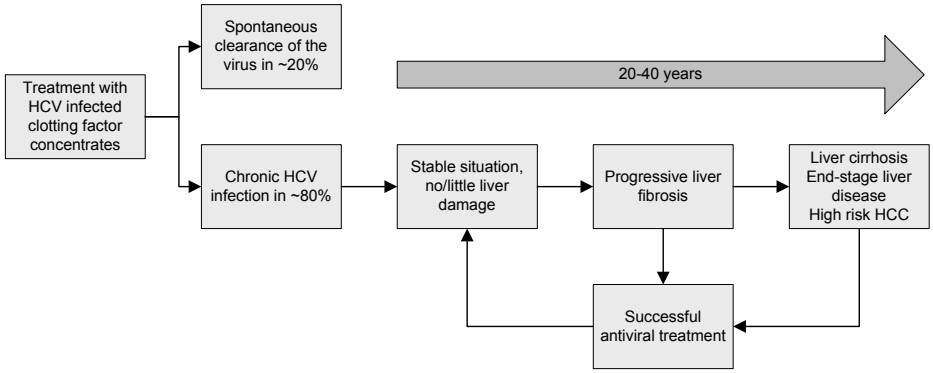


Figure 3. The course of hepatitis C infection over time.
HCV = hepatitis C virus
HCC = hepatocellular carcinoma

In patients with no or limited liver fibrosis, antiviral treatment can be postponed, while in patients with extensive or progressive damage, treatment will be strongly recommended. Liver biopsy is still the golden standard for the assessment of the extent of liver damage. This procedure, however, has many disadvantages. Only a very small part of the liver is assessed, resulting in a risk of sampling error. Liver biopsy also has relatively large intra- and inter-observer variability and carries a risk of significant complications [34-36]. Moreover, because of its invasive nature, regular use of liver biopsies to assess progression of fibrosis is not considered feasible. Because of a high bleeding risk and the need for expensive clotting factor correction during and after the procedure, liver biopsies are relatively contra-indicated in patients with inherited bleeding disorders. A non-invasive alternative for the assessment of liver fibrosis is liver stiffness measurement (LSM) using Fibroscan®. With increasing liver fibrosis, the developing scar tissue increases the stiffness of the liver. This stiffness can be used as a measure of the extent of fibrosis (Figure 4) [37-39].

Liver stiffness measurement is quick and non-invasive, thus avoiding expensive clotting factor substitution in haemophilia patients. LSM has also been reported to be very reliable, especially for the detection of high grades of fibrosis or cirrhosis [37,40] and can be an ideal tool to assess changes in the extent of fibrosis over time, especially in haemophilia patients. Little information is available, however, on the longitudinal use of LSM, either in 'regular' HCV patients or in HCV infected patients with congenital bleeding disorders.

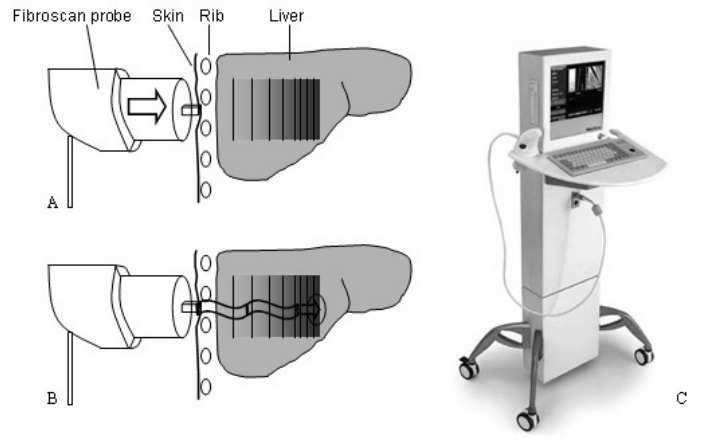


Figure 4. Liver stiffness measurement using Fibroscan®.
A. Transmission of low-frequency radio waves to induce an elastic shear wave in the liver.
B. Transmission of a pulse-echo ultrasound wave measuring the velocity of movement of the shear wave in the liver.
C. Fibroscan® device (Echosens, Paris).

Outline of this thesis

Part I. Age-related co-morbidity

The occurrence of age-related co-morbidity is increasing in haemophilia patients, but little is known about these types of co-morbidity, and the studies which are performed are based on mortality data, or small study cohorts, often with methodological flaws. **Chapter 2** provides an overview of the data that were available on the occurrence of co-morbidity in the ageing haemophilia population in 2009. In **chapter 3**, a retrospective evaluation of the occurrence of cardiovascular disease, malignancies and other types of co-morbidity, using medical records of 408 haemophilia patients born before 01-01-1971, who were treated at the Van Creveldkliniek at any point during the period 1985-2010, is presented.

In 2009, we started an international multicentre prospective study assessing the occurrence of CVD events in 709 haemophilia patients aged 30 years or older from The Netherlands and the UK. **Chapter 4** is a short report on the history of non-fatal CVD in this study cohort. In **chapter 5**, the prevalences of CVD risk factors in this cohort were compared with the general population, and overall risk profiles were calculated using the QRISK®2-2011 cardiovascular risk score. **Chapter 6** provides more detailed data on the prevalence of hypertension in the patients who were enrolled in this prospective study.

Part II. Virus-related co-morbidity

The course of hepatitis C infection, the effect of antiviral treatment and the occurrence of end-stage liver disease in 847 patients with inherited bleeding disorders from the Netherlands and the UK were described by Posthouwer et al in 2007 [28,41]. **Chapter 7** adds an additional six years of follow-up to this cohort, assessing natural history of hepatitis C and treatment effects on the very long term.

Antiviral treatment has many physical and mental side-effects, but studies assessing these side-effects and their psychosocial impact in haemophilia patients are limited. In **chapter 8**, the occurrence and course of various side-effects and changes in health-related quality of life during antiviral treatment in 47 patients with inherited bleeding disorders are described, thus providing an insight in the overall burden of this treatment. In **chapter 9**, the occurrence and course of depression during antiviral treatment is discussed in more detail.

Liver stiffness measurement using Fibroscan® is a promising, non-invasive alternative for performing liver biopsies to assess the extent and progression of fibrosis. Since this method is relatively new, its value and optimal use are still under debate, and follow-up data are lacking. **Chapter 10** describes our experience with the longitudinal use of LSM to assess fibrosis progression in 84 patients with inherited bleeding disorders who were not, or not successfully, treated for their HCV infection. In **chapter 11**, LSM was used to assess improvement of liver fibrosis after successful antiviral treatment.

Because in many countries large proportions of HIV infected haemophilia patients died before the introduction of HAART, long-term follow-up of these patients is often limited. **Chapter 12** describes the course and complications of HIV infection in the 60 HIV infected haemophilia patients who were followed for over 25 years at the Van Creveldkliniek.
Chapter 13 provides a general discussion of the findings described in this thesis.

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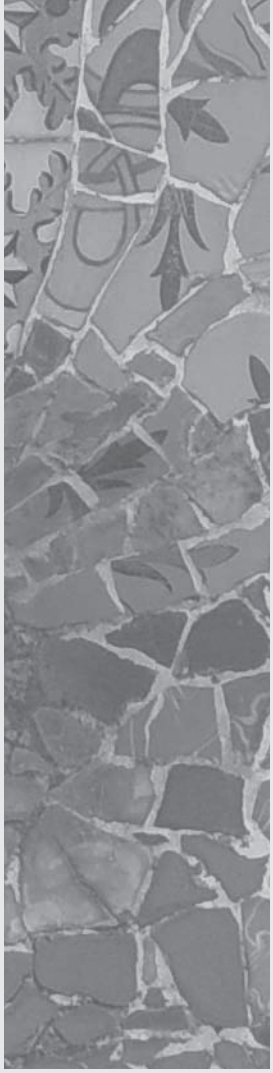
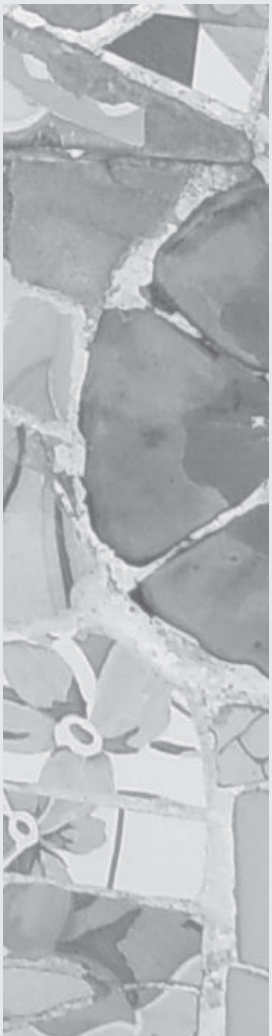


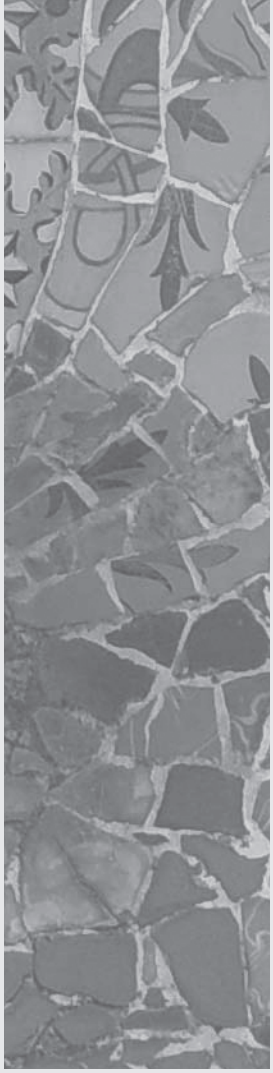
Part I

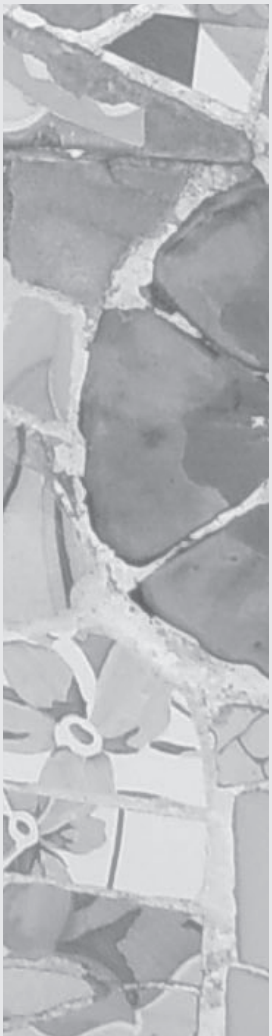
Age-related co-morbidity



Chapter 2

Co-morbidity in the ageing haemophilia patient: the down side of increased life expectancy





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D.E. Fransen van de Putte
R.E.G. Schutgens

Haemophilia 2009; 15: 853-863

Abstract

Because of an increased life expectancy, (age-related) co-morbidity is becoming a common occurrence in haemophilia patients. In this review article, haemophilia-related and non-haemophilia-related medical problems, treatment recommendations and psychosocial consequences in ageing haemophilia patients are discussed.

Haemophilic arthropathy is an important cause of pain and disability, and a frequent indication for surgery in haemophilia patients. In addition, many adult patients are infected with hepatitis C or HIV, the consequences and treatment of which can add to physical and mental discomfort. Moreover, inhibitors against factor VIII can also develop in adulthood, especially in patients with mild haemophilia. Hypertension is reported to occur more often in haemophilia patients than in the general population. Other internal problems, like renal abnormalities, overweight, diabetes mellitus and hypercholesterolemia are discussed. Haemophilia seems to protect against cardiovascular disease, although the incidence is increasing. Recommendations are given on dealing with tooth extractions, surgical interventions and sexuality problems in patients with haemophilia.

In addition to haemophilia in itself, co-morbidity has a major psychological impact, and an important effect on quality of life. It can also result in complex treatment regimens, in which coordination between health care workers is essential.

Introduction

Haemophilia is no longer a disorder of children and young adolescents. Thanks to the introduction of clotting factor concentrates and prophylactic treatment, life expectancy of patients with haemophilia in industrial countries has increased from 7.8 years in 1939 to over 70 years in 2001 [1-3]. When excluding individuals infected with human immunodeficiency virus (HIV) and hepatitis C virus (HCV), life expectancy of Dutch patients with mild and moderate haemophilia approached that of the male population in general (75 years compared to 76 years), and life expectancy of patients with severe haemophilia increased to 71 years [3]. Similar results were reported in studies from the United Kingdom and Greece [1,4].

Nowadays, a substantial proportion (39%) of all patients treated at the Van Creveldkliniek, a large haemophilia treatment centre in the Netherlands, are born before 1967 (Figure 1).

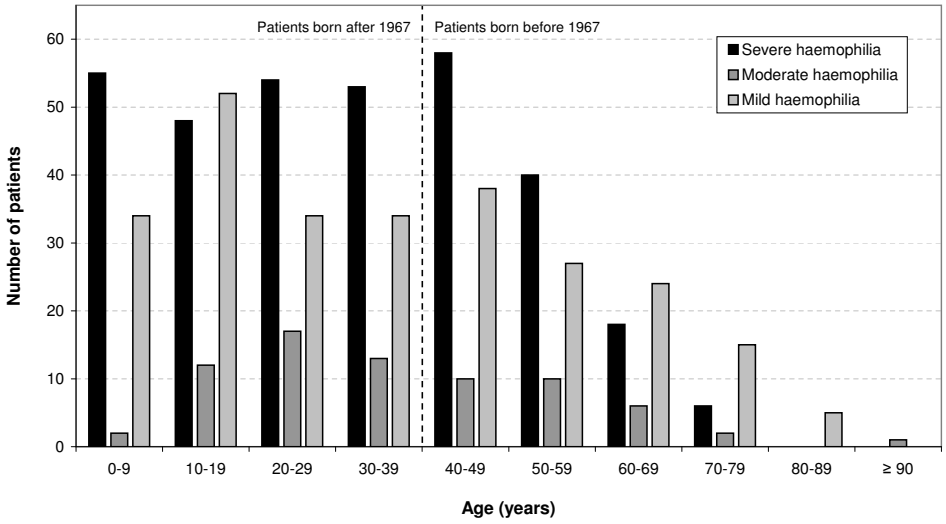

Figure 1. Number of patients, according to age and severity of haemophilia, treated at the Van Creveldkliniek in 2007 (n = 668) [9].

Elderly haemophilia patients have different problems compared with the younger generation. They not only have to live with premature arthropathy due to lack of treatment, and with HCV and/or HIV infection, they are also confronted with age-related ailments. So a growing number of haemophilia patients will, besides co-morbidity related to haemophilia, suffer from co-morbidity like cardiovascular disease, urological problems and cancer. Little is published on haemophilia and co-morbidity, but reports on cardiovascular disease are emerging [5,6]. Several studies report a reduced mortality due to ischemic cardiovascular disease in haemophilia patients compared with the general

age-matched male population, but the number of deaths due to ischemic heart disease is increasing [1,3,4,7,8]. In the Netherlands, between 1972 and 2001, death caused by ischemic heart disease increased from 2% to 6%. An American study demonstrated that the age-specific prevalence of ischemic heart disease in haemophilia patients ranged from 0.05% in those aged under 30 years to 15.2% in those 60 years or older [5].

This review focuses on co-morbidity in the ageing haemophilia patient and its consequences (Table 1). It is partially based on the book 'Aging with haemophilia; medical and psychosocial impact' [9].

Co-morbidity is defined as the effect of all other diseases an individual patient might have, other than the primary disease of interest.

Table 1. Co-morbidity and (medical) problems in ageing haemophilia patients.

Haemophilia-related items	Non-haemophilia-related items
Haemophilic arthropathy	Internal diseases
Chronic hepatitis C infection	Cardiovascular disease
HIV infection	Malignancy and surgical interventions
Inhibitor development	Tooth extraction
Sexual problems	
Psychosocial impact	
Quality of life	

Haemophilic arthropathy

Haemophilia patients born before the introduction of clotting factor concentrates will suffer from haemophilic arthropathy. Patients born before 1950 generally have four to six arthropathic joints [10]. These joints have a limited range of motion and are more or less painful and/or stiff. Patients experience limitations in their activities of daily life and various restrictions in participation in society. Treatment may be complicated and exists of various components, such as analgesics, rehabilitation and surgical interventions.

Pain

Wallny et al studied pain in a group of 91 adult haemophilia patients with a mean age of 43 years. On average, they had four joints with major pain: ankle (45%), knee (39%), elbow (7%) and hip (6%) [11]. Fourteen percent of patients complained of distressing pain in the spine. Fifty percent of patients had pain throughout the day, when no treatment was given. These findings were confirmed by Van Genderen et al, who found that 36% of patients who indicated to have pain used analgesics [12]. Since pain has an

impact on quality of life and daily functioning, it has to be addressed adequately [12,13]. Treatment consists of pain medication, distal traction transcutaneous electrical nerve stimulation (TENS) and hot packs.

Adequate pain medication can be prescribed according to the guideline in Table 2. Since codeine and morphine often lead to constipation, prescription of a laxative drug is mandatory. Morphine may cause nausea, which improves with time and can be treated with rectal metoclopramide.

Diclophenac and other non-steroidal anti-inflammatory drugs are theoretically contra-indicated and should, in general, be avoided, because they may affect platelet function and may increase bleeding tendency. However, some patients with chronic arthropathic pain may benefit from ibuprofen, without bleeding complications [14]. Although others have reported increased bruising and gastrointestinal bleeding [15,16], Rattray et al reported a positive effect of cyclo-oxygenase 2 (COX-2) inhibitors on chronic pain in a small group of patients with haemophilia [17]. When given in commonly used doses, COX-2 inhibitors do not increase the risk of cardiovascular disease [18].

Table 2. Guideline for the use of analgesics for haemophilic arthropathy.

1. Paracetamol (500-1000 mg, max 6 times a day) is the initial medication of choice; if not effective:
2. Paracetamol and a muscle relaxant (diazepam 5 mg, 1-3 times a day), or:
3. Paracetamol and codeine (10-20 mg, max 6 times a day).
4. Tramadol is indicated for very severe pain (50-100 mg, 3-4 times a day).
5. Morphine: use a slow release product, starting with 20 mg 2 times a day, with an escape of a rapid release product 10 mg 4 times a day. Increase the slow release product if the rapid release product is used more than 4 times a day.

Rehabilitation and physical therapy

Physical therapy, under the guidance of an experienced therapist, is an integral component of prevention and treatment of joint arthropathy [19]. The goal of physical therapy is restoration or maintenance of range of motion, muscle strengthening, prevention or treatment of articular contracture, pain management, and improved balance, coordination, and proprioception [20]. In the elderly haemophilia patient, physical therapy will focus on functional training and remedial exercise. Functional training may include hydrotherapy, walking (with or without aid), climbing stairs and cycling. In some cases orthotics and/or adaptations at home or at work may further support good daily functioning [21]. After orthopedic surgery, intensive physical therapy is also indicated. Training and goals are the same as mentioned above.

Orthopedic surgery

If conservative management fails (analgesics, orthotics, physical therapy), and pain and disability are intense, orthopedic intervention is indicated. The most common surgical procedures used for haemophilic arthropathy in the ageing haemophilia patient are arthrodesis (joint fusion), osteotomy and joint arthroplasty [19,22]. The major indication for surgical intervention is pain. Arthrodesis is mostly performed for severe pain in arthropathic ankle joints [22]. Corrective osteotomy may be performed in case of arthropathy and axial deformity, particularly around the hip, knee and ankle [23]. Most total joint replacements in haemophilia patients are performed on hips and knees. After total knee replacement (TKR), pain relief is obtained in the vast majority of patients, but outcome of range of motion is variable [24]. The long-term survival of joint replacement in haemophilia is reported to be 90% at 5 years and 85% at 10 years, which is lower than in patients with rheumatoid arthritis, in whom survival rates are 90-95% after 10-15 years [25-27]. Late infection, often occurring years after surgery, is the most common reason for failure in haemophilia [25,26]. Haemophilic arthropathy develops at a young age, which is reflected by the average age (21-40 years) of patients who have undergone TKR [25,28]. The ultimate outcome in this younger population is not yet known. Elbow and shoulder arthroplasties are also performed in haemophilia patients, but results are not as good as those obtained for hips and knees [22]. Ankle replacement has not been proven to be better than arthrodesis.

Balance dysfunctions and risk of falls

Falls are associated with increased morbidity, mortality and referral to nursing homes. So far, there is little literature on the problem of falling in patients with haemophilia. A study on this subject was initiated by Street et al [29]. The results are still pending. According to Rao et al, risk factors for falls include muscle weakness, a history of falls, arthritis (especially of the knee), and impairment in gait and activities in daily living [30]. In general, patients with haemophilic arthropathy have several of these risk factors. The most effective preventive strategies are multi-factorial interventions targeting identified risk factors, balance training and exercises for muscle strength [29,30].

Osteoporosis

Wallny et al studied 62 male patients with severe haemophilia, with a mean age of 41 years. Reduced bone mineral density (BMD) was found in 43.5% and osteoporosis in 25% [31]. Number and severity of arthropathic joints were associated with lower BMD in the neck of the femur. Painful haemophilic arthropathy with reduced mobility and lack of activity may lead to a reduction of bone mass. Additional risk factors were chronic HCV, low body mass index (BMI) and age. These findings were confirmed in a study by Mansouritorghabeh [32]. Weight-bearing physical activity (sports), physical

therapy, surgery to remobilize patients and calcium and vitamin D supplementation are recommended [33].

Hepatitis C

Chronic hepatitis C infection is a major co-morbidity in patients with inherited bleeding disorders and a leading cause of morbidity and mortality in haemophilia patients [1,3,9]. Before the introduction of adequate viral inactivation methods for HCV in 1992, 98% of patients treated with large pool products and 66% of patients treated with cryoprecipitate were infected with HCV [34-36]. Eighty percent of these patients developed chronic HCV, which may cause liver fibrosis progressing to cirrhosis. In an international cohort consisting of 847 haemophilia patients infected with HCV, Posthouwer et al demonstrated a cumulative incidence of end-stage liver disease (ESLD) of 17.1% after 35 years of infection [37]. In HIV co-infected patients, the cumulative incidence was 35.1%. Fifty-five patients died from HCV-related diseases; 13 patients developed hepatocellular carcinoma.

Therapy for HCV consists of pegylated interferon (PegIFN) 1.5 μg/kg once a week, and ribavirin 800-1200 mg daily [38,39]. Duration and effectiveness vary with HCV genotype, and are the same for patients with haemophilia compared with other populations [40]. In general, patients with genotype 1 or 4 are treated for 48 weeks, with a success rate of 40-50%. Patients with genotype 2, 3 or 5 are treated for 24 weeks, with a success rate of 80-90% [39]. Unfortunately, most haemophilia patients are infected with genotype 1. This implicates that around half of patients are treated without success and are at risk of developing ESLD. For these patients, the development of new antiviral drugs is of particular importance. Recently, HCV protease and polymerase inhibitors have been developed and tested in clinical trials. One of the most promising is VX-950, a HCV NS3.4 protease inhibitor with success rates of 100% after 12 weeks of combination therapy with PegIFN alpha-2a [41,42].

Side-effects of treatment with interferon and ribavirin are common. Flu-like symptoms and weight loss in excess of 5 kg are seen in over 60% of patients. Psychological side-effects, like irritability and concentration problems, occur in more than 80% of patients, and severe depression requiring antidepressant drugs in one fifth of patients [43]. Weight loss and haematological toxicity (anaemia, neutropenia or thrombocytopenia) are the main reasons for dose adjustment. Over 10% of haemophilia patients withdraw from treatment because of side-effects [40]. Flu-like symptoms and fever can be treated with paracetamol. To further improve compliance, erythropoietin can be given to treat anaemia, and antidepressants (in particular selective serotonin reuptake inhibitors, SSRIs) for psychiatric symptoms.

HIV

Haemophilia patients treated with plasma-derived clotting factor concentrates were exposed to HIV between 1978 and 1986. Infection rates vary from 1% in Finland to 42.1% in the USA and even 90% of patients who were intensively treated [44-46]. Before the introduction of highly active antiretroviral therapy (HAART) in 1996, many patients died. Progression to AIDS was most rapid in patients older than 35 years at time of infection [46]. Twenty-seven to 35% of infected haemophilia patients have survived 20 to 25 years after infection [46,47]. Most of them are treated with HAART, but a small minority of infected patients shows little or no clinical or laboratory progression and remains well without therapy [48].

Side-effects of HAART

Most HAART regimens consist of nucleoside analogues (NRTIs), combined with a non-nucleoside analogue (NNRTI) or a protease inhibitor (PI). Long-term side-effects are metabolic complications, such as diabetes mellitus, hyperlipidemia, elevated total cholesterol, lipodystrophy, abnormal fat distribution and osteopenia, and hepatotoxicity [49,50]. Since the introduction of PI, the incidence of ischemic cardiovascular events has increased: the relative rate of myocardial infarction per year of PI exposure was 1.16 (95% CI 1.09-1.23) in a cohort of 23 437 patients infected with HIV. After correction for other cardiovascular risk factors, an incidence of 6.0 per 1000 person years was found for patients exposed for more than six years [49]. All NRTIs can cause lactic acidosis and severe hepatomegaly with steatosis, and some NNRTIs are associated with drug induced hepatitis [50]. Increased bleeding tendency due to PI has been reported in 15% of patients [51-53]. In case of an increased bleeding tendency, prophylaxis with clotting factor concentrates has to be adjusted. When this has no or little effect, discontinuation of the protease inhibitor may be warranted.

Inhibitor development in elderly haemophilia patients

Most inhibitors against factor VIII develop in children with severe haemophilia, within the first 50 exposure days [54]. There is no literature on the development of inhibitors against factor IX in patients with mild haemophilia B, but in patients with mild haemophilia A, inhibitors are predominantly seen in adulthood, especially in patients who were never exposed to factor VIII [55]. This may cause an increasing problem, as haemophilia patients at an advanced age may encounter age-related health problems, which require medical intervention or surgery, for which clotting factor correction is indicated. Therefore, these patients should be monitored carefully, and seen regularly at a haemophilia treatment centre. After each surgery for which clotting factor concentrates were given, inhibitory antibodies against factor VIII or IX should be checked.

Inhibitors in mild haemophilia are often directed against patients' native factor VIII, actually making them severe haemophiliacs. Bleeding pattern, however, is different. Mostly, huge subcutaneous and muscle bleedings occur, whereas joint bleedings are less frequently seen.

Treatment of bleedings

Treatment of bleedings consists of activated prothrombin complex concentrate or recombinant factor VIIa [55-57]. Patients with inhibitors in whom native factor VIII is still present can be treated with DDAVP [58].

Immune tolerance induction

In the majority of patients with mild haemophilia A and inhibitors, antibodies will disappear spontaneously over time [55]. Immune tolerance induction with regular infusion of factor VIII is successful in only 25% of these patients [59,60]. Few reports have been published on the use of rituximab to obtain immune tolerance, with varying results [59,61].

Internal Diseases

Hypertension

Some studies show that haemophilia patients have higher mean blood pressures, have twice as often hypertension and use more antihypertensive medication compared with the general population [62-64]. An explanation might be that the incidence of renal insufficiency is higher in patients with haemophilia [65,66]. This may be caused by renal bleeding in the past, HIV infection, or medication like tranexamic acid. Hypertension increases the risk of intracranial bleeding and myocardial infarction. Since they are at higher risk of developing hypertension, blood pressure in haemophilia patients should be regularly checked and hypertension adequately treated.

Renal abnormalities

Renal abnormalities in haemophilia patients were investigated by Prentice et al in 1971 and Beck and Evans in 1972 [65,67]. Abnormalities on intravenous pyelography and isotope renography were found [65]. The majority of lesions were seen in the upper renal tract and were apparently the result of clot formation. This may be negatively influenced by the use of tranexamic acid during haematuria, as this drug affects physiological fibrinolysis and may cause acute renal obstruction [65]. A more recent publication describes a strong association between hypertension and HIV, and chronic and acute renal disease in haemophilia patients [68]. An association with inhibitors and recent haematuria was described.

Overweight

Between 1992 and 2001, the prevalence of overweight (BMI 25-30 kg/m^2) has increased from 27% to 35% in adult Dutch haemophilia patients [69]. Overweight occurred significantly less frequently in haemophilia patients than in the general Dutch male population, in which the prevalence of overweight was 41% in 1992 and 50% in 2001. The prevalence of obesity (BMI > 30 kg/m^2) doubled in adult haemophilia patients from 4% to 8%. The prevalence of obesity was not significantly decreased compared to the prevalence in the general population of 5% in 1992 and 8% in 2001 [69]. Increased body weight is a risk factor for development of diabetes mellitus (DM), atherosclerosis and cardiovascular disease, and may further damage arthropathic joints due to an increase in weight-bearing. It is important for haemophilia patients not to be overweighed. Therefore, regular physical activity should be advised. If functional limitations limit daily activities, a physical therapist familiar with haemophilia may be of help.

Diabetes mellitus

The prevalence of diabetes mellitus in haemophilia is not well documented. Walsh et al reported a prevalence of 24% in a cohort of 47 patients with mild haemophilia A, compared with 6.1% in 33 control males (mean age in both groups 46 years) [63]. However, there have been no other studies published to confirm these findings. If treatment with insulin is indicated, subcutaneous injections can be applied without bleeding complications.

Cholesterol

Mean cholesterol levels of patients with haemophilia are lower than in the general population. Patients with severe haemophilia had lowest cholesterol levels, which would suggest an association between low cholesterol concentrations and the clotting factor deficiency or its treatment [62]. Another hypothesis is that viral infections influence both the immune system and liver function, and therefore have an effect on cholesterol levels. This is supported by the recent observation that chronic hepatitis C is associated with lower cholesterol concentrations [70].

Cardiovascular disease

Although hypertension is more frequently seen in this patient group, haemophilia seems to protect against ischemic cardiovascular disease. In the 1990s, hardly any deaths from cardiovascular disease were reported [62]. Although the number is increasing, Darby et al and Plug et al reported a lower incidence of deaths due to cardiovascular disease compared with the age-matched general population. Darby et al describe that, in the United Kingdom, the mortality from ischemic heart disease in haemophilia patients was only

62% (95% CI 51-76%) of that in the general population [1,3]. Hospital discharge rates for ischemic heart disease in the USA were lower compared with age-matched males [5]. Walsh et al, however, published a higher prevalence of heart disease compared with a control group: 18% and 9% respectively [63,71].

A possible association between the occurrence of myocardial infarction and administration of clotting factor concentrates has been described by Girolami et al. They report that thrombotic events occurred during or after the infusion of factor VIII concentrates, prothrombin complex concentrates, recombinant factor VIIa preparations or intravenous desmopressin administration in 29 of 42 published cases of myocardial infarction or other arterial occlusion in patients with haemophilia A [72]. With increasing age, more patients are likely to develop cardiovascular disease.

The lower incidence of ischemic heart disease in patients with haemophilia has been attributed to the hypocoagulable state of these patients compared with the general population, leading to a decreased tendency to form occlusive thrombi. It remains unclear whether the deficiency of coagulation factor VIII or IX also exerts a protective effect on the development of atherosclerosis. Studies concerning intima-media thickness in haemophilia patients report conflicting results [73-75]. Using B-mode ultrasound, Srámek et al found no differences in intima-media thickness of the carotid artery between patients with bleeding disorders and healthy controls. Intima-media thickness of the femoral artery was minimally reduced in patients with bleeding disorders compared with healthy controls (adjusted difference -0.078 mm, 95% CI -0.17 to 0.018 mm). Femoral artery walls were thinnest in individuals with moderate to severe haemophilia [75]. Another study, however, showed that the mean intima-media thickness was significantly lower in 50 patients with haemophilia (38 severe, 12 moderate) compared with control subjects [74].

Facing ischemic cardiovascular disease in haemophilia is a major challenge for all specialists involved. It is important to realise that the lack of specific guidelines for treatment of ischemic heart disease should not withhold haemophilia patients from optimal cardiac care. Adequate clotting factor concentrate correction in combination with an adapted anticoagulant and antiplatelet therapeutic schedule tailored to the individual patient profile is required, taking into account severity of haemophilia, severity of cardiovascular disease, age, inhibitor status and renal function. The balance between thrombosis and haemostasis requires a tight cooperation between haemophilia specialists and cardiologists. Sometimes adjusted prophylaxis with clotting factor concentrates is required [6]. Careful follow-up is indicated in all cases.

Stable angina pectoris in patients with mild haemophilia or those on prophylaxis with clotting factor concentrates can be treated with 80 mg aspirin daily. When bleeding frequency increases, aspirin should be stopped. Some haemophilia specialist state that clopidogrel is well tolerated in patients with mild haemophilia [9].

General indications for cardiac interventions can be applied to haemophilia patients. Acute coronary syndromes require adequate correction with clotting factor concentrates. For cardiovascular interventions, complete clotting factor correction is given to prevent bleeding. This should be combined with thrombosis prophylaxis, as in patients without haemophilia. In the event of arterial puncture, this should ideally be performed in the radial artery, to decrease the bleeding risk and minimise the amount of clotting factor concentrates required. In this case, 24 hours of complete clotting factor correction will do.

Tooth extraction

Many elderly haemophilia patients lacked good dental care during their youths, due to their clotting factor deficiencies, and have teeth which are in bad condition, often necessitating tooth extractions. This requires good coordination between dentist or oral surgeon and haematologist. Clotting factor correction is dependent on the number of extractions, the shape of the gingival and complications during extraction. For uncomplicated extractions, a single infusion with clotting factor concentrates, aiming at a peak level of 50%, in combination with tranexamic acid in a dosage of 3 times a day 1-1.5 grams for 7 days, will do. In patients with severe or moderate haemophilia, clotting factor concentrate infusion should be repeated at the first and fifth day after extraction, to prevent renewed bleeding and impaired wound healing [76]. To avoid upper airway haematoma, nerve trunk infiltration and general anaesthesia may only be given after complete clotting factor correction. Local application of antifibrinolitics, like Spongostan oral®, and silk suturing may further prevent bleeding [77,78].

Malignancy and surgical interventions

Except for hepatocellular carcinoma due to chronic HCV infection, mortality rates for cancer are the same in haemophilia patients as in the general population [1,3]. With increasing age, patients with haemophilia will develop malignancies and other diseases like prostate hyperplasia, which require surgical intervention. Haemophilia is not a contra-indication, but adequate clotting factor correction during surgery and postoperatively is required. Duration and dosage depend on the type of surgery. Daily measurement of clotting factor levels helps to optimise therapy with clotting factor concentrates.

Prevention of deep venous thrombosis

Deep venous thrombosis is described in haemophilia patients receiving high doses of clotting factor concentrates during surgical intervention [79,80]. Patients undergoing surgery should be treated with thrombosis prophylaxis with low-molecular-weight heparin according to local protocols, as in patients without haemophilia. However, thrombosis prophylaxis should always start *after* complete clotting factor correction! In addition, compression stockings can be used peri-operatively until the patient is fully mobilised.

Sexuality

For many people, sexuality is an essential part of well being. They continue trying to find satisfactory sexual expression and intimacy. Being old in itself is no reason to give up sex. Haemophilia can be accompanied by sexual dysfunction, which may include lack of sexual desire or excitement (erection) or sexual response (ejaculation) [9,81,82]. Pain, or fear of pain, may affect sexual desire. Haemophilic arthropathy may place limitations on sexual intercourse too. Chronic HCV or HIV itself, or its treatment, can influence sexuality. Fear of transmission, or use of condoms, may decrease sexual desire in a patient or his partner. Hypertension, kidney disease and heart disease may also have a negative effect. Communication between health care professionals and patients is important to detect sexual dysfunction. As patients are mostly too shy to bring up the subject, haemophilia care givers should proactively do so. For counseling the PLISSIT (Permission, Limited Information, Specific Suggestions and Intensive Therapy) model can be used [81]. Analgesics before sexual contact and specific advice, including positions suitable for various joint problems, may further improve sexuality. Erection-enhancing medication may be useful. However, one should be careful with prescribing Viagra®, as this may cause bleedings.

Psychological impact

Associated with the physical aspects of arthropathy and ageing, haemophilia patients become aware of, or suffer from, psychosocial problems [29]. These may be triggered by loss of work, early retirement, decline in health or altered family dynamics. The patients' network may shrink and sometimes they have to give up independent life and move to a nursing home. For haemophilia patients, who have fought for self-determination for many years, this can be very dramatic.

When focusing too much on medical and psychological problems associated with co-morbidity, the senior with a chronic illness may be perceived as a person in a downward spiral. But ageing has positive aspects too; one should realise that many haemophilia

patients learned to overcome problems with their disease during their youths. These experiences will help them to tackle problems they meet when getting older.

Hospitalisation

During hospitalisation, patients may be confronted with unexpected emotional problems due to negative experiences in their youths. This may be aggravated by fear. As most patients are manager of their own disease (haemophilia), lack of control, especially during hospitalisation, may cause additional stress and emotions. Furthermore, the fact that patients are used to self-infusion may be confusing for a medical staff unfamiliar with haemophilia patients. Good information and education by a haemophilia nurse is mandatory.

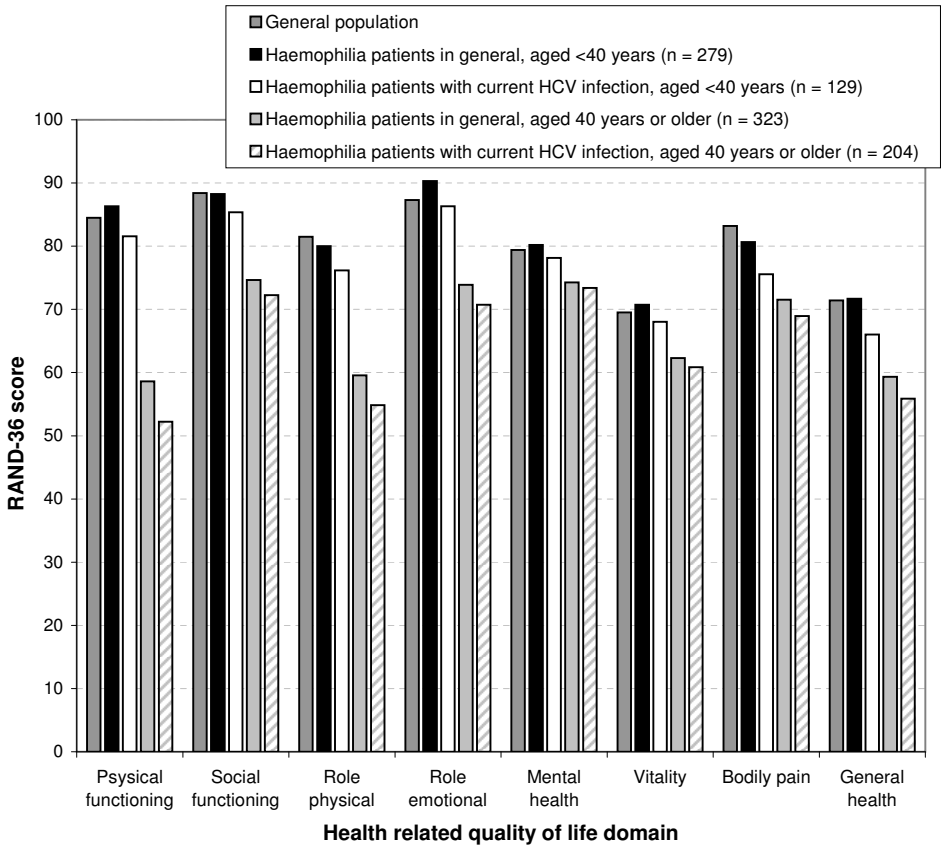

Figure 2. Health related quality of life in haemophilia patients in general and in a subgroup of haemophilia patients with a current HCV infection, divided in different age groups, compared with the general population (adjusted from Posthouwer et al 2005 [85]). Higher RAND-36 scores indicate better quality of life.

Quality of life

Quality of life is an important issue in ageing haemophilia patients. Several studies indicate that quality of life, especially regarding physical functioning, is reduced compared with the general population, even in patients with mild haemophilia [63,83,84]. Factors that negatively influence quality of life in these patients are increasing age, severity of haemophilia, the presence of arthropathy, HCV infection, HIV infection and unemployment [13,85,86]. Health-related quality of life (HQRoL) was determined in a cohort of 602 Dutch haemophilia patients [85]. Figure 2 shows HRQoL in the general population and in haemophilia patients aged < 40 years or > 40 years, with or without HCV infection. HRQoL was measured using the RAND-36 questionnaire, assessing 8 domains of HRQoL: physical functioning, social functioning, role physical (difficulties in daily activities due to physical health problems), role emotional (difficulties in daily activities due to emotional problems), mental health, vitality, bodily pain and general health. Higher scores indicate better quality of life. In haemophilia patients in general, HRQoL was significantly lower for patients aged 40 years or older than for patients aged < 40 years in all domains (p-value of the Mann-Whitney U-test ≤ 0.001). In patients with a current HCV infection, the only non-significant difference between the two age groups occurred in the mental health domain (p-value 0.07). All other domains showed significantly worse HRQoL in HCV infected patients aged 40 years or older, than in patients aged < 40 years (adjusted from Posthouwer et al [85]).

Conclusion

Co-morbidity is quite common in the later phase of human life, and haemophilia patients are no exception. It may lead to an increase in functional limitations, psychosocial complaints and symptoms, social and societal problems, and a decrease in quality of life. In patients with arthropathy, quality of life can be improved by adequate pain medication, rehabilitation, orthopedic interventions and adaptation at home. In HCV infected patients, successful treatment with antiviral therapy may improve quality of life. It is also important to look for ways to prevent or reduce co-morbidity. Haemophilia care givers should play a role in this, and during annual check-ups not only pay attention to haematological aspects of haemophilia, but also to unrelated co-morbidity.

Co-morbidity in haemophilia patients may lead to complex treatment. Mortality rates among haemophilia patients are higher in patients not treated in haemophilia centres [71]. Lack of coordination between various health care workers may result in slow and ponderous health care delivery services, uncontrolled polypharmacy, and loss of patients' well being and quality of life. Haemophilia centres should have an important part in coordinating care for these patients, not only when they are admitted to hospital,

but also by supporting care and needs in the domestic environment. This may help to improve quality of life and to keep independence.

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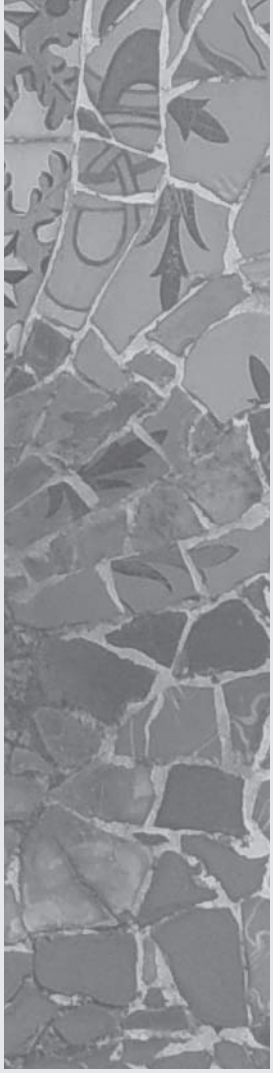
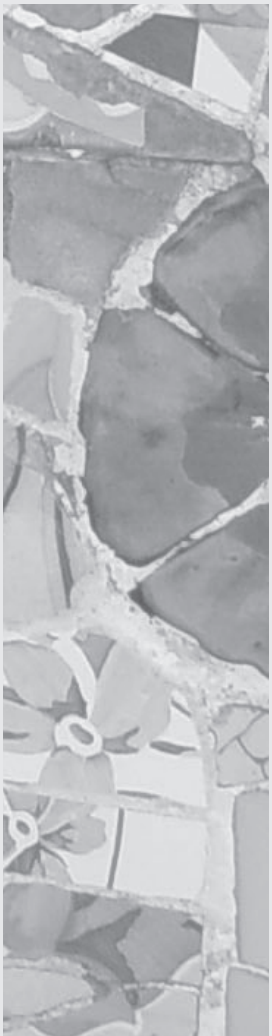
Chapter 3

Non-fatal cardiovascular disease, malignancies and other co-morbidity in adult haemophilia patients





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Abstract

Introduction

With increasing life expectancy, more haemophilia patients will be confronted with age-related problems. To ensure optimal care, it is important to know the occurrence of both fatal and non-fatal cardiovascular disease, malignancies and other types of co-morbidity in these patients. Our aim was to retrospectively assess the occurrence of co-morbidity and causes of death in a substantial birth-cohort of haemophilia patients.

Methods

Data on all types of co-morbidity were collected from medical records of 408 haemophilia patients (204 severe, 204 non-severe) born before 1971, and compared with the Dutch age-matched general male population.

Results

Ten patients had 11 myocardial infarctions, none of which were fatal. The cumulative incidence of non-fatal myocardial infarction was significantly lower in patients with severe haemophilia than in the general population (0.5% versus 4.8%), but was not decreased in patients with non-severe haemophilia (4.4%). Intracranial bleeding occurred significantly more often in haemophilia patients. The occurrence of non-virus related malignancies and other non-virus related co-morbidities was similar in haemophilia patients and the general population. HIV infection was present in 12% of patients and hepatitis C infection in 56%. Seventy-eight patients (19%) were deceased. Main causes of death were malignancies, AIDS, hepatitis C and intracranial bleeding.

Conclusions

Our results showed a decreased occurrence of myocardial infarction in patients with severe haemophilia, suggesting a protective effect of very low clotting factor levels on thrombotic cardiac events. No differences were found between haemophilia patients and the general population in the occurrence of any other type of non-virus related co-morbidity.

Introduction

Life expectancy of haemophilia patients has increased significantly during the past decades and is approaching that of the general population, especially in patients who are not infected with HIV or HCV [1]. Haemophilia patients will therefore, in addition to haemophilia-related problems (arthropathy, inhibitors to factor VIII or IX, viral complications), be confronted more often with age-related co-morbidity, like cardiovascular disease (CVD) and malignancies. In a publication by Darby et al, mortality and causes of death in 6018 English HIV negative haemophilia patients were compared with national mortality over the period 1977-1999. A reduced mortality due to ischemic heart disease was found (standard mortality rate (SMR) 0.62, 95% CI 0.51-0.76) [2]. Other studies also report lower risks of ischemic heart disease in haemophilia patients than in the general population, based on mortality rates [1,3,4] and in one study on hospital discharge data [5].

As expected, the study by Darby et al reported an increased mortality due to bleeding episodes and hepatitis C related problems. An interesting finding was the relatively high mortality due to Hodgkin lymphoma (SMR 4.95, 95% CI 1.35-12.67). In this study, no significant differences between haemophilia patients and the general population were reported in the overall cancer mortality [2]. In a study by Plug et al, no increased mortality due to malignancies other than hepatocellular carcinoma was found either [1]. An earlier Dutch study reported an increased mortality due to malignancies in haemophilia patients compared with the general population over the period 1973-1986 (SMR 2.5, over half of reported malignancies were lung carcinoma, with a similar proportion of smokers in both groups) [3]. Over the period 1986-1992, however, the observed mortality due to malignancies was consistent with the expected mortality [6]. A German study, on the other hand, reported an increased prevalence of malignancies in a small population of 29 haemophilia A patients aged 60 years or older (28%, compared with 5% in the age-matched general population) [7].

Reported data are nearly all based on mortality rates. Non-fatal cardiovascular events and malignancies are not taken into account, while they do have great impact on daily functioning and quality of life. Considering improved treatment strategies for both haemophilia (and viral infections) and age-related co-morbidity, the number of haemophilia patients with multiple problems is expected to increase in the near future. Knowledge on the occurrence of both fatal and non-fatal co-morbidity in the ageing haemophilia population is of great importance, to ensure optimal treatment and monitoring of these patients.

The aim of this study was to retrospectively assess the occurrence of both fatal and non-fatal cardiovascular disease, malignancies, other types of co-morbidity and causes of death in a substantial birth-cohort of haemophilia patients.

Materials and methods

Data collection

All male haemophilia patients who were treated at the Van Creveldkliniek, a large Dutch haemophilia treatment centre, at any time between 1985 and 2010, and were born before 01-01-1971, were extracted from our patient database, thus creating a birth cohort of patients who, when alive, would all have been 40 years or older in 2010. The majority of these patients regularly visit our centre (patients with non-severe haemophilia at least once every 1-2 years, and patients with severe haemophilia at least 2-3 times per year) and medical records have been meticulously kept by their treating physicians and haemophilia nurses from 1972 onwards. Correspondence regarding medical treatment in other hospitals is also kept on file, thus providing a complete record of a patient's medical history. Deaths not occurring in our centre are reported to us by other physicians or patient's relatives. Patients nowadays get a general medical check-up, including measurement of weight and blood pressure and several laboratory parameters like lipid and glucose levels, and are asked about the use of any non-haemophilia related medication at least once every two years at our clinic. These data were not routinely collected in the past, making reliable retrospective assessment of cardiovascular risk factors for patients who are no longer treated at our clinic difficult.

Baseline characteristics, including type and severity of haemophilia, haemophilia treatment characteristics, height and weight, current treatment status (still treated at our centre, treated elsewhere or deceased), cause of death and data on all types of co-morbidity were collected from available medical records, using a structured case report form. When a specific type of co-morbidity was not mentioned in a patient's records, it was assumed to be absent in that patient. Follow-up started at age 18 years and ended at time of either last clinical evaluation before 01-09-2010, or transfer to another haemophilia treatment centre, or death. Patients for whom no records or only very limited information was available were excluded from the analyses.

This study was approved by the medical ethics review board of the University Medical Center Utrecht.

Comparison with the general population

For comparison with the general population, cumulative incidences of specific cardiovascular events (angina pectoris, myocardial infarction, ischemic stroke and intracranial

bleeding, all defined as such when this diagnosis was established by a cardiologist or neurologist, respectively) and malignancies were calculated for our patient group. For CVD, data on the general population from the websites of the Dutch National Institute for Healthcare and Environment (RIVM, www.rivm.nl) and the Dutch Heart Foundation (Nederlandse Hartstichting, www.hartstichting.nl) were used. Cumulative incidences per 1000 people of coronary heart disease (angina pectoris and myocardial infarction) over 2007 were available for men in 5-year age categories. Based on the number of patients in each age category at end of follow-up in our study, a weighted average cumulative incidence for the age-matched general male population for coronary heart disease was calculated. This weighted average cumulative incidence was used as the comparison risk for the age-matched general male population, thus correcting for any age-effect on disease risk. Based on the proportions of patients with coronary heart disease who had either angina pectoris (43.5%) or myocardial infarction (56.5%) that were available from RIVM data, the average cumulative incidences for angina pectoris and myocardial infarction could be calculated separately. Weighted average cumulative incidences for ischemic stroke and intracranial bleeding were calculated using the same method. Because the cumulative incidences we used stated the number of people who were alive at time of the analyses who had experienced a specific event, patients with fatal events in our study population were excluded from both the numerator and the denominator in the calculations. Cumulative incidences of cardiovascular events were also calculated for patients with severe (factor level < 1%) and non-severe (factor level ≥ 1%) haemophilia separately.

For malignancies, data from the Dutch Cancer Registration database (Nederlandse Kankerregistratie, www.ikcnet.nl) over 2003 were used, describing the risk of developing certain types of malignancies for 5-year age categories. Weighted average cumulative incidences were calculated for the age-matched male population.

To calculate the prevalence of other types of co-morbidity in our study population, only patients for whom data on these conditions were available were included in the denominator. Data on the prevalence of hepatitis C and HIV infections in the general population were obtained from the RIVM website. For hepatitis C only a non-gender specific estimation of the occurrence in the general population was available. RIVM data on the prevalence of diabetes mellitus types 1 and 2 in the general male population in 2007 were available for different age categories. Again, weighted averages were calculated to determine the prevalence in the age-matched general population to enable comparison with our patients.

Using most recent data on height and weight when available, body mass indexes (BMI, calculated as weight in kg/(height in m)^2) were calculated for all patients. Data from the

website of the Dutch Central Bureau of Statistics (CBS, www.cbs.nl) for men in 2009 were used for comparison with the general population. BMIs were categorised in 4 groups: < 18.5 kg/m^2 (underweight), 18.5-25.0 kg/m^2 (normal weight), 25.1-30.0 kg/m^2 (overweight) and > 30.0 kg/m^2 (obesity). Data on the prevalence of renal insufficiency requiring dialysis were obtained from the website of the Dutch Kidney Foundation (www.nierstichting.nl), while information on the prevalence of benign prostate hypertrophy was retrieved from a national report on the prevalence of diseases in general practice [8], and data on the prevalence of cataract from the CBS website.

The presence of other types of non-bleeding related co-morbidity was limited or could not be reliably assessed from our retrospective data and was therefore not included in the analyses.

Deceased patients were classified according to causes of death. Data on causes of death in the general male population were retrieved from the CBS website. The proportions of deceased patients with specific causes of death in our study were calculated and compared with the proportions of deceased men with the same causes of death in the general population, using the average proportions over the years 1990, 1995, 2000, 2005 and 2009.

For all cumulative incidences and prevalences 95% confidence intervals were calculated. In all analyses, a statistically significant difference (p-value < 0.05) in occurrence of a specific type of co-morbidity between our study population and the age-matched general male population was assumed when there was no overlap in 95% confidence intervals. Data were analysed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Our database search yielded 421 male haemophilia patients born before 01-01-1971 who were treated at the Van Creveldkliniek in the period 1985-2010. Thirteen patients (3%) were excluded from the analyses because of lack of information (10 with mild and 3 with moderate haemophilia). Baseline characteristics of the remaining 408 patients are shown in Table 1.

Mean number of follow-up years per patient was 35.7, while the total number of patient years was 14574. Most patients had haemophilia A (89%), and were still treated at our haemophilia centre (71%). Exactly 50% of patients had severe haemophilia. Thirty-nine patients (10%) were transferred to another treatment centre at time of analysis, while 78 patients (19%) were deceased. Mean age at the end of follow-up was 53.7 years (range 24-94 years). The mean age of the 291 patients who were still treated at our clinic in

2010 was 54.5 years (range 40-94 years). In this group, the mean age of the 131 patients with severe haemophilia was slightly lower than the mean age of the 160 patients with non-severe haemophilia (52.8 versus 55.8 years, p-value of student's t-test 0.02).

Table 1. Baseline characteristics of our study population.

Total number of patients	408	
Haemophilia A	365	(89%)
Haemophilia B	43	(11%)
Severe haemophilia	204	(50%)
Non-severe haemophilia	204	(50%)
Current situation		
Still treated at our centre	291	(71%)
Treated elsewhere	39	(10%)
Deceased	78	(19%)
Mean age at end of follow-up in years (range)	53.7	(24-94)
Total number of patient years	14574	
Mean follow-up per patient in years (IQR)	35.7	(27-44)

IQR = interquartile range

Ischemic cardiovascular disease

A total of 99 cardiovascular problems occurred in 73 patients. Fifty-three patients had one event (73%), 14 patients had two events (19%) and six patients had three events (8%). Ten patients had a total of 11 myocardial infarctions (MI), while in 19 patients a total of 22 episodes of angina pectoris (AP) were reported. None of these events were fatal. Four patients experienced both MI and AP. Ischemic stroke was present in four patients (in one patient twice, in whom the second stroke was fatal), while transient ischemic attacks were reported in four patients. Other reported cardiovascular problems were intracranial bleeding (35 patients, see below), atrial fibrillation (9 patients), ventricular fibrillation (3), intermittent claudication (2), cardiac decompensation (2), cardiomyopathy (1) and aortic valve insufficiency (1). Pulmonary embolism and deep vein thrombosis were not reported at all in our patients.

Table 2 shows the cumulative incidences of non-fatal myocardial infarction, angina pectoris, ischemic stroke and intracranial bleeding in our study population compared with the age-matched general male population. The occurrence of angina pectoris was similar in haemophilia patients and the general population. Myocardial infarction, however, occurred significantly less often in haemophilia patients than in non-haemophilic males (cumulative incidence 2.5% (95% CI 1.2-4.5) versus 4.8% (95% CI 4.6-4.9)). This overall difference was solely caused by a very low cumulative incidence of myocardial infarction in patients with severe haemophilia (0.5% (95% CI 0.0-2.7)). Ischemic stroke had a similar cumulative incidence in haemophilia patients and the general population.

Only one MI patient was on regular prophylactic treatment when the event occurred, while no ischemic stroke patients were treated prophylactically with factor VIII or IX.

Table 2. Cumulative incidence of non-fatal cardiovascular events in our study population, according to severity of haemophilia, and comparison with the age-matched general male population.

	Number of patients	Overall occurrence in study population (n = 408)		Occurrence in patients with severe haemophilia (n = 204)		Occurrence in patients with non-severe haemophilia (n = 204)		Occurrence in age-matched general male population	
Myocardial infarction	10	2.5%	(1.2-4.5)*	0.5%	(0.0-2.7)*	4.4%	(2.0-8.2)	4.8%	(4.6-4.9)
Angina pectoris	19	4.7%	(2.8-7.2)	3.9%	(1.7-7.6)	5.4%	(2.7-9.4)	3.7%	(3.6-3.8)
Non-fatal ischemic stroke	4	1.0%	(0.3-2.5)	0.5%	(0.0-2.7)	1.5%	(0.3-4.2)	1.4%	(1.28-1.42)
Non-fatal intracranial bleeding	30	7.4%	(5.0-10.3) *	10.8%	(6.9-15.9) *	3.9%	(1.7-7.6) *	0.4%	(0.35-0.43)

Values are numbers or cumulative incidences (95% confidence interval).
* statistically significant difference between our study population and the age-matched general male population.

Intracranial bleeding

Intracranial bleeding occurred 39 times in 35 patients (8.6%). Of these bleedings, 15 were traumatic, 19 spontaneous and five of unknown cause. No inhibitors against factor VIII or IX were detected in any of these patients at the time of intracranial bleeding. Spontaneous intracranial bleedings occurred in significantly older patients than traumatic intracranial bleedings (mean ages 54 and 29 years, respectively, p-value of student's t-test 0.007). Nine intracranial bleedings were fatal (23%). As expected, non-fatal intracranial bleeding occurred more often in haemophilia patients than in the general population (cumulative incidences 7.4% (95% CI 5.0-10.3) and 0.4% (95% CI 0.35-0.43), respectively). The occurrence of intracranial bleeding was highest in patients with severe haemophilia (13.2%). Eight patients (23%) were on regular prophylactic treatment when the intracranial bleeding occurred.

Malignancies

A total of 67 malignancies occurred in 52 patients (13%), of which 20 were fatal (38%). The most common type of malignancy was basal cell carcinoma of the skin (15 tumours in 10 patients), followed by colon carcinoma (8 patients), hepatocellular carcinoma (7 patients, all infected with hepatitis C), prostate carcinoma (6 patients) and lung carcinoma (5 patients). Hodgkin lymphoma and Kaposi sarcoma were both reported once in HIV positive patients, while Non-Hodgkin lymphoma occurred in two patients (one of whom was HIV positive). Other reported malignancies were larynx carcinoma (3), bladder carcinoma (3), esophageal carcinoma (3), squamous cell carcinoma of the mouth (3), skin (2) or rib (1), melanoma (2), gastric carcinoma, parotid carcinoma,

appendix carcinoma and seminoma of the testis (all in one patient). One patient died of metastases of an unknown primary tumour.

Table 3 shows the cumulative incidences and comparison with the age-matched general male population for different types of malignancies for which data from the general population were available. No differences were seen in the occurrence of any type of tumour between haemophilia patients and the general population, except for hepatocellular carcinoma, which occurred significantly more often in haemophilia patients (cumulative incidences 1.7% (95% CI 0.7-3.5) in our study population and 0.08% (95% CI 0.06-0.1) in the general population). The cumulative incidence of Hodgkin lymphoma was similar in our patients and in the general population (0.3 versus 0.14%).

Table 3. Cumulative incidence of malignancies in our study population and comparison with the age-matched general male population.

Type of malignancy	Number of patients	Occurrence in study population (n = 408)		Occurrence in age-matched general male population	
All types of tumours	52	12.8%	(9.7-16.4)	9.7%	(9.47-9.83)
All types, excluding virus-related tumours (HCC, Kaposi sarcoma, HIV-associated lymphoma)	42	10.3%	(7.5-13.7)	9.7%	(9.47-9.83) #
Colon carcinoma	8	2.0%	(0.9-3.8)	1.3%	(1.19-1.33)
Hepatocellular carcinoma	7	1.7%	(0.7-3.5) *	0.08%	(0.06-0.1)
Prostate carcinoma	6	1.5%	(0.5-3.2)	1.7%	(1.64-1.80)
Lung carcinoma	5	1.2%	(0.4-2.8)	1.5%	(1.42-1.58)
Urothelial cell carcinoma bladder	3	0.7%	(0.2-2.1)	0.83%	(0.77-0.89)
Esophageal carcinoma	3	0.7%	(0.2-2.1)	0.28%	(0.25-0.31)
Non-Hodgkin lymphoma	2	0.5%	(0.1-1.8)	0.44%	(0.40-0.48)
Hodgkin lymphoma	1	0.3%	(0.0-1.4)	0.14%	(0.12-0.16)

Values are numbers or cumulative incidences (95% confidence interval).
same cumulative incidence used as for all types of tumours, because specific data on non-virus related tumours were not available. Virus-related tumours will only be a very small proportion of the total number of malignancies in the general population.
* statistically significant difference between our study population and the age-matched general male population.
HCC = hepatocellular carcinoma
HIV = human immunodeficiency virus

Other types of co-morbidity

Transfusion-related infections with hepatitis C and HIV were much more common in our patients than in the general population (in 56% and 12%, respectively, compared to 0.4% and 0.002%). Diabetes mellitus requiring treatment was reported in 25 patients (6.6%; 2 type 1, 22 type 2, 1 type unspecified). Its prevalence was comparable to that in

the general male population. Weight distribution was similar in haemophilia patients and the general population. Forty-nine percent (95% CI 44-54%) of our patients had BMIs > 25 kg/m^2, indicating overweight, versus 52% in the general population. Obesity (BMI > 30 kg/m^2) was present in 8.5% (95% CI 5.5-11.4%) of our patients and 11.2% of the general population. Weight distribution was comparable across haemophilia severities: overweight was present in 37% (95% CI 30-45%) of severe haemophilia patients and in 43% (95% CI 36-51%) of non-severe patients, while obesity was present in 8% and 9%, respectively.

Renal insufficiency requiring dialysis was reported in four haemophilia patients (1.0%, 95% CI 0.02-1.94%). In the general Dutch population, 0.03% of people depended on renal dialysis in 2010 (irrespective of age and gender). Benign prostate hypertrophy was reported in 25 patients (6.1%), and had a similar prevalence (5.7%) in the general Dutch male population. The prevalence of cataract was with 2.2% also comparable to the general population (prevalence 2.7%).

Causes of death

Causes of death for the 78 deceased patients are shown in Table 4. Most common causes of death were virus-related (32%, including hepatocellular carcinoma), malignancies (21%, excluding hepatocellular carcinoma) and intracranial bleeding (12%). Median age at death was 54 years (range 24-89 years), which is younger than the mean age of death of 71.6 years of the general male Dutch population over the period 1985-2009. Death before age 40 years was mainly caused by AIDS. All AIDS-related deaths occurred before 1998. Fatal malignancies mostly occurred in patients aged 60 years or older. Two HIV negative patients committed suicide (3%; age 30 and 37 years, respectively; one with severe and one with moderate haemophilia).

In 20 of 78 patients (26%, 95% CI 16-35%) death was caused by malignancies (including hepatocellular carcinoma). In the general male population, the proportion of deaths caused by malignancies was similar (31%). For heart disease, the proportions of deceased patients in our study and the general population were 0% and 22%, respectively, showing decreased mortality due to heart disease in our patients. The proportion of patients dying of stroke (including both intracranial bleeding and ischemic stroke) was similar in our patients and in the general population (13% (95% CI 5-20%) versus 6.5%), but 90% of fatal strokes were haemorrhagic in our patients, while this proportion is only 21% in the general population. The proportion of patients dying of AIDS or hepatitis C complications (including hepatocellular carcinoma) was significantly increased in haemophilia patients (32% (95% CI 22-42%) versus 0.3% in the general population). Causes of death in the 24 deceased HIV positive patients were AIDS in 14

(58%), a combination of HCV and AIDS in 3 (13%), liver-related in 3 (13%), intracranial bleeding in 2 (8%), sepsis in 1 (4%) and unknown in 1 patient (4%). HCV status was known for 59 of the 78 deceased patients (76%). The remaining patients died before the introduction of HCV testing in the early 1990s. Causes of death in the 46 HCV positive deceased patients were AIDS in 5 (11%), a combination of HCV and AIDS in 4 (9%), liver-related (including HCC) in 9 (20%), infection in 6 (13%), malignancies (excluding HCC) in 5 (11%), intracranial bleeding in 4 (9%), other bleeding in 2 (4%), suicide in 2 (4%), renal insufficiency in 1 (2%), hepatorenal syndrome in 1 (2%) and unknown in 7 patients (15%).

Table 4. Causes of death in 78 adult haemophilia patients born before 1971.

Cause of death	**Number of patients**	**Proportion of deceased patients** (n = 78)	**Remarks**
Haemophilia-related			
Intracranial bleeding	9	12%	Mean age 56.6 yrs, range 27-79
Other bleeding	5	6%	Mean age 46.8 yrs, range 24-73
Virus-related			
AIDS	13	17%	Mean age virus-related death 43.5
HCV complications	9	12%	yrs, range 25-73
End-stage liver disease	5		All AIDS-related deaths occurred
HCC	4		before 1998
Combination AIDS/HCV	3	4%	
Malignancies (excluding HCC)	16	21%	Mean age 64.1 yrs, range 45-89
Cardiovascular events			
Ischemic stroke	1	1%	At age 82 yrs
Other causes of death			
Infection/sepsis	8	10%	
Renal insufficiency	2	3%	
Hepatorenal syndrome	1	1%	
Multi-organ failure	1	1%	
Multimorbidity	1	1%	
Suicide	2	3%	At ages 30 and 37 yrs
Cause of death unknown	7	9%	

AIDS = acquired immune deficiency syndrome
HCV = hepatitis C virus
HCC = hepatocellular carcinoma

Discussion

Our study showed a significantly lower cumulative incidence of myocardial infarction in patients with severe haemophilia than in the age-matched general male population. No fatal myocardial infarctions were seen in this relatively large cohort of haemophilia patients. Intracranial bleeding, however, occurred more often in haemophilia patients than in non-haemophilic males. The cumulative incidences of non-virus related malignancies were comparable in haemophilia patients and the general population. As

expected, liver carcinoma did occur more often in (hepatitis C-infected) haemophilia patients. All comparison data from the general population were age-matched by weighing data for different age groups according to the number of patients in these age groups in our study population, thus correcting for any age-effect on disease risk. This method enabled us to reliably compare the occurrence of disease in all patients, irrespective of whether they died or were lost to follow-up, with the occurrence in men from the general population in the same age group as our patients at the end of their respective follow-up periods.

Ideally, studies on co-morbidity are prospective. The retrospective character of this study will, however, not have induced large ascertainment bias and/or misclassification regarding major events like cardiovascular disease and malignancies in these well documented patients, who regularly visit our haemophilia centre. It is unlikely that such events would not have been noted in their medical records.

A cohort of 408 patients yields relatively large 95% confidence intervals around any results. The fact that, even in this cohort, a statistically significant difference was found between haemophilia patients and the general population in the cumulative incidence of non-fatal myocardial infarction suggests the presence of an actual reduced risk. A decreased occurrence of myocardial infarction in haemophilia patients could be caused by less development of atherosclerosis in patients with low levels of factor VIII or IX, or a lower tendency to form occlusive thrombi, or a combination of both [9]. Our observation of a reduced myocardial infarction risk in patients with severe haemophilia, but no decreased occurrence of angina pectoris, supports the hypothesis that (very) low clotting factor levels are protective against thrombotic cardiac events, as stated by others based on in vitro and animal studies [10-12], but perhaps not against coronary artery atherosclerosis. This is confirmed by another study by our group, in which no differences were found in coronary artery calcification scores on Multi Detector-Row Computed Tomography between 42 patients with moderate or severe haemophilia A aged 59 years or older and 613 age-matched non-haemophilic males [13].

Our results are in accordance with the lower ischemic heart disease mortality rate reported by Darby et al and Plug et al [1,2]. The study by Darby et al, however, reported a similarly decreased risk of (fatal) ischemic heart disease in patients with non-severe haemophilia as in patients with severe haemophilia, which could not be confirmed in our study. Interestingly enough, no fatal myocardial infarctions were present in our cohort over a period of 25 years. All our ischemic heart disease events would therefore have been missed in a study focusing on mortality only.

The prevalence of diabetes mellitus types 1 and 2 was similar in haemophilia patients and the general population. This is in contrast with the findings of Walsh et al, who reported a prevalence of diabetes of 24% in a cohort of 47 patients with mild haemophilia A, compared with 6.1% in 33 control males of similar age [14]. Overweight and obesity did not occur more often in our study population than in the general population, confirming the results of Hofstede et al [15]. Weight distribution was also comparable in patients with severe and non-severe haemophilia in our study.

To confirm our findings on the occurrence of cardiovascular disease, a prospective study in a large, multicentre cohort of haemophilia patients was initiated in 2009 (study identifier NCT01303900 at www.clinicaltrials.gov), in which the presence of certain cardiovascular disease risk factors (hypertension, hypercholesterolemia, smoking, family history of CVD), which could not be reliably determined from our retrospective data, will be assessed as well.

In our study, no differences in the occurrence of non-virus related malignancies were found between haemophilia patients and the general population, confirming the results of others (based on mortality data) [1,2,16]. We could not confirm the increased risk of Hodgkin lymphoma in haemophilia patients reported by Darby et al [2]. The prevalences of hepatitis C and HIV in our study population were similar to those reported before [17,18]. Because of the relatively small numbers, and the lack of HCV- and HIV-status specific comparison data from the general population, no reliable comparison in the occurrence of different types of co-morbidity could be made between HCV positive and HCV negative or HIV positive and HIV negative haemophilia patients, nor between these subgroups and the general population.

Benign prostate hypertrophy, cataract and renal failure requiring dialysis did not occur more often in haemophilia patients than in the general population. For renal failure, no age- and gender-specific data were available for the general population. The difference between haemophilia patients and non-haemophilia males is expected to be even smaller when age-specific prevalences are taken into account.

As expected, the median age at death was lower in our 78 deceased patients than in the general male population (54 versus 71.6 years), because of the risk of death at a relatively young age due to severe bleeding complications or AIDS, especially during the earlier years of our follow-up period. The proportions of deaths caused by malignancies (26%, including HCC) and stroke (13%) in our patients were similar to those in the general population. The proportion of intracranial bleeding in patients dying of stroke, however, was much larger in our study (90% versus 21% in the general population).

Because no fatal myocardial infarctions were present in our study, the proportion of deaths caused by heart disease (0%) was much lower in our patients than in the general population. As expected, the proportion of deaths caused by AIDS or hepatitis C was, on the other hand, significantly higher in our study population (32% when including hepatocellular carcinoma). In a study on survival in 226 haemophilia patients from Austria (33% HIV positive), 96 patients (42.5%) died in the period 1983-2006. Death was caused by HIV/hepatitis C in 54%, heart disease in 6% and cancer in 5% [19]. Mortality in 415 Italian haemophilia patients over the period 1980-2007 was caused by HIV/hepatitis C in 59%, cardiovascular disease in 4.8% and cancer in 7.7% [4]. The relatively low cancer mortality in these studies could be explained by a large competing risk of death from AIDS or hepatitis C complications. In our study, overall cancer mortality was similar to that in the general population. Excluding the five fatal virus-related malignancies, however, resulted in only 19% of deaths caused by malignancies in our study population, compared with 31% in the general male population. The low heart disease mortality we found in our study could (partly) be explained by the same competing risks principle, but could also (partly) reflect an actual lower cardiovascular disease risk in patients with low clotting factor levels, as is also suggested by the low cumulative incidence of non-fatal myocardial infarction we found in our study. Unfortunately, the large competing risk of death from HCV or HIV complications is part of haemophilia history, and can not be avoided when studying historic birth cohorts of haemophilia patients. In more recent, prospective cohorts, like that of our CVD study, this effect, especially regarding HIV/AIDS, will be less prominent.

Conclusion

Myocardial infarction occurred significantly less often in our cohort of 408 haemophilia patients born before 1971 than in the age-matched general male population, and no fatal myocardial infarctions were present at all in this study population. Although based on retrospective data and relatively limited patient numbers, our results support the protective effect of severe haemophilia against thrombotic cardiac events. Intracranial bleeding, on the other hand, occurred more often in haemophilia patients. The occurrence of any other type of non-virus related co-morbidity was comparable to the general population. Larger, prospective studies will have to be performed to confirm these results.

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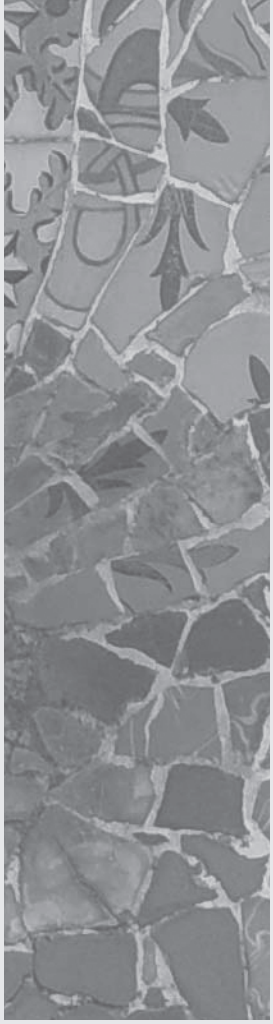
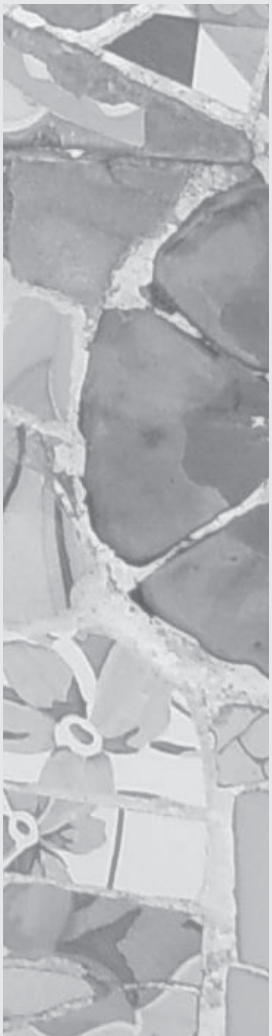


Chapter 4

History of non-fatal cardiovascular disease in a cohort of Dutch and British patients with haemophilia



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Abstract

Objective

Cardiovascular disease (CVD) mortality is reported to be lower in haemophilia patients than in the general population, but information on the occurrence of non-fatal CVD is lacking. The aim of our study was to assess CVD history in a cohort of living haemophilia patients.

Methods

Retrospective data on the occurrence of myocardial infarction, angina pectoris, ischemic stroke and intracranial bleeding in 709 living Dutch and British haemophilia patients aged 30 years or older were analysed and compared with the general age-matched male population.

Results

There was a trend towards a lower cumulative incidence of myocardial infarction (1.7 versus 4.0%) and ischemic stroke (0 versus 1.5%) in patients with severe haemophilia than in the general population, while the occurrence of angina pectoris was similar (3.2 versus 3.7%). As expected, the cumulative incidence of intracranial bleeding was, on the other hand, significantly increased in haemophilia patients (1.6% versus 0.4% in the general population).

Conclusion

Our results suggest a protective effect of severe haemophilia against acute ischemic cardiovascular disease.

Introduction

During the past decades, life expectancy of haemophilia patients has increased significantly [1,2]. Haemophilia patients are therefore likely to be confronted more often with age-related disorders, such as cardiovascular disease (CVD). CVD mortality has been reported to be lower in haemophilia patients than in the general population [1-3]. Data on the occurrence of non-fatal CVD are, however, lacking. This article reports on the medical history of non-fatal cardiovascular events in 709 haemophilia patients from the Netherlands and the United Kingdom (UK) participating in a multicentre, prospective study on CVD and its risk factors.

Patients and methods

All male haemophilia patients aged 30 years or older who visited one of the six participating haemophilia treatment centres (in Utrecht and Groningen in The Netherlands, and Sheffield, Glasgow, London (Royal Free Hospital) and Cardiff in the UK) between January 2009 and July 2011 were asked to participate in a prospective CVD study. The study was approved by the Medical Ethics Review Boards of all participating hospitals and is registered at www.clinicaltrials.gov (identification number NCT01303900). All participating patients provided written informed consent. An analysis of retrospective data on the history of non-fatal cardiovascular events obtained at inclusion in the study is presented in this article.

A history of cardiovascular disease (myocardial infarction, angina pectoris, ischemic stroke or intracranial bleeding) was considered positive when a specific diagnosis was made by a medical specialist and recorded as such in a patient's medical records.

Data on the cumulative incidences of specific CVD events in our study population were compared with data from the general male population, adjusted for age and country of origin by calculation of weighted averages. 95% confidence intervals (CI) for the reference risks were determined using the number of patients in the studies on which the reference data were based. A statistically significant difference (p-value < 0.05) was assumed when there was no overlap between these 95% CIs and those calculated around the cumulative incidences in our study population. Subanalyses were performed according to haemophilia severity (severe: factor level $< 1\%$, non-severe: factor level $\geq 1\%$). For The Netherlands, reference data were obtained from the website of the Dutch National Institute for Healthcare and Environment (RIVM, www.rivm.nl) and for the UK from the website of the British Heart Foundation (www.heartstats.org). Data were analysed using SPSS 15.0 statistical software (SPSS Inc., Chicago, IL, USA).

Results

Of the 733 patients who were approached, 709 (97%) agreed to participate in our study. The baseline characteristics of these patients are shown in Table 1. Mean age at inclusion was 49.8 years (range 30-94). Most patients (84%) had haemophilia A and 48% had severe haemophilia. In the 365 patients with non-severe disease, median factor level was 13% (range 1-48%). Patients with severe haemophilia were younger at inclusion than patients with non-severe disease (46.9 versus 52.4 years, p-value < 0.001) and were more often infected with hepatitis C and/or human immunodeficiency virus (HIV). A total of 223 patients (31%) used prophylactic clotting factor concentrate treatment at inclusion in the study (209 with severe haemophilia and 14 with non-severe disease). The estimated amount of clotting factor concentrates used per year was < 100 000 units in 13%, 100 000-150 000 units in 13%, 150 000-200 000 units in 34%, 200 000-250 000 units in 16% and > 250 000 units in 24% of patients.

Table 1. Baseline characteristics of our study population overall and according to haemophilia severity.

	Overall		**Severe haemophilia**		**Non-severe haemophilia**	
Number of patients	709		344		365	
Mean age at inclusion in years (range)	49.8	(30-94)	46.9	(30-88)	52.4	(30-94)
Haemophilia A	594	(84%)	283	(82%)	311	(85%)
Prophylactic treatment	223	(31%)	209	(61%)	14	(4%)
Active chronic hepatitis C infection	228	(32%)	160	(47%)	68	(19%)
HIV infection	76	(11%)	74	(22%)	2	(1%)
Dutch patients	388	(55%)	202	(59%)	186	(51%)
British patients	321	(45%)	142	(41%)	179	(49%)

HIV = human immunodeficiency virus

The cumulative incidences of non-fatal myocardial infarction, angina pectoris, ischemic stroke and intracranial bleeding overall and according to haemophilia severity and comparison data for the general age-matched male population are shown in Table 2. Myocardial infarction occurred in 19 patients (2.7%, six with severe haemophilia), at a mean age of 61 years (range 25-88, in five patients before the age of 50 years). The mean age at diagnosis of myocardial infarction in the general Dutch male population in 2008 was similar (64 years). One patient with mild haemophilia had two myocardial infarctions (age 55 and 58 years). In patients with severe haemophilia, the cumulative incidence of myocardial infarction was about half the cumulative incidence found in patients with non-severe disease (1.7 versus 3.6%). The occurrence of myocardial infarction in patients with severe haemophilia was much lower than in the general population (1.7% (95% CI 0.6-3.8) versus 4.0% (95% CI 3.5-4.6)), but this difference did not reach statistical significance.

Table 2. Cumulative incidences of cardiovascular events overall and according to haemophilia severity, compared with the age-matched general male population.

	Overall (n = 709)		**Severe haemophilia** (n = 344)		**Non-severe haemophilia** (n = 365)		**General age-matched male population**	
Myocardial infarction	2.7%	(1.6-4.2)	1.7%	(0.6-3.8)	3.6%	(1.2-6.0)	4.0%	(3.5-4.6)
Angina pectoris	4.2%	(2.9-6.0)	3.2%	(1.6-5.7)	5.2%	(3.2-8.0)	3.7%	(3.2-4.3)
Stroke overall	2.1%	(1.2-3.5)	2.0%	(0.8-4.2)	2.2%	(1.0-4.3)	1.9%	(1.6-2.4)
Ischemic stroke	0.6%	(0.2-1.4)	0.0%	(0.0-0.9)*	1.1%	(0.3-2.8)	1.5%	(1.2-1.9)
Intracranial bleeding	1.6%	(0.8-2.8)*	2.0%	(0.8-4.2)*	1.1%	(0.3-2.8)	0.4%	(0.2-0.6)

Values are proportions (95% confidence interval).
* statistically significant difference between haemophilia patients and the general age-matched male population.

One patient who had a myocardial infarction (5%) was treated prophylactically with clotting factor concentrates, while the other 18 patients received on demand treatment. The occurrence of myocardial infarction was not associated with haemophilia type or HCV or HIV infection. The cumulative incidence of angina pectoris was similar in haemophilia patients and the general population (4.2 versus 3.7%) and also across haemophilia severities. Of the 30 patients who experienced angina pectoris, six (20%) were treated prophylactically with clotting factor concentrates. Stroke occurred in 15 patients (2.1%), four of whom had ischemic stroke and 11 intracranial bleeding. One of the ischemic stroke cases had a history of atrial fibrillation. All ischemic stroke patients received on demand clotting factor treatment. The cumulative incidence of ischemic stroke was lower than that in the general population (0.6 (95% CI 0.2-1.4) versus 1.5% (95% CI 1.2-1.9)), but the difference was only statistically significant for patients with severe haemophilia, in whom no cases of ischemic stroke were reported at all. Intracranial bleeding, on the other hand, occurred significantly more often in patients with severe haemophilia than in the general population (2.0% (CI 0.8-2.4) versus 0.4% (0.2-0.6)). Of the 11 patients with intracranial bleeding, three (27%) were on prophylactic clotting factor treatment. The cumulative incidences of cardiovascular events were similar in Dutch and UK patients (data not shown). No transient ischemic attacks were reported in the study population. Atrial fibrillation occurred in 17 patients (2.4%, five with severe haemophilia). Other reported cardiovascular problems were aortic stenosis/aortic valve replacement (5 patients), peripheral vascular disease (4), pericarditis/endocarditis (3), ventricular fibrillation (2), heart failure (1) and cardiomyopathy (1).

Discussion

The results of our study show a low cumulative incidence of myocardial infarction and ischemic stroke in patients with severe haemophilia. The design of the study enabled us to retrospectively assess the occurrence of non-fatal cardiovascular events using

baseline data from a relatively large cohort of haemophilia patients. Because patient participation was very high (97%), this study cohort reliably represents the current population of living haemophilia patients in The Netherlands and the UK, with limited selection bias. This retrospective analysis of non-fatal CVD in a cohort of living haemophilia patients is unaffected by the competing risks of dying from bleeding, AIDS or complications of hepatitis C infection, and therefore provides a more reliable estimate of CVD occurrence in the current haemophilia population than many other (retrospective) cohort studies in which both living and deceased patients were included. A smaller difference in CVD occurrence between haemophilia patients and the general population in our study than in some other studies could be (partly) explained by this phenomenon. In a retrospective analysis of CVD history in a birth cohort of 408 haemophilia patients born before 1971 from one of the centres participating in the current study, for example, the cumulative incidence of non-fatal myocardial infarction was significantly lower in patients with severe haemophilia than in the general age-matched male population [4]. In that study, 19% of patients were deceased, and death occurred at a relatively young age (median 54 years), resulting in a lower number of patients reaching the age CVD events usually occur. A limitation of any retrospective analysis is the possibility of misclassification. However, because patients are intensively followed and records are meticulously kept at the participating treatment centres, this type of bias is likely to be limited, especially for solid end points like major cardiovascular events.

In our retrospective assessment of CVD, we found a trend towards a lower cumulative incidence of non-fatal myocardial infarction in patients with severe haemophilia compared with the general age-matched male population. Because of still relatively low patient numbers, this difference did not reach statistical significance. The occurrence of myocardial infarction was twice as high in patients with non-severe haemophilia as in patients with severe disease, suggesting the highest protective effect in patients with the lowest factor levels. The fact that myocardial infarction was reported in 19 haemophilia patients (mainly in patients with non-severe haemophilia), however, indicates that this is not a rare occurrence and that haemophilia doctors should be aware of this. The cumulative incidence of ischemic stroke was significantly lower in patients with severe haemophilia than in the general population. Our results suggest that the decreased mortality from ischemic CVD reported in other studies [1-3] can not be (solely) explained by a more benign course of disease, but (at least partly) reflects a lower risk of developing acute ischemic events. Recent studies suggest that any protective effect of low clotting factor levels on CVD occurrence may be a result of a lower tendency to form occlusive arterial thrombi, and not of decreased development of atherosclerosis [5-8]. This would be compatible with the finding of a lower occurrence of myocardial infarc-

tion, in combination with an unaltered occurrence of angina pectoris in haemophilia patients, as was found in the present study.

Theoretically, prophylactic treatment with clotting factor concentrates could attenuate a possible protective effect of low clotting factor levels on CVD risk, especially in patients with severe haemophilia. In our study, seven of the 17 patients with severe haemophilia who experienced ischemic cardiovascular events (41%) were on prophylactic treatment. The effect of clotting factor treatment on CVD risk will be further evaluated in the prospective part of the study.

Because data collection on cardiovascular disease risk factors was cross-sectional at inclusion in our CVD study, no information was available on the prevalence of such risk factors at the time of occurrence of the reported cardiovascular events. We were, therefore, unable to correlate the occurrence of events with specific cardiovascular risk profiles.

In conclusion, the retrospective results from our study suggest a protective effect of (very) low clotting factor levels against acute ischemic cardiovascular events. The prospective part of the study will provide more answers on the actual occurrence of CVD events and the association with risk factors in the ageing haemophilia population.

Acknowledgements

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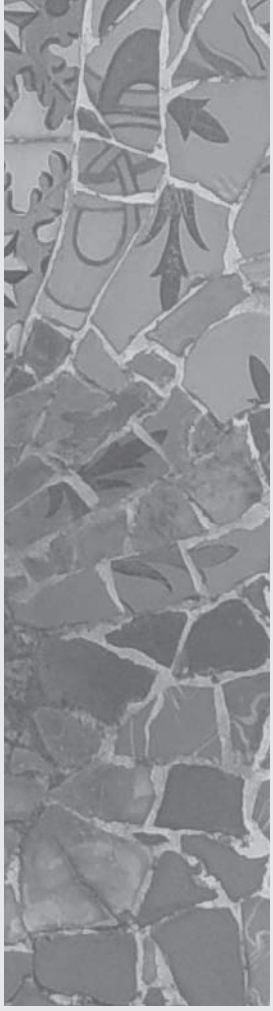
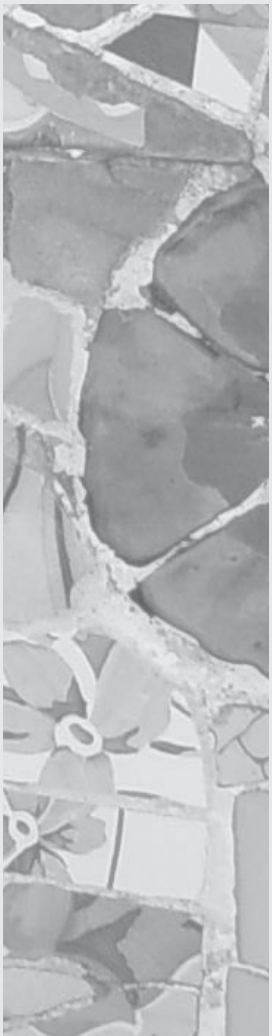
Chapter 5

Unfavourable cardiovascular disease risk profiles in a cohort of Dutch and British haemophilia patients





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Thrombosis and Haemostasis - in press

Abstract

Introduction

Cardiovascular disease (CVD) mortality is reported to be decreased in haemophilia patients, but reports on the prevalence of CVD risk factors are conflicting. A cross-sectional assessment of CVD risk profiles was performed in a large cohort of haemophilia patients.

Methods

Baseline data on CVD risk factors of 709 Dutch and British haemophilia patients aged ≥ 30 years were analysed and compared with the general age-matched male population. CVD risk profiles were assessed using the QRISK®2-2011 and SCORE algorithms. Although QRISK®2 was only validated in the UK, comparison with SCORE indicated similar properties of QRISK®2 in both Dutch and British patients (correlation 0.86).

Results

Mean age was 49.8 years. Hypertension was more common in haemophilia patients than in the general population (49% versus 40%), while the prevalences of obesity and hypercholesterolemia were lower (15 versus 20% and 44 versus 68%, respectively), and those of diabetes and smoking were similar. The predicted 10-year QRISK®2 risk was significantly higher in haemophilia patients than in the general population (8.9 versus 6.7%), indicating more unfavourable cardiovascular disease risk profiles. This increased risk became apparent after the age of 40 years.

Conclusion

Our results indicate an increased prevalence of hypertension and overall more unfavourable CVD risk profiles in haemophilia patients compared with the general age-matched male population.

Introduction

Haemophilia is a hereditary bleeding disorder, caused by low levels of clotting factor VIII (haemophilia A) or IX (haemophilia B). Life expectancy of haemophilia patients has improved considerably during the past decades and is approaching that of the general population, especially in patients who are not infected with HIV or hepatitis C [1,2]. Haemophilia patients are therefore likely to be confronted with age-related disorders such as cardiovascular disease (CVD), in addition to their primary illness and related diseases. Due to the delicate balance between bleeding and coagulation in haemophilia patients, treatment of CVD events is challenging in this population. In theory, low clotting factor levels could affect the risk of ischemic cardiovascular events. CVD mortality has indeed been reported to be lower in haemophilia patients than in the general population [1-3]. Prospective data on the occurrence of non-fatal CVD are, however, lacking. To really appreciate the effect of haemophilia on CVD risk, it is also important to know whether CVD risk profiles are similar in haemophilia patients and non-haemophilic males. Reports in this area are conflicting. In 1990, Rosendaal et al reported that haemophilia patients were more often hypertensive than a reference population, while they had significantly less fatal CVD [4]. Higher prevalences of hypertension in haemophilia patients were also reported by other studies on cardiovascular risk factors [5-7], while yet other study groups reported similar prevalences of hypertension in haemophilia patients and the general population [8-10]. Rosendaal also reported lower mean cholesterol levels in his group of Dutch haemophilia patients [4]. The prevalences of overweight and obesity appear to be similar in adult haemophiliacs and non-haemophilic males [11], but reports on the prevalences of diabetes mellitus are conflicting [10,12]. Because many reports on cardiovascular risk factors in haemophilia patients are based on relatively small numbers, sometimes limited by methodological flaws, the actual prevalence of most of these risk factors in this patient population remains unclear.

To address the issues described above, a multicentre, prospective study on cardiovascular disease and its risk factors in haemophilia patients from the Netherlands and the United Kingdom (UK) was started in 2009. This paper reports on baseline cardiovascular disease risk profiles of the 709 patients participating in this study.

Materials and methods

All male haemophilia patients aged 30 years or older who attended one of the participating haemophilia treatment centres (in Utrecht or Groningen in The Netherlands, and Sheffield, Glasgow, London (Royal Free Hospital) or Cardiff in the UK) between January 2009 and July 2011 were asked to participate in a prospective CVD study. At inclusion, haemophilia characteristics, ethnic background, data on medical history, medication use, smoking habits, alcohol use and family history of CVD were collected from patient

files and by direct interviews. Height, weight, blood pressure, non-fasting total and HDL cholesterol levels, creatinine and glucose levels and platelet counts were measured during a regular clinic visit. Data are stored anonymously in a central database at the University Medical Center Utrecht. This study was approved by the Medical Ethics Review Boards of all participating hospitals. All participating patients provided written informed consent. The study is registered at www.clinicaltrials.gov (identification number NCT01303900).

Baseline data obtained at inclusion in the CVD study were used for the analyses described in this paper. During follow-up periods of 2, 5 and 10 years, data on the occurrence of cardiovascular events will be prospectively collected.

Parameter definitions

Hypertension was defined as recorded blood pressure higher than 140/90 mmHg (using the mean value of at least two measurements when available) and/or use of antihypertensive medication. Weight distribution was assessed using the body mass index (BMI, weight/(height)^2) in four categories: underweight (< 18.5 kg/m^2), normal weight (18.5-25.0 kg/m^2), overweight (25.1-30.0 kg/m^2) and obesity (> 30.0 kg/m^2). Active chronic hepatitis C infection was defined as current positive HCV-RNA levels during a period of at least 6 months. Non-fasting cholesterol levels of all patients, regardless of statin use, were included in the analyses to enable comparison with similar data from the general population.

Comparison with the general population

Cross-sectional data on the prevalences of CVD risk factors in the study population were compared with data from the general male population, adjusted for age and country of origin by calculation of weighted averages according to the number of patients in different categories in our study population. Around the reference risks thus calculated, 95% confidence intervals (CI) were determined using the number of patients in the studies on which the reference data were based. For The Netherlands, reference data were obtained from the websites of the Dutch National Institute for Healthcare and Environment (RIVM, www.rivm.nl), the Dutch Heart Foundation (Nederlandse Hartstichting, www.hartstichting.nl) and the Dutch Central Bureau of Statistics (CBS, www.cbs.nl). Because no age-specific reference data on total and HDL cholesterol levels were available, no weighted averages could be calculated for these two variables in Dutch patients. For the UK, data were obtained from the website of the British Heart Foundation (www.heartstats.org) and a publication by Primatesta et al on the prevalence of hypertension [13].

Cardiovascular risk profiles: QRISK®2 and SCORE

The QRISK®2-2011 cardiovascular risk score was used to assess individual patients' risk profiles. This score was developed and validated in the UK [14-16]. The algorithm assesses

the risk of having a heart attack or stroke within the next 10 years, based on a number of variables (Table 1). It also provides a 10-year comparison risk for a typical person with the same age, gender and ethnicity, which can be used for comparison with the general age-matched male population. QRISK®2-2011 risks can be calculated for anyone aged 30-84 years, without a history of cardiovascular disease, who is not using statins to control hypercholesterolemia. For patients from outside the UK, the UK postcode can be left blank. When other fields of the algorithm are left blank (in case of missing data), the program substitutes an average value. To ensure optimal reliability of our results, QRISK®2-2011 2-, 5- and 10-year risks were only calculated for patients who did not have any missing data. In the algorithm, smoking is coded in categories (non-smoker, ex-smoker, light, moderate or heavy smoker). When the exact number of cigarettes smoked per day was unknown for a smoking patient, he was categorised as a moderate smoker.

Table 1. QRISK®2-2011 and SCORE cardiovascular disease risk algorithms.

	QRISK®2-2011	**SCORE**
Available at	www.qrisk.org	www.heartscore.org
Outcome	Risk of fatal or non-fatal heart attack or stroke	Fatal CVD
Period	1-10 years	10 years
Age range	30-84 years	40-65 years
Exclusion criteria	History of CVD Use of statins	History of CVD Diabetes
Determinants	Age Gender Smoking (never, former, light, moderate, heavy) Systolic blood pressure Total cholesterol HDL cholesterol Ethnicity Postal code (UK only) Diabetes Angina/heart attack in 1st degree relative < 60 yr Chronic kidney disease Atrial fibrillation Use of blood pressure treatment Rheumatoid arthritis Body mass index	Age Gender Smoking (yes/no) Systolic blood pressure Total cholesterol HDL cholesterol
Developed and validated in	UK	Europe
Remarks	Provides 10-year comparison risk for person of same age, gender and ethnicity	Provides aim (risk in absence of controllable risk factors)

CVD = cardiovascular disease
HDL = high density lipoprotein
UK = United Kingdom

Because QRISK®2 was developed and validated in the UK only, its results were compared with those of the SCORE algorithm. SCORE was developed for European popula-

tions and is subcategorised into high-risk and low-risk countries [17]. The Netherlands and the UK are both in the high-risk category. SCORE provides a 10-year risk of death from CVD based on several parameters (Table 1) and can be calculated for anyone aged 40-65 years without a history of CVD or diabetes. The algorithm also provides a 'risk aim' for a person of the same age and gender, which can be interpreted as the risk in the absence of controllable risk factors. In the SCORE calculations, systolic blood pressures were rounded to the nearest even number and cholesterol levels to the nearest decimal number.

Statistical analyses

The prevalences of cardiovascular risk factors and their 95% CIs were calculated for the study population. Missing data were not considered in the analyses. Separate analyses were performed according to country (using country-specific reference data) and haemophilia severity (severe: factor VIII/IX level < 1%, non-severe: ≥ 1%). A statistically significant difference (p-value < 0.05) was assumed when there was no overlap in 95% CIs. Univariate linear regression was used to assess the association between hepatitis C infection and cholesterol levels.

QRISK®2 has not been validated for the Dutch population, while SCORE was validated in large European populations. To asses the performance of QRISK®2 in Dutch patients, Spearman's correlations of 10-year QRISK®2 risk and SCORE were calculated for Dutch and UK patients and compared. In addition, the association between QRISK®2 and SCORE was assessed using linear regression, adjusted for country of origin, to study whether this association was different for Dutch and UK patients. As a final step, the proportions of patients with elevated risks (higher than comparison risk or 'aim') were compared according to country and scoring system using the chi-square test.

The difference between the mean 10-year QRISK®2-2011 risk in our patients and the mean risk for the age-matched general male population (as provided by the algorithm) was tested with Wilcoxon's Signed Rank Test. Subanalyses according to country and severity were performed using the Mann-Whitney U-test. Mean QRISK®2-2011 risks were also analysed for different age groups. The relative 10-year risk was calculated by dividing the 10-year risk in our study population by the comparison 10-year risk for the general population. Linear regression analyses corrected for age and haemophilia severity were performed to assess the effect of haemophilia type (A versus B), active chronic hepatitis C infection and HIV infection on 10-year QRISK®2 risks. Data were analysed using SPSS 15.0 statistical software (SPSS Inc., Chicago, IL, USA).

Results

Of the 733 patients who were approached, 709 (97%) agreed to participate in the study. The baseline characteristics of these patients according to haemophilia severity and country of origin are shown in Table 2. Mean age at inclusion in the study was 49.8 years (range 30-94). The majority of patients had haemophilia A (84%), and nearly half (48%) had severe haemophilia. The proportion of patients with severe haemophilia was lower in UK patients than in Dutch patients (44 versus 52%, p-value 0.04). Active chronic hepatitis C infection was present in 228 patients (32%), while 76 patients (11%) were infected with HIV. The prevalence of HIV infection was higher in UK patients than in Dutch patients, and also in patients with severe haemophilia. Patients with severe haemophilia also more often had active chronic hepatitis C infection (47 versus 19%) and were younger at inclusion in the study (46.9 versus 52.4 years, p-value < 0.001) compared with non-severe patients. The other baseline characteristics were similar across haemophilia severities and countries of origin.

Table 2. Baseline characteristics of our study population overall and according to haemophilia severity and country of origin.

	Overall		**Severe haemophilia**		**Non-severe haemophilia**		**Dutch patients**		**UK patients**	
Number of patients	709		344		365		388		321	
Mean age at inclusion in years (range)	49.8	(30-94)	46.9	(30-88)	52.4	(30-94)	49.5	(30-94)	50.2	(30-88)
Haemophilia A	594	(84%)	283	(82%)	311	(85%)	335	(86%)	259	(81%)
Severe haemophilia	344	(48%)	344	(100%)	-		202	(52%)	142	(44%)
Moderate haemophilia	83	(12%)	-		83	(23%)	46	(12%)	37	(12%)
Mild haemophilia	282	(40%)	-		282	(77%)	140	(36%)	142	(44%)
Prophylactic treatment	225	(32%)	209	(61%)	16	(4%)	141	(36%)	84	(26%)
Active chronic hepatitis C infection	228	(32%)	160	(47%)	68	(19%)	127	(33%)	101	(31%)
HIV infection	76	(11%)	74	(22%)	2	(1%)	27	(7%)	49	(15%)
Caucasian background	681	(96%)	321	(93%)	360	(99%)	376	(97%)	305	(95%)

HIV = human immunodeficiency virus

Individual CVD risk factors

The prevalences of various cardiovascular disease risk factors are shown in Table 3. Hypertension data were available for 706 patients (99.6%). In 65% the mean value of at least two blood pressure measurements could be used. Hypertension was present in 347 patients (49%, 95% CI 46-53%), which was significantly more than in the general population (40%, CI 38-43%). Antihypertensive medication was used by 177 patients (25% in both countries). The prevalence of hypertension was similar across haemophilia severities, but higher in Dutch patients than in UK patients (52 versus 46%), although

this difference was not statistically significant. While mean systolic blood pressure was higher in Dutch patients (139 versus 133 mmHg, p-value < 0.001), mean diastolic blood pressures were similar in both groups (82 and 81 mmHg, p-value 0.13). In both groups, hypertension was present more often than in the general population, with statistical significance in Dutch patients only.

Table 3. Prevalence of cardiovascular disease risk factors overall and according to haemophilia severity and country of origin, and comparison with the age-matched general male population (when data were available).

	Overall (n = 709)		**Severe haemophilia** (n = 344)		**Non-severe haemophilia** (n = 365)		**General age-matched male population**		**Dutch patients** (n = 388)		**General Dutch population**		**UK patients** (n = 321)		**General UK population**	
Hypertension	49%	(46-53)*	50%	(44-55)*	48%	(43-53)	40%	(38-43)	52%	(47-57)*	43%	(40-46)	46%	(41-52)	40%	(39-42)
Use of antihypertensive medication	25%	(22-29)	25%	(21-30)	25%	(21-30)	-		25%	(21-30)	-		25%	(20-30)	-	
Current smoker	28%	(24-31)	30%	(25-35)	26%	(21-30)	26%	(25-27)	31%	(27-36)	30%	(28-31)	23%	(19-28)	22%	(21-23)
Ex-smoker	32%	(29-36)	28%	(23-33)	36%	(32-42)	-		35%	(31-40)	-		29%	(24-34)	-	
Heart disease in 1st degree relative before age 60 years	18%	(15-21)	15%	(12-20)	20%	(16-24)	-		20%	(16-24)	-		14%	(11-19)	-	
Diabetes mellitus	6.1%	(4.4-8.1)	7.0%	(4.5-10.2)	5.2%	(3.2-8.0)	6.3%	(5.7-7.0)	5.9%	(3.8-8.8)	6.4%	(5.7-7.1)	6.2%	(3.9-9.5)	6.2%	(5.6-7.0)
Overweight (25.1-30.0 kg/m²)	43%	(40-47)	42%	(37-47)	45%	(39-50)	41%	(40-42)	44%	(39-49)*	37%	(37-38)	42%	(37-48)	41%	(40-42)
Obesity (> 30.0 kg/m²)	15%	(12-18)*	12%	(9-16)*	18%	(14-22)	20%	(19-21)	9%	(6-12)	12%	(12-13)	22%	(18-27)	20%	(19-21)
Total cholesterol ≥ 5.0 mmol/l	44%	(40-48)*	38%	(33-43)*	49%	(44-55)*	68%	(67-70)	48%	(43-53)*	70%	(66-74)	39%	(34-45)*	67%	(65-68)
HDL cholesterol ≤ 1.0 mmol/l	32%	(28-36)*	35%	(30-40)*	29%	(24-34)*	8%	(7-9)	35%	(31-40)*	8%	(5-10)	28%	(23-33)*	8%	(7-9)
Use of statins	12%	(9-14)	7%	(5-11)	16%	(12-20)	-		10%	(7-13)	-		14%	(10-18)	-	

Values are proportions (95% confidence interval).
HDL = high density lipoprotein
* statistically significant difference between haemophilia patients and the general age-matched male population.

Twenty-eight percent of patients were current smokers. Forty-three patients were diagnosed with diabetes mellitus (6.1%, 3 type 1 and 40 type 2). The prevalences of smoking and diabetes were similar to those in the general population. Smoking and heart disease in a first degree relative before the age of 60 years were present more often in Dutch than in UK patients, but these differences did not reach statistical significance. Obesity was present significantly less often in haemophilia patients than in the general population (15 versus 20%), which was mainly caused by a low prevalence of obesity in patients with severe haemophilia (12%). Obesity was present in significantly more UK than Dutch patients (22 versus 9%), but there was also more obesity in the general UK population than in the general Dutch population (20 versus 12%). The prevalence of increased BMI (> 25.0 kg/m^2) was significantly lower in HIV positive patients than in HIV negative patients (38%, CI 27-50 versus 61%, CI 57-64) and also significantly lower in patients with chronic HCV infection than in patients without such an infection (49%, CI 43-56 versus 63%, CI 58-67).

Total and HDL cholesterol levels were significantly lower in haemophilia patients than in the general population. This difference was most striking for patients with severe haemophilia: 38% (CI 33-43) of these patients had increased total cholesterol levels, compared with 68% (67-70) in the general population. Use of statins was also significantly less common in patients with severe haemophilia than in non-severe patients (7 versus 16%), and mean total and HDL cholesterol levels were lower (4.7 versus 5.0 and 1.13 versus 1.19 mmol/l, p-values 0.006 and 0.03, respectively). No other differences were seen between patients with severe and non-severe haemophilia. Mean total cholesterol levels and total/HDL cholesterol ratios were lower in patients with active chronic hepatitis C infection than in patients without such an infection (4.4 versus 5.1 mmol/l, p-value < 0.001, and 4.2 versus 4.6, p-value 0.001, respectively) and linear regression analyses showed significant associations between active chronic hepatitis C infection and these two variables (p-values <0.001 and 0.001, respectively). In the 476 patients without active chronic hepatitis C infection, however, the prevalences of increased total cholesterol levels (52%, CI 47-56%) and low HDL cholesterol levels (28%, CI 24-33%) were still significantly different from those in the general population (68%, CI 67-70% and 8%, CI 7-9%, respectively). A similar, but less significant effect on cholesterol levels was seen for HIV infection (prevalence of increased total cholesterol levels 33%, CI 23-45% in 76 HIV positive patients and 45%, CI 41-49% in 623 HIV negative patients).

QRISK®2 risk profiles

QRISK®2-2011 risks could be calculated for 595 patients (84%). The risk could not be calculated because of a history of CVD in 44 patients (6%), use of statins in 50 (7%), age over 84 years in 4 (1%) or missing data in 16 patients (2%). The results of the QRISK®2

risk assessment are shown in Table 4. Mean estimated 2-, 5- and 10-year risks were 1.7, 4.3 and 8.9% (CI 8.1-9.8), respectively, in our patients, while the mean 10-year comparison risk for the age-matched general male population was lower (6.7%, CI 6.1-7.2, p-value < 0.001). Overall, the absolute 10-year risk in our patients was 2.2% (CI 1.7-2.8%) higher than in the general population, while the 10-year relative risk was 1.39 (CI 1.28-1.50). As expected, CVD risks increased significantly with age (Figure 1). In all except the youngest age group (age 30-40 years), statistically significant differences in 10-year risks were seen between haemophilia patients and the general population.

Table 4. Results of QRISK®2-2011 and SCORE cardiovascular disease risk assessment overall and according to haemophilia severity and country of origin.

	Overall (n = 709)		**Severe haemophilia** (n = 344)		**Non-severe haemophilia** (n = 365)		**Dutch patients** (n = 388)		**UK patients** (n = 321)	
QRISK®2 available	595	(84%)	307	(89%)	288	(79%)	343	(88%)	252	(79%)
Age of patients with available QRISK®2	47.2		45.7		48.8		47.7		46.6	
2-year QRISK®2 risk	1.7%	(1.5-1.8)	1.5%	(1.3-1.7)	1.8%	(1.6-2.1)	1.9%	(1.6-2.1)	1.4%	(1.1-1.6)
5-year QRISK®2 risk	4.3%	(3.8-4.7)	3.8%	(3.2-4.4)	4.7%	(4.0-5.4)	4.8%	(4.2-5.4)	3.6%	(3.0-4.2)
10-year QRISK®2 risk	8.9%	(8.1-9.8)	8.1%	(7.0-9.2)	9.8%	(8.5-11.1)	10.0%	(8.8-11.1)	7.5%	(6.3-8.7)
10-year QRISK®2 comparison risk (general population)	6.7%	(6.1-7.2)	5.8%	(5.1-6.5)	7.6%	(6.7-8.6)	7.0%	(6.2-7.7)	6.2%	(5.4-7.1)
10-year QRISK®2 risk difference between patients and general population	2.2%	(1.7-2.8)	2.3%	(1.6-3.1)	2.2%	(1.4-2.9)	3.0%	(2.2-3.7)	1.3%	(0.6-1.9)
10-year relative QRISK®2 risk	1.39	(1.28-1.50)	1.44	(1.29-1.59)	1.34	(1.18-1.50)	1.49	(1.35-1.63)	1.26	(1.08-1.44)
Increased 10-year QRISK®2 risk	259	(44%)	126	(44%)	123	(43%)	164	(48%)	95	(38%)
SCORE available	378	(53%)	180	(52%)	198	(54%)	210	(54%)	168	(52%)
SCORE 10-year risk	3.5%	(3.1-3.8)	3.5%	(2.9-4.1)	3.5%	(3.0-3.9)	4.1%	(3.6-4.7)	2.7%	(2.3-3.0)
SCORE 10-year aim	3.0%	(2.8-3.2)	2.8%	(2.5-3.1)	3.2%	(2.9-3.5)	3.2%	(2.9-3.5)	2.8%	(2.5-3.1)
Difference between SCORE and aim	0.5%	(0.2-0.7)	0.7%	(0.3-1.1)	0.2%	(0.0-0.5)	0.9%	(0.6-1.3)	-0.2%	(-0.4-0.0)
SCORE above aim	123	(33%)	65	(36%)	58	(29%)	85	(41%)	38	(23%)

Values are numbers (proportion) or means (95% confidence interval).

Patients with severe haemophilia were significantly younger than those with non-severe disease (mean age 45.7 versus 48.8 years, p-value 0.001). 10-year QRISK®2 risks were lower in severe than in non-severe patients (8.1% (CI 7.0-9.2) and 9.8% (8.5-11.1), respectively), but because of the lower age, the 10-year comparison risk for the general population was also significantly lower in these patients (5.8% (5.1-6.5) versus 7.6% (6.7-8.6)). This resulted in similar 10-year risk differences and relative risks compared with the general population across haemophilia severities. Haemophilia type and infection with hepatitis C or HIV were not independently associated with 10-year QRISK®2 risks (data not shown). Compared with UK patients, Dutch patients appeared to have higher QRISK®2 risks overall. The mean 10-year risk was significantly higher in Dutch patients than in UK patients (10.0% (CI 8.8-11.1) versus 7.5% (6.3-8.7), p-value 0.011). The 10-year risk difference between haemophilia patients and the general population and the relative 10-year risk were also higher in Dutch than in UK patients (p-value for both variables < 0.001). The mean ages of Dutch and UK patients were similar (47.7 versus 46.6 years, p-value 0.26).

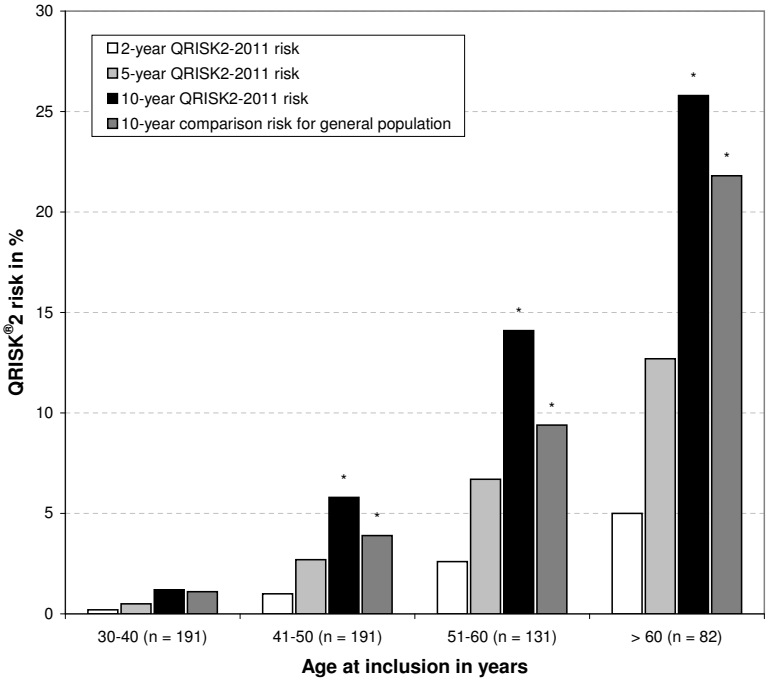

Figure 1. QRISK®2-2011 cardiovascular disease risks in 595 haemophilia patients according to age category.
Values represent mean risks in %, indicating the risk of developing myocardial infarction or stroke during a specific time period.
* statistically significant difference between haemophilia patients and the general age-matched male population.

Comparison of QRISK®2 and SCORE

Of the 433 patients who were in the right age group (40-65 years, 61% of the total study population), SCORE could be calculated in 378 (87%). The remaining patients had a history of CVD (17 patients), diabetes (25) or missing data (13). Mean estimated 10-year SCORE risk was 3.5%, while the mean aim was 3.0% (Table 4).

There was a strong correlation between QRISK®2 10-year risks and SCORE results (Spearman's rho correlation coefficient 0.87 overall, p-value < 0.001). This was the case irrespective of country of origin (correlation coefficient 0.86, p-value < 0.001 for each group when analysing Dutch and UK patients separately). A multivariate linear regression model also showed a significant association between QRISK®2 and SCORE (beta 0.35, p-value < 0.001), while country of origin had no significant effect (beta -0.3, p-value 0.22). For both QRISK®2 and SCORE, the proportions of patients with elevated risks were significantly higher in Dutch patients than in UK patients (48 versus 38%, p-value 0.009 for QRISK®2, and 41% versus 23%, p-value < 0.001 for SCORE, respectively), indicating comparable behaviour of the two algorithms. The difference between Dutch and UK patients even appeared to be slightly higher for SCORE than for QRISK®2. Both for QRISK®2 and for SCORE, results were similar in patients with severe and non-severe haemophilia.

Discussion

The results of our study show a higher prevalence of hypertension and lower prevalences of hypercholesterolemia and obesity in haemophilia patients, and an overall more unfavourable CVD risk profile, compared with the general age-matched male population. The design of the study allowed a cross-sectional assessment of the prevalence of CVD risk factors using baseline data from a relatively large cohort of haemophilia patients. Patient participation was very high (97%), resulting in a reliable representation of the current population of living haemophilia patients in The Netherlands and the UK, without major selection bias. To our knowledge, this cross-sectional analysis of CVD risk factors is the largest reported so far. The use of the QRISK®2 risk score enabled us to compare risk profiles with the general age-matched male population, and adjust for these risk profiles in the planned prospective analyses.

The prevalence of hypertension was significantly higher in the haemophilia patients than in the general population, confirming several other reports [4-7]. Multiple blood pressure measurements were available for 84% of Dutch and 41% of UK patients, making the assessment more reliable in Dutch patients. In 10% of hypertensive patients, this diagnosis was solely based on a single blood pressure measurement (as opposed to the mean value of multiple measurements and/or the use of antihypertensive medica-

tion). This was mainly the case in UK patients, who showed lower mean blood pressures than Dutch patients. It is possible that, because of regular follow-up visits and close surveillance, hypertension is diagnosed and antihypertensive treatment started earlier in haemophilia patients than in the general population. This could have led to a higher prevalence of hypertension according to the study definition. Primatesta and Poulter reported that about half of hypertensive individuals in the UK use antihypertensive medication (48% overall, 45% in men) [13]. In our study population, a slightly higher proportion of hypertensive patients was on antihypertensive treatment (51% overall, CI 46-56%, 48% in Dutch and 55% in UK patients), but this could, in our opinion, not solely explain the large increase in the prevalence of hypertension. Another possible explanation for the increased prevalence of hypertension in haemophilia patients could be the presence of (mild) renal problems due to repetitive renal bleeding and/or the effects of hepatitis C, HIV or antiretroviral treatment on the kidney. This will be subject of further study.

The prevalences of smoking and diabetes mellitus were similar in haemophilia patients and the general population. Obesity, however, occurred less often in our patients, especially in those with severe haemophilia, which is in contrast with the findings of a retrospective study we performed in a large birth cohort of haemophilia patients [12] and those of others [11,18], but in agreement with a report by Lim et al [7]. The relatively low weight in patients with severe haemophilia appears to be associated with both HIV and hepatitis C infection, but could also (partly) be caused by muscle atrophy secondary to arthropathy. The relatively low cholesterol levels we found in our patients, especially in those with severe haemophilia, confirm the findings Rosendaal et al reported over 20 years ago [4] and those of a more recent study [18]. They might partly be explained by the high prevalence of chronic hepatitis C in patients with severe haemophilia, which is reported to reduce cholesterol levels [19,20]. Indeed, in our study, active chronic hepatitis C infection was significantly associated with total cholesterol levels and total/HDL cholesterol ratios. In patients without active chronic hepatitis C infection, however, cholesterol levels were still significantly lower than in the general population, indicating that hepatitis C infection can only explain part of the reduction in these levels. HIV infection also appeared to reduce cholesterol levels.

Overall, 10-year QRISK®2-2011 predicted risks were higher in our patients than in the general population. The relative 10-year risk was 1.39, indicating a 39% higher risk of CVD over a 10-year period in haemophilia patients. For an average person from our study population, this resulted in an increase in his risk of developing myocardial infarction or stroke within the next 10 years from 6.7 to 8.9%. Patients aged 30-40 years had low risks overall and similar results to the general population, mainly because many

risk factors are rare in this age group. In patients older than 40 years, risks increased with age, and in all age groups significantly higher risks were seen in haemophilia patients than in the general population. Major contributors to the high CVD risks were hypertension, low HDL cholesterol levels and a positive family history of heart disease before the age of 60 years. Because the QRISK®2-2011 uses a computer algorithm to calculate risk scores, we were, however, unable to analyse the exact contributions of individual risk factors.

In this study, the QRISK®2-2011 risk score was preferred as the main outcome measure over other well known risk scores like Framingham and SCORE, because it was developed and validated in the UK, where a large part of our patient population is from, it enables risk calculations for different time periods and comparison with the general population, it includes a large number of relevant risk factors, and its endpoints include both fatal and non-fatal cardiovascular disease (Table 1) [21,22]. Because QRISK®2 was not validated in The Netherlands, we compared its results in Dutch and UK patients with those of the more widely used (but more limited) SCORE algorithm. Our results showed significant association and strong correlation between 10-year QRISK®2 risks and SCORE results, independent of country of origin. Any differences between Dutch and UK patients in QRISK®2 were similar to (or even smaller than) differences seen in SCORE. Based on these results, we concluded that QRISK®2 could be reliably used in both UK and Dutch patients. The fact that, according to SCORE, The Netherlands and the UK are both classified in the same high risk category further supports this conclusion. It should be noted though, that neither QRISK®2-2011 or SCORE, nor any other risk score, has been specifically tested or validated in the haemophilia population. It is also important to realise that all CVD risk scores represent risk estimates at a specific point in time, which are largely dependent on determinants which could vary over time (such as weight, smoking, blood pressure and cholesterol levels).

Interestingly, quite some differences in CVD risk factors were observed between haemophilia patients from The Netherlands and the UK. The prevalences of hypertension, smoking and heart disease in a first degree relative before the age of 60 years were higher in Dutch patients, while the prevalence of obesity was lower. Some differences did not reach statistical significance, but the first three did contribute to the more unfavourable QRISK®2 risks that were seen in Dutch patients. It should be noted that the prevalence of smoking was also significantly higher in the general Dutch population compared to the general UK population, while the prevalence of obesity was much lower. Although the effect was larger in Dutch patients, the more unfavourable QRISK®2 profile was present across countries.

The prospective study that will follow these patients for 10 years will provide more answers on the actual occurrence of CVD events in the ageing haemophilia population, while taking the risk profiles into account. The first results of this study are expected in about five years' time. If the prospective follow-up indeed shows a lower occurrence of CVD in patients with (severe) haemophilia, this would be present in spite of more unfavourable risk profiles, suggesting a large protective effect of very low clotting factor levels on CVD risk.

Conclusion

CVD risk factors, especially hypertension, are highly prevalent in haemophilia patients and this is reflected in significantly increased 10-year CVD risk estimates compared with the general population. We recommend that screening for, and treatment of these risk factors should be integrated into haemophilia care, especially in patients aged 40 years or older.

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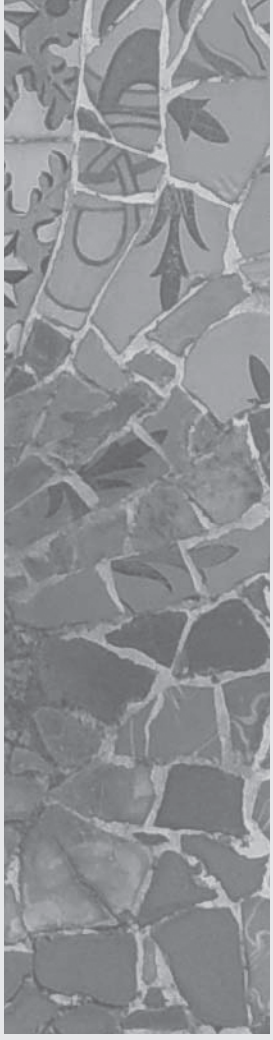
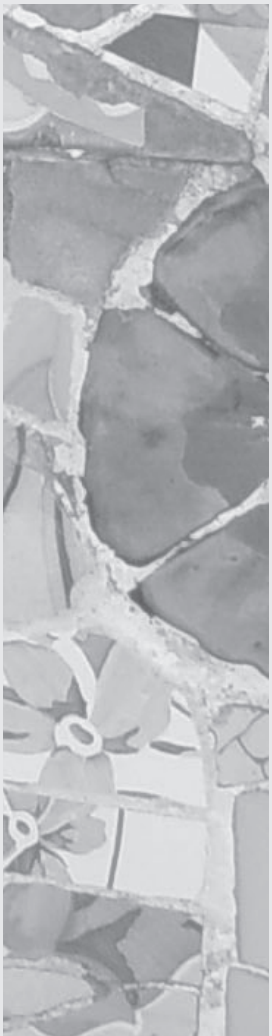
Chapter 6

Increased prevalence of hypertension in haemophilia patients





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Thrombosis and Haemostasis - in press

Abstract

Introduction

An increased prevalence of hypertension is reported in haemophilia patients, but data from large, unbiased studies are lacking. The aim of our study was to cross-sectionally assess the prevalence of hypertension in a large cohort of 701 haemophilia patients.

Methods

Blood pressure (BP) measurements performed in 386 Dutch and 315 UK haemophilia patients aged 30 years or older were analysed and compared with the general age-matched male population. Mean values of up to three BP measurements were used when available. Hypertension was defined as BP over 140/90 mmHg and/or the use of antihypertensive medication.

Results

49% of patients had severe haemophilia. Mean age was 49.8 years. The prevalence of hypertension was significantly higher in haemophilia patients (49%, 95% CI 45-53) than in the general population (40%, 95% CI 37-43). The prevalence of hypertension was higher in patients with severe haemophilia than in those with non-severe disease, but similar across haemophilia types and in Dutch and UK patients. Multiple BP measurements were available for 70%. The prevalence of hypertension was similar in patients with multiple BP measurements and the complete cohort. Hypertension was not significantly associated with renal function, a history of renal bleeding or infection with hepatitis C or HIV, but it was associated with overweight/obesity and age.

Conclusion

The prevalence of hypertension is higher in haemophilia patients than in the general population. The cause of this increased prevalence is unknown. Blood pressure measurements should be part of standard care in haemophilia patients aged 30 years or older.

Introduction

Several studies have reported a protective effect of haemophilia on cardiovascular disease (CVD), based on a decreased mortality due to CVD in haemophilia patients compared to the general population [1-3]. This could be due to the occurrence of less atherosclerosis with lower levels of factor VIII or IX, or a lower tendency to form occlusive thrombi, or both [4]. To really appreciate the effect of haemophilia on CVD risk, it is important to compare risk factors for cardiovascular disease between haemophilia patients and the general population. In 1990, Rosendaal et al reported that haemophilia patients were more often hypertensive than a reference population, and used antihypertensive drugs twice as often, while they experienced significantly less CVD [5]. Higher prevalences of hypertension in haemophilia patients were reported in several other studies [6-8], while yet others did not find significant differences in the occurrence of hypertension between haemophilia patients and the general population [9-11]. These results are based on relatively small numbers (between 14 and 100 haemophilia patients) and some studies have methodological flaws, so the actual prevalence of hypertension in haemophilia patients remains unclear.

The aim of our study was to assess the occurrence of hypertension in a large cohort of haemophilia patients aged 30 years or older.

Materials and methods

Data collection

Between January 2009 and July 2011, data on cardiovascular disease history and the presence of cardiovascular risk factors and other medical problems were collected for a large group of male haemophilia patients aged 30 years or older from The Netherlands (Utrecht and Groningen) and the United Kingdom (UK) (Sheffield, Glasgow, London and Cardiff), who are participating in a multicentre prospective study assessing cardiovascular disease and its risk factors (study identifier NCT01303900 at www.clinicaltrials.gov). All male haemophilia patients aged 30 years or older who are treated at the participating haemophilia treatment centres were approached for inclusion in this ongoing study, irrespective of haemophilia type and severity and of medical history, and 97% of approached patients agreed to take part in the study. Height, weight, blood pressure and various laboratory parameters, including creatinine levels, were measured at inclusion. The study was approved by the institutional review boards of all six hospitals. Written informed consent was obtained from all participating patients.

Blood pressure measurements were performed electronically in Utrecht, Sheffield, Glasgow and Cardiff, and by hand in Groningen and London. Measurements were performed in a sitting position with the cuff at heart level, after several minutes of rest, using validated devices

and standard cuffs when possible. The use of antihypertensive medication was extracted from records on co-medication collected at inclusion in the study. Antihypertensive medication was prescribed according to national guidelines by general practitioners or medical specialists. Hypertension was defined as systolic blood pressure over 140 mmHg and/or diastolic blood pressure over 90 mmHg and/or the use of antihypertensive medication.

Statistical analyses

Baseline blood pressure measurements provided a cross-sectional evaluation of the prevalence of hypertension in our study population. Patients for whom no blood pressure measurements were available were excluded. At inclusion in the study, only single blood pressure measurements were performed in most patients. To increase the reliability of our results, mean values of up to three blood pressure measurements were used for patients with multiple measurements available between three years before and two years after the baseline measurement. Mean systolic and diastolic blood pressures and the proportion of patients who had hypertension were calculated.

The prevalence of hypertension in the general population was calculated using two different control populations. In both populations, the same definition of hypertension was used as in our study population. First, a paper by Kearney et al on the global burden of hypertension provided prevalences of hypertension for men for all established market economies together, for different age categories, based on a population of about 50000 men [12]. Using these prevalences and the age-distribution of the patients in our study population, we calculated a weighted average prevalence of hypertension, representing the prevalence in an age-matched general male population comparable to our study population. Secondly, to correct for country effects, we calculated weighted average prevalences of hypertension based on data from the Dutch and UK general populations separately, and combined them to calculate an overall prevalence in an age-matched general male population comparable to our study population. Data for these calculations were derived from a report of the Dutch National Institute for Healthcare and Environment (RIVM), using data of 886 Dutch men [13], and from a paper by Primatesta and Poulter on the Health Survey for England 2003, performed in 4279 men from the UK [14]. Data on body mass index (BMI) distribution in the general population were derived from the website of the Dutch Central Bureau of Statistics (www.cbs.nl) for The Netherlands and from the website of the British Heart Foundation (www.heartstats.org) for the UK. Again, weighted averages were calculated to represent the general age-matched male population.

Around the prevalences for the general age-matched male population 95% confidence intervals (CI) were calculated. Student's t-tests were used to assess differences in continuous variables between different subgroups of patients (severe (factor level < 1%) versus non-severe (factor level ≥ 1%) haemophilia, haemophilia A versus haemophilia B,

prophylactic versus on demand clotting factor treatment and Dutch versus UK patients), while Fisher's exact chi-square test was used for comparison of categorical variables. Because patients with severe haemophilia were significantly younger than patients with non-severe disease, age-adjusted multivariate logistic regression analysis was performed to assess the association between haemophilia severity and hypertension. To validate our overall results, the prevalence of hypertension in the whole study cohort was compared with the prevalence in patients with multiple blood pressure measurements only.

To assess the association between renal function and hypertension, univariate logistic regression analysis was performed with creatinine levels in quartiles as the covariate. Logistic regression analysis was also performed to asses the association between age, BMI, a history of renal bleeding (self-reported overt macroscopic haematuria), current chronic hepatitis C infection and HIV infection on one hand, and hypertension on the other hand. The prevalence of hypertension in different age categories was compared with the general population using the country-specific data described above. P-values < 0.05 and non-overlapping 95% CIs were considered statistically significant. Data were analysed using SPSS version 15 (SPSS Inc. Chicago, IL, USA).

Results

A total of 709 patients aged 30 years or older were enrolled in our prospective study. For eight patients (1%) no blood pressure measurements were available, so they were excluded from the analyses. Baseline characteristics of the remaining 701 patients are shown in Table 1. Mean age at inclusion was 49.8 years (range 30-94 years). Nearly a quarter of patients (24%) were 60 years or older at time of inclusion. Eighty-four percent had haemophilia A, and 49% had severe haemophilia. 399 patients (57%) had chronic hepatitis C infection, 169 of whom had undergone successful antiviral treatment. Seventy-five patients (11%) were infected with HIV.

Table 1. Baseline characteristics of our study population.

Total number of patients	701	
Mean age at inclusion in years (range)	49.8	(30-94)
Haemophilia A	588	(84%)
Severe haemophilia	340	(49%)
Dutch patients	386	(55%)
UK patients	315	(45%)
Active chronic hepatitis C infection	230	(33%)
Successfully treated hepatitis C infection	169	(24%)
HIV infection	75	(11%)
On HAART	69	

HIV = human immunodeficiency virus
HAART = highly active antiretroviral therapy

Table 2 shows the results of blood pressure measurements overall and according to haemophilia severity. Overall, mean systolic blood pressure was 136 mmHg (range 95-198), while mean diastolic blood pressure was 81 mmHg (range 48-113). Hypertension was present in 343 patients (49%). Twenty-five percent of patients (n = 174) used antihypertensive medication. Patients with severe haemophilia were significantly younger than patients with non-severe haemophilia (mean age 46.9 versus 52.6 years, p-value < 0.001) and had slightly but significantly higher mean diastolic blood pressure (83 versus 80 mmHg, p-value 0.005), while other blood pressure characteristics were similar across haemophilia severities (hypertension present in 50% and 48%, respectively). Age-adjusted logistic regression, however, showed a significantly higher prevalence of hypertension in patients with severe haemophilia than in patients with non-severe disease (adjusted odds ratio 1.75, 95% CI 1.25-2.47, p-value 0.001). UK patients had significantly lower mean systolic blood pressure (133 versus 139 mmHg, p-value < 0.001), but the difference was small and not clinically relevant. There were no differences in mean diastolic blood pressure, use of antihypertensive medication or the prevalence of hypertension between Dutch and UK patients. Hypertension profiles were similar across haemophilia types (A versus B) and treatment strategies (prophylactic versus on demand) as well. In 99 of 174 treated patients (57%) blood pressure was still higher than 140/90 mmHg.

Table 2. Results of blood pressure measurements overall and according to haemophilia severity.

	Overall (n = 701)		**Severe haemophilia** (n = 340)		**Non-severe haemophilia** (n = 361)		**P-value** *
Age at inclusion (years)	49.8	(39-59)	46.9	(37-54)	52.6	(41-63)	<0.001
Systolic BP (mmHg)	136	(124-146)	136	(125-146)	135	(123-145)	0.44
Diastolic BP (mmHg)	81	(74-88)	83	(76-89)	80	(73-88)	0.005
Systolic BP over 140 mmHg	252	(36%)	126	(37%)	125	(35%)	0.53
Diastolic BP over 90 mmHg	122	(17%)	67	(20%)	55	(15%)	0.14
Use of antihypertensive medication	174	(25%)	85	(25%)	89	(25%)	0.93
BP over 140/90 and/or use of antihypertensive medication	343	(49%)	170	(50%)	173	(48%)	0.60

Values are means (interquartile range) or numbers (proportion).
BP = blood pressure
* differences between severe and non-severe haemophilia assessed by independent samples t-test for continuous variables and Fisher's exact chi-square test for categorical variables.

For 488 patients (70%) two (400 patients) or three (88 patients) blood pressure measurements could be analysed, while for 213 patients (30%) only single blood pressure measurements were available. Additional blood pressure measurements were collected during clinic visits within two years of inclusion in 461 of the 488 patients who had multiple measurements (94%) and extracted from medical records dating up to three

years before inclusion in the remaining 6%. Patients with multiple blood pressure measurements significantly more often had hypertension than patients with single measurements only (54 versus 37%, p-value <0.001).

Both methods that were used to calculate the prevalence of hypertension in the age-matched general male population yielded very similar results. Using data for men in established market economies, the calculated prevalence of hypertension in the age-matched general male population was 40% (95% CI 39-41%). Using individual Dutch and UK data, this prevalence was also 40% (95% CI 37-43%). In our study population, hypertension was present significantly more often: in 49% of patients (95% CI 45-53%).

In our study population, diagnosis of hypertension was based on the use of antihypertensive medication and/or the mean of at least two blood pressure measurements in 310 of 343 hypertensive patients (90%). In 33 patients (10%), this diagnosis was only based on single blood pressure measurements. In patients with multiple blood pressure measurements, the prevalence of hypertension was slightly, but not significantly higher than in the complete study cohort (54%, 95% CI 50-59 versus 49%, 95% CI 45-53), indicating valid results overall. Patients who underwent electronic blood pressure measurements more often had blood pressures over 140/90 than patients in whom blood pressures were measured manually (42% versus 23%).

Mean serum creatinine level was 80 μmol/l in our study population (range 45-202 μmol/l). Low or normal creatinine levels were present in 684 patients (98%), while 11 patients (2%) had increased levels (normal range 74-120 μmol/l). 230 patients (33%) reported a history of renal bleeding. Mean BMI was 26.3 kg/m^2 (range 17.3-51.3). In 301 patients (43%) BMI was 25.1-30.0 kg/m^2 (overweight) and 105 patients (15%) had BMI higher than 30.0 kg/m^2 (obesity). BMI distribution was comparable to that in the age-matched general male population (41% overweight and 20% obesity, respectively). There was a tendency towards a lower prevalence of obesity in haemophilia patients (15% versus 20%), but this difference was not statistically significant.

Logistic regression analyses did not show statistically significant associations between hypertension and serum creatinine levels (p-value 0.31) or a history of renal bleeding (odds ratio 1.35, 95% CI 0.99-1.84, p-value 0.05, suggesting a trend towards a higher risk of hypertension in patients who had a history of renal bleeding). There were no significant associations either between current chronic hepatitis C infection or HIV infection and hypertension (p-values 0.69 and 0.94, respectively). The presence of overweight or obesity (BMI > 25.0 kg/m^2) (odds ratio 1.8, 95% CI 1.3-2.4, p-value < 0.001) and age (odds ratio 1.08, 95% CI 1.06-1.10 per year increase, p-value < 0.001) were, on the other hand, associated with hypertension. Figure 1 shows the prevalence of

hypertension in different age categories, compared with the general age-matched male population. As expected, there was a clear and significant increase in the prevalence of hypertension with increasing age. Hypertension was present in 23.5% of patients aged 30-40 years and in 82% of patients aged 60 years or older. Despite the relatively small numbers, the prevalence of hypertension was still significantly higher than in the general population in the age categories 40-49 years and 60 years or older (41% (95% CI 34-48) versus 29% (CI 26-32) and 82% (CI 75-88) versus 71% (CI 68-74), respectively).

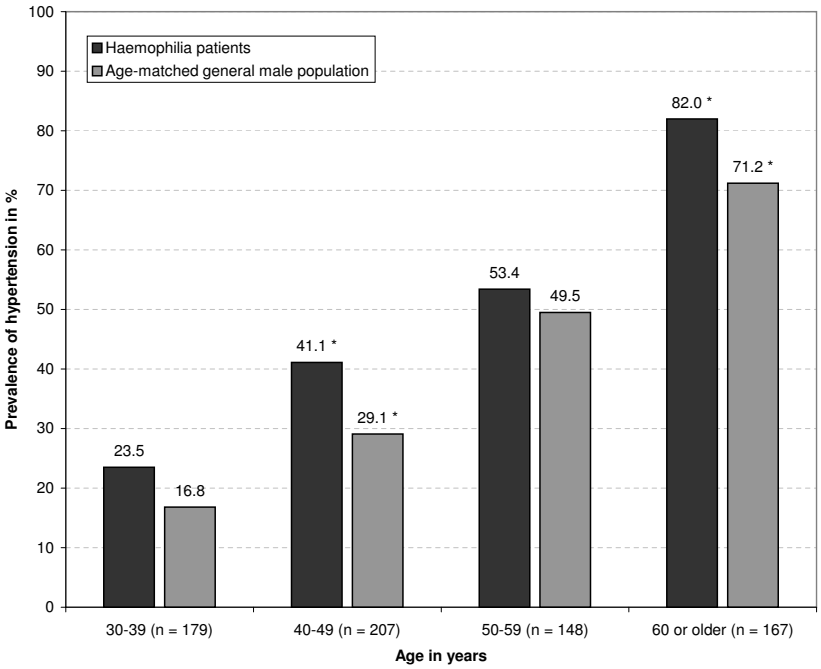

Figure 1. Prevalence of hypertension in 701 haemophilia patients according to age and comparison with the general age-matched male population.
* statistically significant difference between haemophilia patients and the general age-matched male population.

Discussion

In this large cohort of haemophilia patients, the prevalence of hypertension was significantly higher than in the age-matched general male population (49%, 95% CI 45-53 versus 40%, 95% CI 37-43). When adjusted for age, the prevalence of hypertension was significantly higher in patients with severe haemophilia than in patients with non-severe disease. It is not clear why haemophilia patients more often have hypertension than non-haemophilic males. A possible explanation for the increased prevalence of hypertension could be reduced renal function in haemophilia patients, either due to renal bleeding or possibly to the effects of hepatitis C, HIV or antiretroviral treatment on the kidney [5,15].

In our study, the presence of hypertension was not associated with serum creatinine levels, but there was a trend towards an association with a history of renal bleeding. Rosendaal did not find an association between creatinine levels and hypertension in his study either [5]. Since patients with abnormal renal function can have normal creatinine levels, the glomerular filtration rate may be a more reliable parameter of actual renal function. Unfortunately, glomerular filtration rates were not available for our study population and therefore this parameter could not be included in our analyses. This would be an interesting subject for further study. There was no association between hepatitis C or HIV infection and hypertension in our patients. As expected, higher age and higher BMI were associated with hypertension in our study population. BMI distribution was, however, comparable to that in the age-matched general male population, and there even was a tendency towards a lower prevalence of obesity in haemophilia patients. Haemophilia patients (especially those with severe haemophilia) could engage in less physical activity than healthy males, either due to arthropathy or because of fear of inducing bleeds, which might (partly) explain the higher prevalence of hypertension. Unfortunately, data on physical activity were not available for our study population.

It is possible that, because of regular follow-up visits, hypertension is more easily diagnosed in haemophilia patients and antihypertensive treatment started earlier than in the general population, accounting for a relatively large proportion of patients using antihypertensive medication in our study population, and thus a higher prevalence of hypertension according to our definition. Primatesta and Poulter described in their paper that about half of hypertensive individuals in the UK were using antihypertensive medication (48% overall, 45% in men) [14]. In our study population, a similar proportion of hypertensive patients was on antihypertensive treatment (174 of 343 patients, 51%, 95% CI 45-56%), indicating that there was no statistically significant difference and thus no disproportionately high use of antihypertensive medication in our haemophilia patients.

Because of the unavailability of a study-specific control population, data from different literature sources on the occurrence of hypertension in the general population were used. Weighted averages were calculated using available data for different age categories based on the age-distribution of patients in our study population. The two methods we used yielded very similar results for the prevalence of hypertension in the general age-matched male population, indicating reliable comparison data.

A drawback of our study is that for a proportion of patients (30%) only single blood pressure measurements were available, while using the mean of two or three measurements would have resulted in more reliable results. These were, however, mainly pa-

tients who did not have hypertension. In 33 hypertensive patients (10%), this diagnosis was based on single blood pressure measurements only. This could have led to either over- or underestimation of the prevalence of hypertension in our study population. The significantly higher proportion of patients with hypertension in patients with multiple measurements compared with patients with single measurements (54 versus 37%) may point towards confounding by indication reflecting clinical practice: patients with higher blood pressures are more likely to be subjected to more blood pressure measurements at their haemophilia treatment centres. Our sensitivity analysis showed, however, that the overall prevalence of hypertension was similar in patients with multiple measurements and the complete study cohort, indicating reliable overall results. Including the patients with single measurements only marginally changed (reduced) this prevalence.

Patients with electronic measurements had significantly higher blood pressures than those with manual measurements (42% versus 23% of these patients had blood pressures over 140/90 mmHg). Since electronic measurements are considered more reliable than manual measurements, the actual prevalence of hypertension might even be higher in our study population than indicated by our results.

Clinical implications

Considering the increased risk of hypertension, blood pressure measurements should be part of standard care in haemophilia patients. Since the prevalence of hypertension was already 24% in patients aged 30-40 years, we recommend regular blood pressure measurements in all patients aged 30 years or older. Based on our results, patients with severe haemophilia, those who are overweight or obese and perhaps patients who have a history of renal bleeding should be monitored more closely for the presence of hypertension. Hypertension is a well known risk factor for cardiovascular disease, and increases the risk of intracranial bleeding, which could be especially relevant in patients with bleeding disorders such as haemophilia [16]. Antihypertensive treatment should be started in all patients who have repetitive elevated blood pressures, aiming at levels below 140/90 mmHg.

Conclusion

An increased prevalence of hypertension was present in haemophilia patients compared with the general age-matched male population. The reason for this increased prevalence remains largely unknown. Regular blood pressure measurements should be part of clinical follow-up in the ageing haemophilia population and antihypertensive medication should be started when warranted to decrease CVD risk.

Acknowledgements

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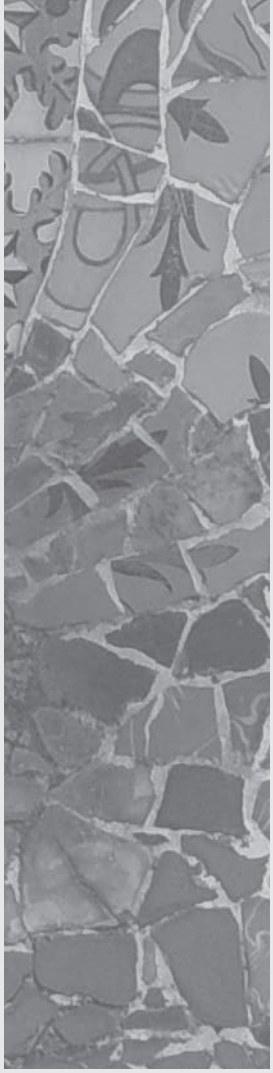
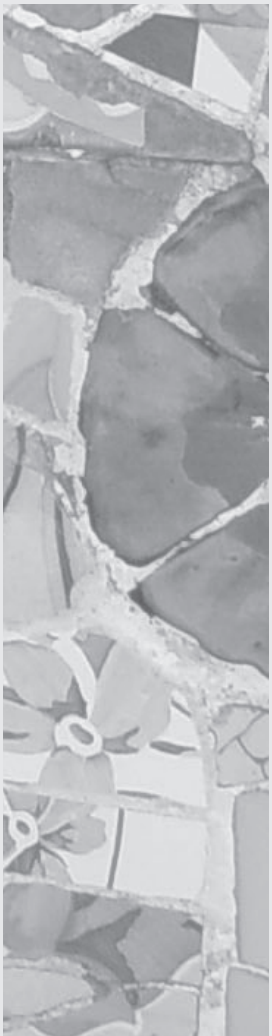


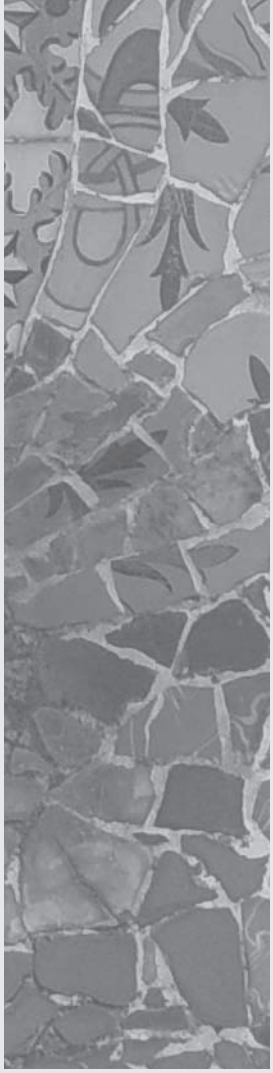
Part II

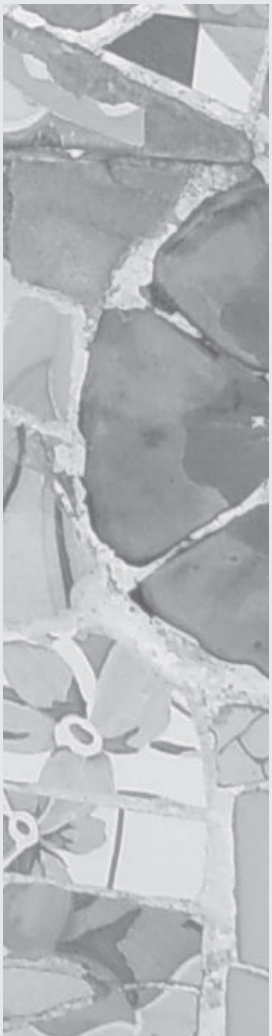
Virus-related co-morbidity



Chapter 7

Long-term follow-up of hepatitis C infection in a large cohort of patients with inherited bleeding disorders





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Abstract

Introduction

Patients with inherited bleeding disorders are an interesting group to study the long-term course of chronic hepatitis C virus (HCV) infection, because of their uniform mode of infection and reliable follow-up. Our aim was to assess the long-term occurrence of adverse liver-related events in these patients.

Methods

The occurrence and determinants of end-stage liver disease (ESLD) were assessed using retrospective data of 863 HCV infected patients with inherited bleeding disorders from the Netherlands and the UK.

Results

Median follow-up since HCV infection was 31 years, while 30% of patients had > 35 follow-up years. Nineteen percent of patients spontaneously cleared the virus and 81% developed chronic HCV infection. Of the 700 patients with chronic HCV, 90 (13%) developed ESLD. Determinants of ESLD development were age at infection (hazard ratio (HR) 1.09 per year increase), HIV co-infection (HR 10.85), history of alcohol abuse (HR 4.34) and successful antiviral treatment (HR 0.14). Liver cirrhosis was diagnosed in 20% of patients with chronic HCV and hepatocellular carcinoma (HCC) in 3%. 41% of HCC cases were diagnosed in the last six years.

Conclusion

After over 30 years of HCV infection, ESLD occurred in a significant proportion of HCV infected patients with inherited bleeding disorders. HCC appears to be an increasing problem. Haemophilia doctors should be aware of these important issues.

Introduction

Hepatitis C virus (HCV) infection is a major co-morbidity in adult patients with inherited bleeding disorders. Due to contamination of blood-derived clotting factor products, nearly all patients who were treated with large-pool donor products before the early 1990s were infected with HCV, and 80% developed chronic hepatitis C [1,2]. In HCV infected patients, a stable situation with little or no liver damage can remain for several decades. However, at long-term follow-up, progressive liver damage (fibrosis and cirrhosis) may develop, which can lead to end-stage liver disease (ESLD) with decompensated liver cirrhosis, hepatocellular carcinoma and death. The aim of antiviral treatment is to eradicate the hepatitis C virus and stop progression of liver fibrosis to ESLD. Antiviral treatment with interferon (IFN) monotherapy first became available in 1987, and ribavirin was added in 1995. Until recently, optimal antiviral treatment consisted of a combination of pegylated interferon (PegIFN) and ribavirin, which is successful in about 50% of patients with HCV genotypes 1 or 4 and 80-90% of patients with genotypes 2 or 3 [3,4]. New treatment regimens including protease inhibitors are currently being implemented in clinical care [5-7]. Because of the many side-effects [8], and the relatively low success rate in genotype 1, which is most prevalent in patients with inherited bleeding disorders, many patients are reluctant to start antiviral treatment. Antiviral treatment is, however, strongly indicated in patients who have extensive or progressive liver fibrosis [9]. To avoid liver biopsies, which are invasive and costly (because of the need of clotting factor correction during and after the procedure), non-invasive liver stiffness measurements (LSM) using Fibroscan® are increasingly performed in patients with inherited bleeding disorders to determine the extent of liver damage and indication for antiviral treatment [10-13].

The majority of patients with inherited bleeding disorders were infected with HCV at the time of their first infusion with clotting factor concentrates [14]. Because the onset of infection can therefore be relatively reliably estimated, patients with inherited bleeding disorders represent a unique model for studying the natural history of HCV infection and the potential beneficial effect of antiviral treatment on the occurrence of ESLD. In 2007, Posthouwer et al reported the first results of a study on the occurrence of ESLD using data until 2005 of 847 HCV infected patients with inherited bleeding disorders from the Netherlands and the United Kingdom (UK) [15,16].

The current study extends the follow-up of this cohort by six years. Our aim was to assess the occurrence of ESLD in HCV infected patients with inherited bleeding disorders, to assess the effect of HCV infection and determinants of ESLD development on the very long term.

Methods

Our study cohort consists of all HCV infected patients with inherited bleeding disorders who were treated during the past decades at the Van Creveldkliniek haemophilia treatment centre (University Medical Center Utrecht, The Netherlands), the Sheffield Haemophilia and Thrombosis Centre (Royal Hallamshire Hospital, Sheffield, UK) and the Katharine Dormandy Haemophilia Centre and Thrombosis Unit (Royal Free Hospital, London, UK). Patients were seen at their haemophilia treatment centres on a regular basis and liver-related laboratory tests, abdominal ultrasound examinations and physical examinations were routinely performed. Data on type and severity of the bleeding disorder, alcohol use, viral co-infections, HCV genotype, antiviral treatment history, medical history, date and cause of death, abdominal ultrasound examinations, liver-related laboratory tests and liver stiffness measurements were retrospectively collected from medical files using a structured case report form. For patients who were deceased or lost to follow-up at the 2005 evaluation, no additional information could be obtained. Data of patients with chronic hepatitis C who joined one of the participating centres after 2005 were collected as well. The study was approved by the medical ethics review boards of all three participating centres.

Parameter definitions

The date of first exposure to large-pool clotting factor concentrates or cryoprecipitate was assumed to be the date of HCV infection. In patients for whom this date was unknown, the median date of HCV infection of the patients from the same treatment centre was used (January 1970 for Utrecht, January 1972 for Sheffield and July 1977 for London). For patients who were born after that date, the date of infection was assumed to be the date of their first birthday. Overall follow-up started on the date of HCV infection and ended at last clinical evaluation before April 2012 or death. ESLD-free follow-up ended at the moment of diagnosis of ESLD, last clinical evaluation or death. ESLD was defined as the occurrence of decompensated cirrhosis (clinical liver failure or bleeding esophageal varices) or hepatocellular carcinoma. Liver cirrhosis was defined as the presence of cirrhosis on abdominal ultrasound examination, liver biopsy or post-mortem examination or result of liver stiffness measurement stage F4 (≥ 12.5 kPa). Liver stiffness measurements were performed as described previously, using a Fibroscan® device (EchoSens, Paris, France) [10]. The median result of at least 10 successful measurements, with a success rate of at least 60% and interquartile range/median result below 30% was considered representative of liver stiffness [17,18].
Alcohol abuse was defined as intake of more than 20 units of alcohol per week.
Spontaneous clearance of the hepatitis C virus was defined as positive antibodies against HCV, but absence of HCV-RNA, without antiviral treatment. Successful antiviral treatment (sustained virological response, SVR) was defined as the absence of HCV-RNA at the end and six months after completion of antiviral treatment.

Statistical analyses

Baseline characteristics were described overall and for patients with chronic HCV infection and spontaneous clearance separately. Differences between these two groups were assessed using Mann-Whitney U-test or Fischer's exact test for continuous and categorical variables, respectively. Patients with missing data for a variable were not considered for that specific analysis. The proportions of patients undergoing various types of antiviral treatment and their treatment effects were analysed. The cumulative incidences and 95% confidence intervals (CI) of various adverse liver-related outcomes were calculated overall and according to HCV status. Univariate and multivariate Cox proportional hazard models were used to assess determinants of ESLD in all patients with chronic HCV, using determinants which were known to be important from literature. The models yield hazard ratios (HR), representing the ratio of the occurrence of ESLD with a specific determinant over the occurrence without that determinant. Age at infection and age at start of antiviral treatment were included as continuous variables. The cut-off for inclusion in the multivariate analysis was a univariate p-value below 0.15.

Kaplan-Meier survival analysis for ESLD-free survival was performed according to HCV status. Data were censored at the date of diagnosis of ESLD or end of follow-up. Overall, data were censored at 45 years of follow-up, because of a low number of patients (< 10) with more follow-up years in different strata.

Overall, p-values < 0.05 and non-overlapping 95% CIs were considered statistically significant. Data were analysed using SPSS 15.0 statistical software (SPSS Inc., Chicago, IL, USA).

Results

The baseline characteristics of our study population are shown in Table 1. The 863 participating patients included the 847 from the 2005 evaluation plus 16 new patients (15 with chronic HCV and one with spontaneous clearance). For 551 of these patients, a total of 3833 additional follow-up years were available since 2005. The large majority of patients were male (94%) and had haemophilia A (76%). Mean age at the end of follow-up was 46.2 years (range 10-93). Overall, 163 patients (19%) spontaneously cleared the hepatitis C virus, while 700 (81%) developed chronic HCV infection. HCV genotype 1 was most prevalent (43% overall, 67% of known genotypes). HCV genotype was unknown in 307 patients (35%). This was mainly the case in patients who spontaneously cleared the virus and in patients who underwent successful antiviral treatment in the early 1990s. Two hundred and twelve patients (25%) were co-infected with HIV, while 16 (2%) had hepatitis B co-infection. In April 2012, 239 patients (28%) were deceased. The median follow-up per patient was 31.4 years. Four-hundred and eighty patients (56%) had more than 30 follow-up years, while for 258 patients (30%) more than 35 years of follow-up were available. The total number of follow-up years was 26216.

Table 1. Baseline characteristics of our study population.

	Overall (n = 863)		**Spontaneous clearance** (n = 163)		**Chronic HCV infection** (n = 700)	
Male gender	815	(94%)	153	(94%)	662	(95%)
Haemophilia A	654	(76%)	112	(68%)	542	(77%)
Haemophilia B	129	(15%)	31	(19%)	98	(14%)
Von Willebrand disease	53	(6%)	14	(9%)	39	(6%)
Other diagnoses	27	(3%)	6	(4%)	21	(3%)
Severe bleeding disorder	544	(65%)	91	(57%)	453	(67%)
Mean age in years (range)						
At HCV infection	16.2	(0-76)	15.8	(0-61)	16.3	(0-76)
At end of follow-up	46.2	(10-93)	44.8	(10-93)	46.5	(13-90)
HCV genotype						
1	372	(43%)	5	(3%)	367	(52%)
2	70	(8%)	1	(0.5%)	69	(10%)
3	92	(11%)	1	(0.5%)	91	(13%)
4	14	(2%)	-		14	(2%)
5	8	(1%)	-		8	(1%)
unknown	307	(35%)	156	(96%)	151	(22%)
Co-infection with HIV	212	(25%)	21	(13%)	191	(27%)
Co-infection with hepatitis B	16	(2%)	7	(4%)	9	(1%)
History of alcohol abuse	77	(10%)	10	(8%)	67	(10%)
Deceased	239	(28%)	26	(16%)	213	(30%)
Total number of follow-up years	26216		4815		21401	

HCV = hepatitis C
HIV = human immunodeficiency virus

Significant differences between patients with spontaneous clearance and patients with chronic HCV were the proportion of HIV co-infected patients (13%, 95% CI 8-19 versus 28%, CI 24-31) and the proportion of deceased patients (16%, CI 11-23 versus 30%, CI 27-34).

Antiviral treatment

A total of 361 patients underwent antiviral treatment: 268 once, 66 twice, 24 three times, 2 four times and 1 five times, resulting in a total of 485 treatment episodes. The proportion of patients reaching SVR was 53% overall. This success percentage was 25% for IFN monotherapy, 41% for IFN plus ribavirin and 50% for PegIFN plus ribavirin.

Overall, 195 patients underwent a total of 207 courses of treatment with PegIFN plus ribavirin (183 full courses, 19 courses which were discontinued prematurely because of side-effects and five courses which were ongoing at the end of follow-up). Three patients underwent two full courses of treatment. The most recent full course of antiviral treat-

ment with PegIFN plus ribavirin was successful in 104 of 180 cases (58% overall, 43% of 112 patients with HCV genotype 1 and 82% of 68 patients with other genotypes).

Of the 700 patients with chronic HCV, 260 patients (37%) underwent optimal conventional antiviral treatment (any type of treatment resulting in SVR or an unsuccessful full course of treatment with PegIFN plus ribavirin). Of the 487 patients with chronic HCV who were still alive at the end of follow-up, 250 (51%) had received such optimal treatment. Of the remaining 237 patients, some were not eligible for treatment because of high age or specific types of co-morbidity, but in many there were still treatment options within the conventional treatment regimen. Only five of these patients were undergoing PegIFN plus ribavirin treatment at the last evaluation. New treatment regimens including protease inhibitors were not yet implemented in the participating treatment centres at the end of the current follow-up period.

End-stage liver disease

The cumulative incidences of various adverse liver-related outcomes in our study population, overall and according to HCV status, are summarised in Table 2. A total of 91 patients developed ESLD after a median infection duration of 23 years (IQR 18-30, range 7-46), at a mean age of 49 years (range 23-86). ESLD occurred in 90 of 700 patients with chronic HCV (13%) and in 88 of 510 patients without (successful) antiviral treatment (17%). Liver cirrhosis (either compensated or decompensated) was detected in 141 patients (16%) after a median infection duration of 23 years (IQR 17-29, range 2-45), while the cumulative incidence of decompensated cirrhosis was 9% (77 patients). Of the 215 patients who had successful liver stiffness measurements, 29 patients (14%) had results indicating cirrhosis (stage F4). The first sign of decompensated cirrhosis was clinical liver failure in 69 patients and bleeding esophageal varices in eight patients. Both ESLD and liver cirrhosis were significantly more common in untreated and unsuccessfully treated patients than in patients with successful treatment or spontaneous clearance. ESLD was also seen significantly more often in HIV positive than in HIV negative patients (22 versus 7%). ESLD occurred in one patient with spontaneous clearance (HCC) and two patients after successful antiviral treatment (HCC in one and bleeding esophageal varices in the other).

Thirteen patients with ESLD underwent liver transplantations. In April 2012, six of these patients (46%) were deceased (four from ESLD and two from other causes), two were lost to follow-up and five (38%) were still alive. Unfortunately, failure of the transplanted liver was diagnosed in two of these living patients, and a third patient had lung metastases of his HCC.

Table 2. Adverse liver-related outcomes in 863 HCV infected patients with inherited bleeding disorders.

	Overall (n = 863)		**Chronic HCV infection** (n = 700) *		**Spontaneous clearance** (n = 163)		**Never treated** (n = 344)		**Unsuccessful treatment** (n = 166)		**Successful treatment** (n = 190)	
ESLD	11%	(9-13)	13%	(10-16)	0.6%	(0-3)	16%	(13-21)	19%	(14-26)	1%	(0.1-4)
Liver cirrhosis	16%	(14-19)	20%	(17-23)	1%	(0.2-4)	21%	(17-26)	37%	(29-45)	3%	(1-6)
Decompensated cirrhosis	9%	(7-11)	11%	(9-13)	0.6%	(0-3)	14%	(11-18)	16%	(10-22)	0.5%	(0-3)
Hepatocellular carcinoma	3%	(2-4)	3%	(2-5)	0.6%	(0-3)	3%	(2-6)	5%	(2-9)	0.5%	(0-3)
Liver transplantation	1.5%	(0.8-3)	2%	(1-3)	0%	(0-2)	2%	(0.6-4)	4%	(1-8)	0.5%	(0-3)
Liver-related death	8%	(6-10)	9%	(7-12)	0.6%	(0-3)	13%	(10-17)	11%	(7-17)	0%	(0-2)

Values are proportions (95% confidence interval).
ESLD = end-stage liver disease
* includes all patients without spontaneous clearance of the hepatitis C virus.

Liver-related death occurred in 66 patients (28% of all 239 deceased patients). The proportion of deceased patients with liver-related death was much higher in untreated patients (46/166, 28%) and patients who underwent unsuccessful treatment (19/43, 44%) than in patients with successful treatment (0/4, 0%) or spontaneous clearance (1/26, 4%). Other important causes of death were HIV/AIDS (32%), non-HCC malignancies (5%), infection (5%) and haemorrhage (4%).

Table 3 shows the univariate and multivariate Cox proportional hazard analyses to assess determinants of ESLD development in the 700 patients with chronic HCV. Higher age at infection (adjusted HR 1.09), HIV co-infection (HR 10.85) and history of alcohol abuse (HR 4.34) all independently increased the risk of developing ESLD, while successful antiviral treatment significantly decreased this risk (HR 0.14).

The ESLD-free survival according to HCV status is shown in Figure 1. The median ESLD-free follow-up was 31 years. Patients with SVR and spontaneous clearance did equally well (two cases and one case of ESLD, respectively). Patients with unsuccessful treatment initially appeared to do better than untreated patients, but after about 30 years of infection, the ESLD-free survival was similar. The median time between end of unsuccessful antiviral treatment and ESLD development was 3.1 years.

Hepatocellular carcinoma

A total of 22 patients developed HCC, after a median infection duration of 29 years (IQR 21-35, range 11-45), at a mean age of 57 years (range 42-77). Nine new HCC cases (41%) were diagnosed since the 2005 evaluation.

Table 3. Univariate and multivariate Cox proportional hazard analyses of determinants of end-stage liver disease in 700 patients with inherited bleeding disorders and chronic hepatitis C.

	Univariate analysis			**Multivariate analysis**		
	Crude HR (95% CI)		*p-value*	*Adjusted HR (95% CI)*		*p-value*
Male gender	1.46	(0.46-4.63)	0.52	-		-
Age at HCV infection (per year)	1.05	(1.04-1.07)	<0.001	1.09	(1.07-1.11)	<0.001
Age at end of follow-up (per year)	1.01	(0.99-1.03)	0.20	-		-
HCV genotype 1	1.80	(1.00-3.24)	0.05	1.56	(0.81-2.99)	0.18
HIV co-infection	5.14	(3.40-7.78)	<0.001	10.85	(6.06-19.44)	<0.001
History of alcohol abuse	2.57	(1.56-4.23)	<0.001	4.34	(2.33-8.06)	<0.001
Successful antiviral treatment	0.04	(0.01-0.17)	<0.001	0.14	(0.03-0.58)	0.007

HR = hazard ratio
CI = confidence interval
HCV = hepatitis C virus
HIV = human immunodeficiency virus

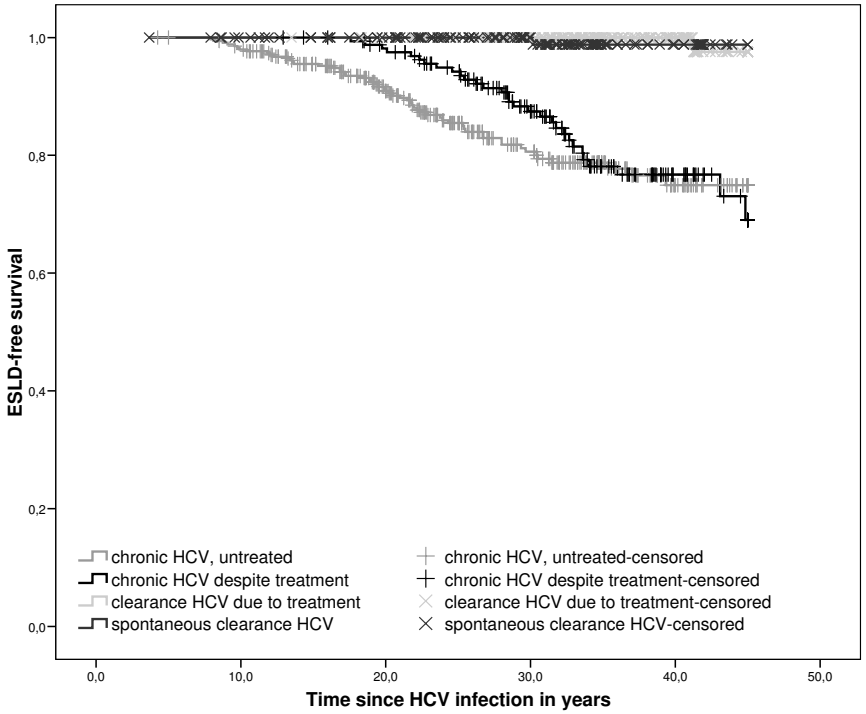

Figure 1. ESLD-free survival in 863 HCV infected patients with inherited bleeding disorders according to infection status.
ESLD = end-stage liver disease
HCV = hepatitis C virus

Seven of the 22 HCC patients (32%) were known alcohol abusers, while three patients (14%) were co-infected with HIV and one (5%) with hepatitis B. In 20 patients (91%) liver cirrhosis was present at the time of HCC diagnosis. One HCC developed in a patient who spontaneously cleared the hepatitis C virus, but subsequently developed ESLD due to alcohol abuse. One HCC developed in a patient five years after successful antiviral treatment. He also was a heavy alcohol abuser, who was diagnosed with cirrhosis before starting antiviral treatment. He underwent a successful liver transplantation shortly after HCC diagnosis and was still alive six months later. The other HCC cases were 12 untreated patients and eight patients who underwent unsuccessful treatment. Five HCC patients (23%) underwent liver transplantations. Twenty HCC patients (91%) were deceased at the end of follow-up. In 19 of these patients (95%) cause of death was HCC or coexistent liver failure. The last patient died 15 years after a successful liver transplantation from an unrelated cause.

Discussion

Studies assessing the natural history in non-haemophilic hepatitis C populations are generally limited by difficulties in reliably determining disease onset, the need for very long follow-up periods because of slow disease progression, and the presence of confounding factors and lack of compliance in for example populations containing a large proportion of intravenous drug users. Because in HCV infected patients with inherited bleeding disorders, who have a uniform mode of infection, time of infection can be reliably estimated, infection has been present for several decades and reliable follow-up is ensured by regular visits to haemophilia treatment centres, they are a very interesting population in which to study the natural history of HCV infection. The relatively large patient number in our cohort provided us with enough power to reliably analyse determinants of ESLD development. Our study describes the complete cohort of HCV infected patients at three large haemophilia centres, including patients who spontaneously cleared the virus, thus eliminating the effect of selection or ascertainment bias (emphasising more serious outcomes) or survival bias.

In our study, 863 patients contributed over 26000 follow-up years. Spontaneous clearance of the HCV virus was reported in 19% of patients, which is similar to other reports [1,19]. Overall, 361 patients underwent antiviral treatment, resulting in SVR in 53%. A total of 180 patients underwent at least one full course of antiviral treatment with PegIFN plus ribavirin, with a success percentage of 58%, which is similar to the success percentages of 54-62% described by others [3,4,20]. Of the patients with chronic HCV who were still alive at the end of follow-up, 51% achieved SVR on any type of treatment or underwent unsuccessful treatment with PegIFN plus ribavirin, leaving 237 patients in whom the decision to start (conventional) antiviral treatment was still pending.

New treatment regimens for HCV with protease inhibitors in addition to PegIFN plus ribavirin are being introduced in clinical practice. They are reported to have increased success rates, especially in patients with HCV genotype 1, but they also appear to have slightly more side-effects [5,6]. At the time of this report, none of our patients had been exposed to protease inhibitors.

ESLD was reported in 13% of patients with chronic HCV, after a median follow-up of 31 years. Other studies report cumulative incidences of ESLD of 7-14% after 12-30 years of HCV infection in HCV infected patients with inherited bleeding disorders [21-24]. The proportion of HIV co-infected patients varied significantly in these studies (0-66%). In our study, ESLD occurred in 22% of HIV positive and 7% of HIV negative patients, confirming the effect of HIV infection on liver disease progression reported by others [1,23,25]. The accelerated progression to liver failure in HIV co-infected patients has been hypothesised to be a result of suppression of the immune response against HCV [26]. Besides HIV co-infection, male gender, older age at infection, HCV genotype and chronic alcohol use are reported to be associated with more rapid fibrosis progression [2,22,27]. In our study, age at infection and a history of alcohol abuse did indeed increase the risk of ESLD development, while successful antiviral treatment had a large protective effect. We did not, however, find any association between male gender and development of ESLD in our predominantly male cohort, nor could we confirm the effect of HCV genotype. Steatosis has also been reported to be an independent risk factor for progression of fibrosis [28], especially in patients with HCV genotype 3 [29], and heavy smoking was associated with both steatosis and severe fibrosis in a Greek study [30]. Unfortunately, the availability of recent abdominal ultrasound examination results was limited in our study cohort, and data on smoking habits were not available at all, so we could not include these variables in our analyses.

In patients with SVR or spontaneous clearance, only very few cases of ESLD were reported. In patients with unsuccessful treatment, ESLD initially appeared to develop about 10 years later than in untreated patients (median infection duration at ESLD diagnosis 20 versus 29 years). This could indicate that antiviral treatment, even when unsuccessful, postpones ESLD development, but does not have a long-term beneficial effect. The median time between end of unsuccessful antiviral treatment and ESLD development was, however, only 3.1 years, rendering this hypothesis less likely. A more likely explanation is that in the group of untreated patients, more patients were censored because of death than in the unsuccessfully treated patients (48% versus 26%), and this censoring occurred at an earlier time because of a higher proportion of AIDS-related deaths in untreated patients (64 of 166 deaths, 39%, 95% CI 31-46 versus 7 of 43 deaths, 16%, CI 7-31). A slightly, but not significantly, higher proportion of HIV

infected patients in the group of untreated patients (35% versus 27%) could also have contributed to earlier ESLD development. The fact that the difference in ESLD-free survival appears to diminish during the time period in which HAART was introduced, around 25-30 years after HCV infection, also suggests an important effect of HIV infection on this difference.

In our study, HCC was diagnosed in 22 patients (3%). The fact that 41% of HCC cases were diagnosed during the past six years confirmed our clinical impression that HCC development is an increasing problem in HCV infected patients who did not undergo (successful) antiviral treatment. Tagliaferri et al also reported an increase in the occurrence of virus-related tumours in Italian haemophilia patients during the period 2001-2010 compared with earlier time periods [31]. In 20 HCC patients (91%), liver cirrhosis was evident at the time of HCC diagnosis. The incidence of HCC in patients with liver cirrhosis is reported to be 1-4% per year [32,33]. The ultimate treatment option for HCC is liver transplantation, which was performed for that indication in five patients. Indications, outcome and long-term survival after liver transplantation are reported to be similar in HCV patients with and without haemophilia [34-36]. Because the transplanted liver is able to produce normal levels of factor VIII and IX, the added benefit of liver transplantation is that patients no longer suffer from haemophilia. Unfortunately, recurrence of HCV in the transplanted liver is common, both in patients with and without haemophilia, and is often associated with accelerated disease progression and limited long-term survival [36,37]. This is reflected by a large proportion (46%) of patients in our study with liver-related death or liver failure after transplantation.

Liver cirrhosis was present in 20% of patients with chronic HCV in our study. In a study by Poynard et al in 2235 HCV infected patients without inherited bleeding disorders, the rate of progression of fibrosis was assessed using serial liver biopsies or one biopsy and known data of infection. The median time to cirrhosis was 30 years and there were slow, intermediate and rapid fibrosers in their cohort. Based on differing rates of progression, 33% would be expected to reach cirrhosis within 20 years of infection, which is substantially more than we found in our study, while 31% would take 50 years or more to become cirrhotic [38].

Using data from 1305 HCV infected Canadian haemophilia patients in a Markov prediction model, the predictive cumulative incidences of cirrhosis, HCC and liver-related death were 16.6, 4.3 and 1.4%, respectively, for 2010 and 28.9, 8.6 and 10.1% for 2020. These predicted cumulative incidences were higher than those predicted in non-haemophilic patients, mainly because of a relatively large prevalence of HIV co-infection in the haemophilia cohort [39]. A down side of this model is that it assumes that there is

no regression between fibrosis stages, while this has been observed both in serial liver biopsy and in serial LSM studies [10,40,41]. This could have led to an overestimation of the predicted cumulative incidences. The predicted cumulative incidences for 2010 for cirrhosis and HCC were, however, very similar to those we found in our 2012 evaluation in patients without spontaneous clearance (20% and 3%, respectively), while the occurrence of liver-related deaths in our study was closer to the predicted occurrence for 2020 (9%). Further follow-up of our own study cohort will have to assess the accuracy of the 2020 predictions for cirrhosis and HCC provided by this Markov model.

In HCV infected patients without inherited bleeding disorders, liver cirrhosis has been reported to occur in 7-55%, HCC in 1-23% and liver-related death in 1-15% after 8-29 years of infection [1]. Comparison of our data with those from studies in HCV infected patients without bleeding disorders is, however, difficult, because of population differences and a relatively large proportion of other factors contributing to adverse outcomes (such as alcohol or drug abuse) in these other study cohorts. Because of the benefits of our study mentioned above, our data will provide a more reliable estimate of ESLD occurrence than many studies in HCV infected patients without inherited bleeding disorders.

After successful antiviral treatment, HCC screening is still recommended for patients with severe fibrosis or cirrhosis [42]. A problem in the implementation of this recommendation is that fibrosis stage is not known in many patients with inherited bleeding disorders, especially not in patients who were successfully treated a long time ago. Liver stiffness measurement could be used in these patients to assess and monitor the extent of fibrosis, as proposed by our group and the group of Kitson et al [12,43]. We observed only one case of HCC in our study in a patient after SVR, who was a known alcohol abuser with long-term liver cirrhosis before starting antiviral treatment.

The increasing number of patients diagnosed with ESLD and the large beneficial effect of successful antiviral treatment on reducing ESLD risk underline the importance of careful follow-up of the extent of liver damage and timely consideration of antiviral treatment in HCV infected patients with inherited bleeding disorders. In many patients who previously declined treatment or who underwent unsuccessful treatment in the past, treatment options could be reconsidered in the light of the availability of new treatment regimens with increasing success rates.

Conclusion

ESLD was diagnosed in a substantial proportion of HCV infected patients with inherited bleeding disorders with a median follow-up of over 30 years. HCC occurrence

especially appeared to be an increasing problem. The risk of developing ESLD increased with higher age at infection, HIV co-infection and alcohol abuse, but was substantially reduced after successful antiviral treatment. The fact that 49% of living patients with chronic HCV in our cohort did not undergo optimal conventional antiviral treatment and the introduction of new therapies including protease inhibitors both indicate a significant potential for antiviral treatment administration to try and limit ESLD occurrence in the future.

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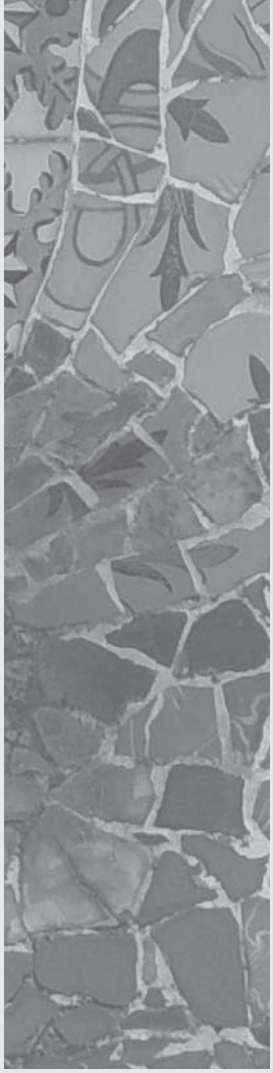
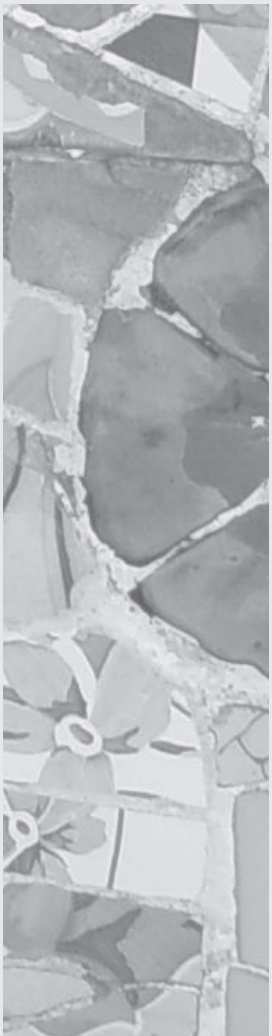


Chapter 8

The burden of HCV treatment in patients with inherited bleeding disorders



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Haemophilia 2011; 17: 791-799

Abstract

Introduction

Many patients with inherited bleeding disorders are infected with hepatitis C virus (HCV). Antiviral treatment, consisting of pegylated interferon and ribavirin, has many side-effects. The aim of this study was to prospectively assess the occurrence and course of side-effects and changes in health-related quality of life (HRQoL) during antiviral treatment in patients with inherited bleeding disorders and chronic HCV.

Methods

Forty-seven patients were followed during antiviral treatment. Side-effects of treatment were recorded, and the Beck Depression Inventory and the RAND-36 HRQoL questionnaire were administered at regular intervals.

Results

Frequently reported side-effects were fatigue (100%), headache (94%), pruritus and skin rash (94%), concentration problems (89%), decreased appetite (89%), fever, irritability and hair loss (all 85%). Many side-effects disappeared soon after end of treatment, but 4 weeks after cessation fatigue, concentration problems and sleeping problems were still present in more than 30% of patients. Dose reduction was necessary in 21 patients (45%), mostly because of decreasing weight or haemoglobin levels. Two patients stopped treatment prematurely because of side-effects. Depression was present in 28 patients (60%). HRQoL decreased significantly during treatment in all RAND-36 domains, and increased again within 4 weeks after treatment. Major side-effects were similar in patients with successful (n = 31, 66%) and unsuccessful antiviral treatment.

Conclusion

In patients with inherited bleeding disorders and chronic HCV, antiviral treatment has many, but mostly transient side-effects, and a significant impact on quality of life. Careful follow-up and management of side-effects will ensure optimal compliance and treatment results.

Introduction

Hepatitis C virus (HCV) infection is an important co-morbidity in adult haemophilia patients. Before the introduction of virus-inactivated clotting factor products, many patients were infected, and about 80% developed chronic HCV [1], progressing to end-stage liver disease after more than two decades in 10-20% of these patients [2]. The objective of antiviral treatment is to eradicate HCV and stop progression of liver damage. According to current guidelines, optimal antiviral treatment consists of a combination of pegylated interferon (PegIFN) and ribavirin. In HCV mono-infected patients, treatment duration is 24 or 48 weeks, depending on HCV genotype and treatment effect. Success rates vary from 50% in genotypes 1 and 4 to 80-90% in genotypes 2 and 3 [3,4]. Successful antiviral treatment has been shown to reduce liver damage, even in patients who have been infected with HCV for a long time and in patients who have significant fibrosis or even cirrhosis [5-7].

In our experience, many haemophilia patients are reluctant to start antiviral treatment, mainly because of expected side-effects and relatively low success rates, especially in HCV genotype 1, which is the most prevalent genotype in this population. Most frequent reported side-effects of antiviral treatment in HCV patients without bleeding disorders are fatigue, headache, influenza-like symptoms, haematological abnormalities and neuropsychiatric symptoms [3,4]. Although a few papers report side-effects of antiviral treatment with PegIFN and ribavirin in patients with inherited bleeding disorders [8-14], little is known about the course of such side-effects and their effect on patients' quality of life in this patient group. It is important for both patients and treating physicians to be aware of side-effects that may occur during antiviral treatment, their time of occurrence, and their impact on daily functioning and quality of life.

The aim of the present study was to prospectively assess the occurrence and course of side-effects and changes in quality of life during antiviral treatment with PegIFN and ribavirin in patients with inherited bleeding disorders and chronic hepatitis C.

Materials and methods

Patients

Forty-seven patients with inherited bleeding disorders, who were treated for HCV in the period 2003 - 2007 at the Van Creveldkliniek, Department of Haematology of the University Medical Center Utrecht, were included in this study. Patients were treated with a combination of PegIFN alpha-2b (1.5 µg/kg once a week) and ribavirin (800-1400 mg per day, depending on body weight). Patients with HCV genotypes 2 or 3 were treated for 24 weeks and patients with genotypes 1 or 4 for 48 weeks. In patients who

had no response to antiviral therapy (less than 2 $\log_{10}$ reduction of viral load in week 12 or HCV RNA detectable in week 24), treatment was discontinued.

Dose reduction of PegIFN was based on the presence of severe physical side-effects, weight loss and/or laboratory parameters (white blood count below 1.5 x 10^9/l and/or platelet count below 50 x 10^9/l), while dose reduction of ribavirin was based on weight loss and/or haemoglobin levels below 6.0 mmol/l.

Data collection

Baseline characteristics and data on treatment duration, treatment efficacy and side-effects of treatment were collected. Data on side-effects were self-reported and collected at baseline (start of treatment), at all visits to our clinic during treatment and 4 weeks after the end of treatment, using a structured list of symptoms of which patients could indicate the presence or absence at that specific time point. Weight was measured at every visit and laboratory parameters (haemoglobin levels, platelet counts, white blood counts) were determined using standard laboratory techniques. Thyroid function was not routinely tested in our patients.

Haemophilia A and B were classified according to factor activity levels as mild (6-40% activity), moderate (1-5%) or severe (< 1%). Early virological response (EVR) was defined as absence of HCV-RNA in serum after 12 weeks of antiviral treatment. Successful antiviral treatment (sustained virological response, SVR) was defined as absence of HCV-RNA in serum six months after completing therapy. Relapse was defined as absence of HCV-RNA in serum at the end of treatment, but a positive value six months later. Non-response was defined as a positive HCV-RNA value both at the end of treatment and six months later.

Depression was assessed using the Beck Depression Inventory (BDI) at baseline, weeks 4, 12, 24 and 48 of treatment and 4 weeks after treatment, as described previously [15]. The BDI is a 21-item self-report depression inventory questionnaire, measuring symptoms of depression during the previous week [16]. Patients are classified according to their total score: no depression (0-9 points), mild (10-16 points), moderate (17-29 points) or severe depression (30-63 points).

Health related quality of life (HRQoL) was assessed using the Dutch version of the RAND-36 questionnaire, which comprises of 36 items, assessing eight domains of HRQoL: physical functioning, social functioning, 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, vitality, bodily pain and general health [17,18]. Each domain is scored form 0 to 100, with higher scores indicating better quality of life. RAND-36 scores were determined at baseline, weeks 24 and 48 and 4 weeks after cessation of treatment.

The study was approved by the medical ethics review board of the University Medical Center Utrecht, and all patients provided written informed consent.

Data analysis

The proportions of patients reporting specific side-effects were calculated at baseline, at weeks 4, 24 and 48 (only for patients with treatment duration of 48 weeks) of treatment and 4 weeks after cessation of treatment. The proportion of patients reporting a specific side-effect at any time during treatment and the overall proportion of observations in which a side-effect was reported (total number of times a side-effect was reported divided by the total number of observations of the presence or absence of that side-effect) were calculated. For all proportion calculations, only patients with available data for a specific side effect were included. At the time points mentioned above, the proportions of patients with weight loss of 5 kg or more, anaemia, leucopenia and thrombocytopenia were determined.

Mean RAND-36 scores were calculated. Changes in RAND-36 scores of 5 points or more were considered clinically relevant [19].

Paired samples t-tests were used to compare laboratory parameters at different time points.

Univariate regression analyses were performed to study the association between the effect of antiviral treatment (SVR versus relapse/no response) as well as of treatment duration (24 versus 48 weeks) and the occurrence of specific side-effects, decrease in weight and laboratory parameters, and dose reduction. The association between the effect of antiviral treatment and the changes in RAND-36 scores in all domains between different time points was assessed by univariate regression analyses. To see whether HRQoL could be improved during treatment by reducing or treating specific side-effects, the associations between weight loss, decrease in haemoglobin levels and highest BDI scores during treatment and changes in the three RAND-36 domains with the largest decreases in scores were analysed. P-values < 0.05 were considered statistically significant. Data were analysed using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics of the 47 participating patients are summarised in Table 1. The one female patient was a carrier of haemophilia A. Thirty-three patients (60%) had severe haemophilia. One patient had (mild) Von Willebrand disease type 2B. HCV genotype 1 was most prevalent (62%), followed by genotypes 3 (23%), 2 (13%) and 4 (2%). Ten patients (21%) were previously treated for HCV (six patients once, two patients twice and one patient three times). Two patients (4%) were co-infected with hepatitis B virus, whereas no patients were co-infected with HIV. Mean age at start of antiviral treatment was 37.7 years (range 17-65 years). Twenty patients (43%) were treated for 24 weeks, 24 (51%) for 48 weeks and in three patients (6%) therapy was discontinued at various other time points (see below).

Table 1. Baseline characteristics of study population.

Total number of patients	47	
Male gender	46	(98%)
Haemophilia A	39	(83%)
Haemophilia B	7	(15%)
Von Willebrand disease	1	(2%)
Severe haemophilia	33	(60%)
Mean age at start of treatment in years (range)	37.7	(17-65)
HCV genotype		
1	29	(62%)
2	6	(13%)
3	11	(23%)
4	1	(2%)
Treatment duration		
24 weeks	20	(43%)
48 weeks	24	(51%)
other	3	(6%)
Early virological response at week 12 of treatment	37	(79%)
Successful antiviral treatment	31	(66%)
Relapse	10	(21%)
No response	6	(13%)
Previously treated for HCV	10	(21%)

HCV = hepatitis C virus

Early virological response at week 12 of treatment was present in 37 patients (79%). Antiviral treatment was successful (SVR) in 31 patients (66%) (in 15 of 29 patients with HCV genotype 1 (52%) and 16 of 17 patients with genotypes 2 or 3 (94%)). Ten patients had a relapse after antiviral treatment (eight patients with HCV genotype 1, one with genotype 3 and one with genotype 4) and six patients showed no response (all with genotype 1). Of the 10 patients without EVR, six had no response to treatment, three had a relapse and one had SVR.

The total number of visits during which side-effects were assessed was 353, and the median number of assessments per patient was 7 (range 3-15).

Side-effects

The occurrence of specific side-effects during antiviral treatment is presented in Table 2. Figure 1 shows the proportions of patients reporting specific side-effects at baseline, at different time points during treatment and 4 weeks after cessation of treatment. At baseline, only irritability was reported in more than 10% of patients. During treatment, all patients reported fatigue. Other frequently reported side-effects were headache (present at any time during antiviral treatment in 94% of patients), pruritus and skin rash (94%), concentration problems (89%), decreased appetite (89%), fever (85%), irritability (85%), hair loss (85%) and sleeping problems (83%). Most side-effects started during the first weeks of antiviral treatment. Only hair loss occurred in less than 10% of patients after 4 weeks. Four weeks after cessation of treatment, most side-effects were reported in less than 20% of patients. Only fatigue (59%), pruritus and skin rash (42%), concentration problems (39%) and sleeping problems (34%) were still present in a large number of patients.

Table 2. Reported side-effects during antiviral treatment.

Side-effect	Proportion of patients in whom side-effect was present at any time during treatment (%)	Overall % of observations in which side-effect was reported
Fatigue	100	94
Headache	94	66
Pruritus/skin rash	94	65
Concentration problems	89	73
Decreased appetite	89	61
Irritability	85	61
Hair loss	85	38
Fever	85	36
Sleeping problems	83	52
Dizziness	72	34
Nausea	70	34
Diarrhea	64	33
Pain in right upper abdomen	57	27

Interpretation: 94% of patients reported headache at least once during antiviral treatment, whereas headache was present in 66% of all evaluations (observations) in which patients were asked about its presence. So nearly all patients experienced headache, but only 66% of the time.

Other reported side-effects (not shown in Table 2 or Figure 1) were dyspnoea in 20 patients (43%), cough in 10 patients (21%), dry mouth and thirst in nine patients (19%), dry and painful eyes in six patients (13%), changes in sensation of taste in five patients (11%) and memory loss in four patients (9%).

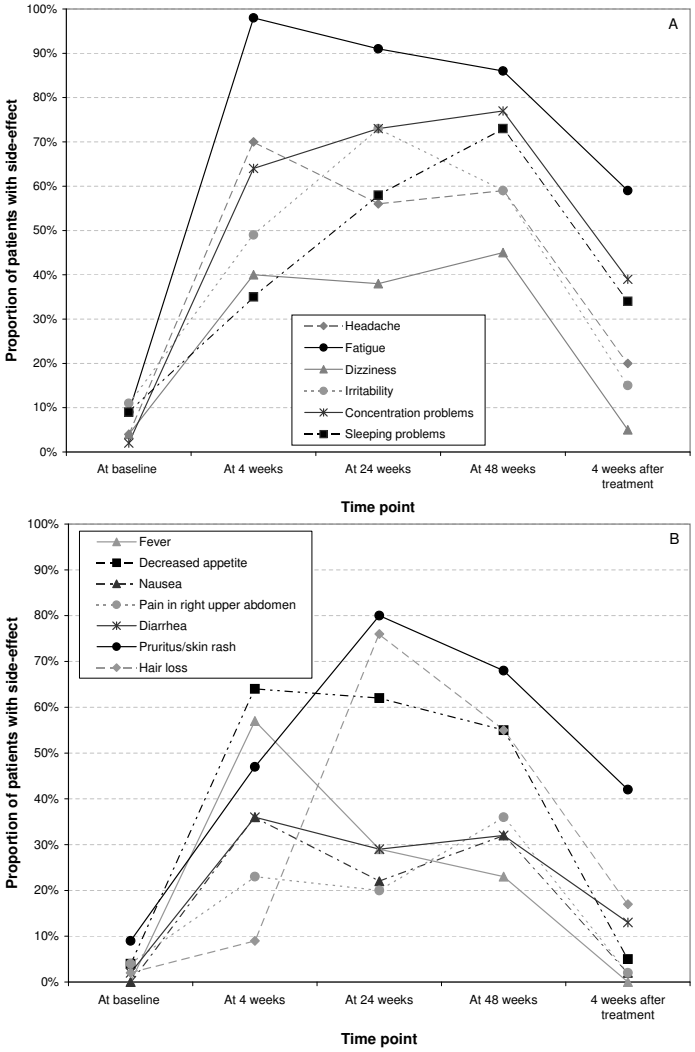

Figure 1. Proportion of patients experiencing various side-effects of antiviral treatment at baseline, at 4, 24 and 48 weeks and 4 weeks after cessation of treatment.

A. Headache, fatigue, dizziness, irritability, concentration problems and sleeping problems.

B. Fever, decreased appetite, pain in right upper abdomen, diarrhea, pruritus/skin rash and hair loss.

Note: assessment at 48 weeks included only the 24 patients who were treated for 48 weeks.

Table 3 shows patients' weight, laboratory parameters, the occurrence of depression and work participation during and 4 weeks after treatment. Weight loss was an important problem: 60% of patients lost more than 5 kg, and the overall mean decrease in weight was 6.7 kg (range 0-19 kg). Mean decrease in haemoglobin levels was 2.4 mmol/l (range 0.6-4.1 mmol/l), whereas white blood count showed a mean decrease

of 4.3 x10^9/l (range 2.1-9.0 x10^9/l) and platelet count of 82 x10^9/l (range 0-186 x10^9/l). A large decrease in haemoglobin levels occurred during the first 4 weeks of treatment, continuing during the first 24 weeks. Between weeks 24 and 48, haemoglobin levels remained stable (p-value 0.60), to significantly rise again after cessation of treatment. In four patients (9%, all treated for more than 24 weeks), haemoglobin levels dropped below 6.0 mmol/l (lowest value 5.5 mmol/l).

Table 3. Weight, laboratory parameters, depression and work participation during and 4 weeks after antiviral treatment.

	At baseline	**At 4 weeks**	**At 24 weeks**	**At 48 weeks**	**4 weeks after treatment**	**At any time during treatment**
Physical side-effects						
Weight loss compared to baseline in kg	-	2.1 (0.3-4.0)	4.9 (2.0-7.0)	7.7 (3.8-10.8)	5. (1.3-7.0)	6.7 (4.0-8.0)
Weight loss ≥ 5 kg	-	13%	49%	67%	-	60%
Laboratory parameters						
Decrease in Hb level compared to baseline in mmol/l	-	1.6 (1.0-2.2)	2.1 (1.8-2.5)	2.0 (1.4-2.7)	0.7 (0.2-1.0)	2.4 (1.8-3.0)
Anaemia *	0%	70%	79%	76%	13%	87%
Hb levels < 6.0 mmol/l	0%	0%	0%	5%	0%	9%
Decrease in WBC compared to baseline x10^9/l	-	3.2 (2.2-4.1)	3.9 (2.8-4.5)	3.9 (2.9-4.6)	0.9 (-0.1-2.1)	4.3 (3.1-5.1)
Leucopenia **	4%	81%	88%	95%	13%	98%
WBC < 1.5 x 10^9/l	0%	2%	7%	5%	0%	9%
Decrease in platelet count compared to baseline x10^9/l	-	50 (20-82)	70 (51-91)	64 (48-81)	3 (-18-26)	82 (57-103)
Thrombocytopenia #	9%	38%	58%	50%	13	66%
Platelet count < 50 x 10^9/l	0%	0%	0%	0%	0%	2%
Psychosocial side-effects						
Depression ##	11%	32%	57%	59%	17%	60%
Mild	11%	23%	36%	32%	17%	34%
Moderate	0%	9%	21%	22%	0%	23%
Severe	0%	0%	0%	5%	0%	2%
Mean participation in work/ study (% of full-time)	87%	36%	39%	45%	68%	-

Values are means (interquartile range) or proportions.
Hb = haemoglobin
WBC = white blood count
* anaemia: haemoglobin levels < 8.1 mmol/l in men and < 7.4 mmol/l in women.
** leucopenia: white blood count < 4.0 x10^9/l in both men and women.
# thrombocytopenia: platelet count < 150 x10^9/l in both men and women.
depression was assessed using the Beck Depression Inventory (BDI). BDI-score 10-16 points indicates mild depression, 17-29 points moderate depression and 30-63 points severe depression.
Note: assessment at 48 weeks included only the 24 patients who were treated for 48 weeks.

White blood counts showed a similar pattern of change to haemoglobin levels, with four patients who had levels below 1.5 $x10^9$/l. Platelet counts also showed a significant decrease during the first months of treatment, but seemed to increase somewhat between weeks 24 and 48 (p-value 0.04), whilst showing a larger and more significant increase after cessation of treatment (p-value <0.005). Only one patient had a platelet count below 50 $x10^9$/l.

A total of 28 patients (60%) had depression (BDI score of 10 points or more) during treatment. Of the 42 patients who did not have depression at baseline, 23 (55%) developed depression during treatment (14 mild, eight moderate and one severe). Mean work participation was 87% of full-time at baseline and decreased to 36% within 4 weeks. Four weeks after cessation of treatment, work participation was still almost 20% lower than at baseline (68%). Mean work loss was 61% (range 0-100%). Only four patients worked full-time during antiviral treatment. The proportion of patients who did not work at all was 5% at baseline, 30% at end of treatment and 16% 4 weeks after treatment.

Dose reduction and premature discontinuation of treatment

The course of antiviral treatment in our patients is shown in Figure 2. Nineteen patients (40%) completed the full intended course of treatment, without any dose reductions or premature cessation of treatment. Of these patients 17 (89%) had SVR, while two (11%) relapsed. Dose reduction was necessary in 21 patients (45%) and occurred between weeks 2 and 39 of treatment (median week 12). Reasons for dose reduction were weight loss in 13 patients (62%), anaemia in five (24%), leucopenia in two (10%) and thrombocytopenia in one patient (5%). Three patients needed second dose reductions, in weeks 26, 41 and 46, respectively. Dose reduction resulted in improvement of laboratory parameters in all patients. Of the 21 patients who had dose reductions, 13 (62%) had SVR, while 8 (38%) relapsed. In eight patients (17%), antiviral treatment was stopped prematurely, including five patients with HCV genotype 1 who were non-responders and were therefore treated for 24 weeks instead of the intended 48 weeks. In three patients, treatment was discontinued at various other time points (19, 38 and 36 weeks respectively), because of physical side-effects (fatigue, concentration problems, irritability, sleeping problems, shortness of breath, earache, throat ache) in combination with leucopenia in the first patient, physical side-effects (fatigue, cough, pruritus) in the second patient and insufficient response to therapy in the third patient. The first patient also needed dose reduction of PegIFN because of weight loss in week 16. He had a relapse. The second patient had SVR.

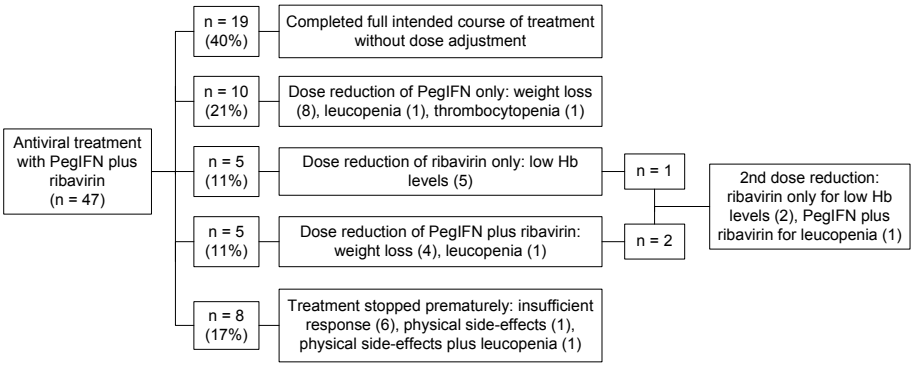

Figure 2. Course of antiviral treatment with PegIFN plus ribavirin in 47 patients with inherited bleeding disorders and chronic hepatitis C.
PegIFN = pegylated interferon
Hb = haemoglobin

Quality of life

At baseline, highest RAND-36 scores were seen in the domains social functioning, role emotional and mental health (all higher than 80 points), while HRQoL was lowest in the domains vitality and general health (both 65 points). At week 24 of treatment, a dramatic reduction of HRQoL was observed in all domains, most notably in the domains role physical, role emotional and vitality (Figure 3). In all domains, a decrease in mean RAND-36 scores of more than 5 points was observed (mean decrease 22.5 points, range 5.5 points in general health to 50 points in role physical). Between weeks 24 and 48 of treatment, mean RAND-36 scores continued to decrease with more than 5 points in all domains except for general health (mean decrease 9.7 points, range 4.7 points in general health to 18.3 points in role emotional). In the four weeks after cessation of treatment, large improvements in mean RAND-36 scores were seen in all domains (mean increase in RAND-36 scores 19.9 points, range 9.0 points in general health to 32.2 points in role physical), with results approaching, and sometimes even exceeding baseline levels in the domains physical functioning, mental health, vitality, bodily pain and general health.

Side-effects did not seem to be predictive of treatment effect. Univariate regression analyses showed no statistically significant association between successful antiviral treatment (SVR) and any of the side-effects mentioned in Table 2, decrease in weight, haemoglobin levels, white blood counts or platelet counts or dose reduction, nor between treatment duration and these variables. No statistically significant association was found between the effect of antiviral treatment and changes in any of the RAND-36 domains.

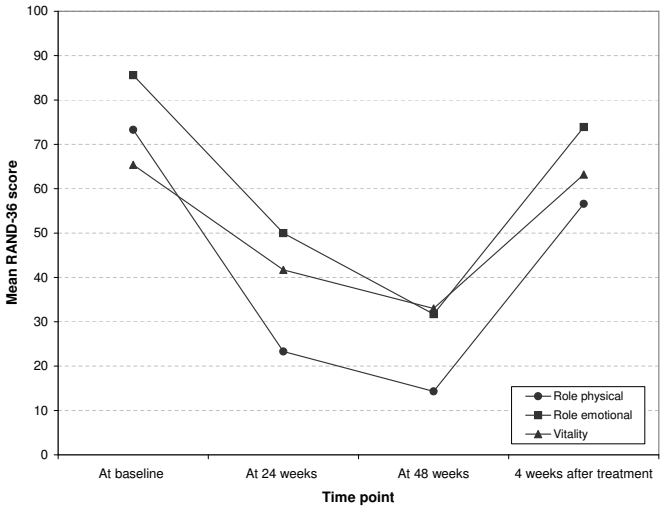

Figure 3. RAND-36 scores in the domains role physical, role emotional and vitality during and 4 weeks after antiviral treatment. Higher RAND-36 scores indicate better health-related quality of life.
Note: assessment at 48 weeks included only the 24 patients who were treated for 48 weeks.

There were no significant associations between decrease in weight or haemoglobin levels and decrease in RAND-36 scores in the three most affected domains (role physical, role emotional and vitality) during antiviral treatment either. The presence of EVR, however, was associated with significantly higher RAND-36 scores at week 24 in the domains role emotional (p-value 0.01) and vitality (p-value 0.04), but not in the domain role physical. There was also a significant association between highest BDI scores during treatment and decrease in RAND-36 scores in the domains role emotional (p-value 0.003) and vitality (p-value 0.002), but not in the domain role physical (p-value 0.11).

Discussion

Our study showed that antiviral treatment puts a large burden on patients with inherited bleeding disorders and chronic HCV. Frequently reported side-effects were fatigue, headache, pruritus and skin rash, concentration problems, decreased appetite, fever, irritability, hair loss and sleeping problems. Depression occurred in 60% of patients and there was significant impairment in all domains of health-related quality of life. Mean decrease in work participation was 61%. Side-effects were reason for dose reduction in 45% of patients. Overall, though, 83% of patients completed the full course of treatment (40% without any dose reductions). In only two patients (4%), side-effects were reason for cessation of treatment. This remarkable achievement might, at least partly, be explained by a high rate of compliance in these patients, who are used to dealing with chronic disease and its treatment [10], in combination with the close monitoring of pa-

tients during antiviral treatment by a comprehensive care team. Although compliance was not measured in our patients, attendance at regular follow-up visits during antiviral treatment was almost 100%, and guidance and support from physicians, nurses and a social worker were routinely scheduled.

The aim of our study was to assess the occurrence and course of side-effects of antiviral treatment and its effect on health-related quality of life in patients with inherited bleeding disorders and chronic HCV. Inherent to all studies in this specific population, analyses are based on relatively small numbers. Many side-effects were self-reported, but structured questionnaires were used. We therefore think we were able to provide a reliable assessment of the burden of antiviral treatment in our patient population.

Side-effects

It turned out to be difficult to compare the occurrence of specific physical side-effects with results of other published papers, mainly because they do not describe how their numbers were derived. These studies report the occurrence of side-effects in a certain proportion of patients, but it is unclear how, when and how often this was assessed, making reliable comparisons impossible.

Laboratory parameters (haemoglobin levels, white blood counts and platelet counts) decreased significantly during the first 24 weeks of treatment, but this decrease seemed to level off and was no longer significant between weeks 24 and 48 for haemoglobin levels and white blood counts. The significant increase in platelet counts between weeks 24 and 48 might be caused by dose reductions of antiviral treatment in several patients. Mean decreases in laboratory parameters in our patients were comparable to those described by Katsarou et al in 50 patients with congenital coagulation disorders [9], and dose reduction was similar to the study of Mancuso et al, in which 44% of haemophilia patients required dose reduction of either PegIFN, ribavirin or both [10].

Depression occurred in 55% of patients who did not show depressive symptoms at baseline, whereas moderate to severe depression, requiring antidepressant treatment, occurred in 21%. The occurrence and course of depression during antiviral treatment in this specific cohort is described in more detail elsewhere [15]. Treating physicians should be alert for signs and symptoms of this important side-effect.

Several side-effects (fatigue, skin problems, concentration problems and sleeping problems) remained present in a large number of patients 4 weeks after cessation of treatment, and work participation was still 19% lower than at baseline. Our experience is that it often takes a few months for patients to return to normal after antiviral

treatment. It is important for both physicians and employers to realise that the end of antiviral treatment does not necessarily mean the end of treatment-related problems. We advise patients to slowly build up their normal daily routines during the first weeks to months after the end of treatment, to ensure optimal recuperation.

Antiviral treatment was successful in 66% of our patients (95% confidence interval (CI) 52-80%). This is in accordance with the 63% (95% CI 51-75%) SVR reported in 64 haemophilia patients and the 56% (CI 47-65%) and 54% (CI 49-58%) reported in non-haemophilic populations treated with PegIFN-alpha-2b plus ribavirin [4,10,20]. We did not find any significant associations between the effect of antiviral treatment and the presence of specific side-effects or dose reduction in this small cohort. We could not confirm the hypotheses of others that the presence of severe side-effects influences compliance to antiviral treatment and thus reduces the chance of successful treatment [21] or could reflect a larger impact of antiviral treatment on the body, thus increasing the chance of achieving SVR [22,23].

Quality of life

Both haemophilia and hepatitis C have been reported to reduce health-related quality of life [24-27]. At baseline, mean RAND-36 scores varied from 65 to 86 points in different domains in our study population. During antiviral treatment, large decreases were seen in all HRQoL domains. Similar results were reported by Hassanein et al in 453 non-haemophilic patients treated with pegylated interferon and ribavirin. In this study, the effects on the domains physical functioning, social functioning, role physical, role emotional, vitality and bodily pain were most prominent [28]. The relatively small effect in the domain bodily pain we found in our study could reflect the fact that our patients are used to pain caused by arthropathy, thus reducing the impact of antiviral treatment in this domain. Similar to our study, other reports in HCV patients without inherited bleeding disorders show largest decreases in RAND-36 scores during antiviral treatment in the domain role physical [28-30], indicating that the main problem for patients during treatment is impairment in work or daily activities due to physical side-effects. Work participation was indeed significantly reduced in our patients. We did not find any association between the effect of antiviral treatment and changes in RAND-36 scores during antiviral treatment, although such associations have been reported in other studies [28,30,31]. EVR at week 12, however, was associated with better RAND-36 scores at week 24 in the domains role emotional and vitality, implying that knowing that the hepatitis C virus is responding well to treatment has a positive effect on HRQoL.

Clinical implications

In order to maintain compliance and avoid unnecessary or unnecessarily severe side-effects, regular monitoring and optimal support of patients during antiviral treatment by a multidisciplinary team is essential. Dose reduction of PegIFN, ribavirin or both should be instigated when indicated, and treatment of side-effects started. The lack of association between decrease in HRQoL and weight loss or decrease in haemoglobin levels makes targeted interventions in these areas to improve quality of life during antiviral treatment difficult. There was, however, an association between high BDI scores and decreased quality of life, confirming that treatment with antidepressants (preferably selective serontonin-reuptake inhibitors) should be offered to patients experiencing significant depressive symptoms [15,32,33]. Prophylactic treatment with antidepressants could also be considered, especially in patients with a history of depression or other psychiatric problems [15].

New HCV treatment strategies with better success rates are being developed. Protease inhibitors like telaprevir and boceprevir will probably be available within a few years. Combination treatment with PegIFN, ribavirin and telaprevir, with a treatment duration of 24 weeks for all HCV genotypes, has been shown to increase success rates in patients with genotype 1 [34,35]. A large advantage of this strategy, besides the higher success rate, is the much shorter treatment duration in patients with HCV genotypes 1 or 4, while the down side appears to be an increased occurrence of severe skin problems like rash and eczema during treatment [34,35]. Further research will have to determine the effect and side-effects of new treatment strategies in HCV patients with and without inherited bleeding disorders.

Conclusion

Antiviral treatment puts a large burden on patients with HCV and inherited bleeding disorders. Side-effects are frequent, may require dose reduction or even discontinuation of treatment and have a large impact on quality of life. However, many patients completed the full course of therapy. Most side-effects disappeared shortly after cessation of treatment, but limitations like fatigue, concentration problems and sleeping problems were still present in a large proportion of patients 4 weeks after the end of treatment. Close observation of patients by a multidisciplinary team and treatment of side-effects during antiviral treatment are warranted to ensure optimal compliance and treatment results and to minimise patient burden.

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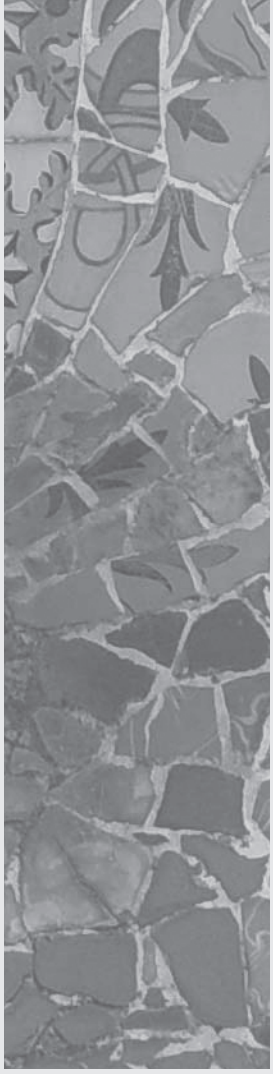
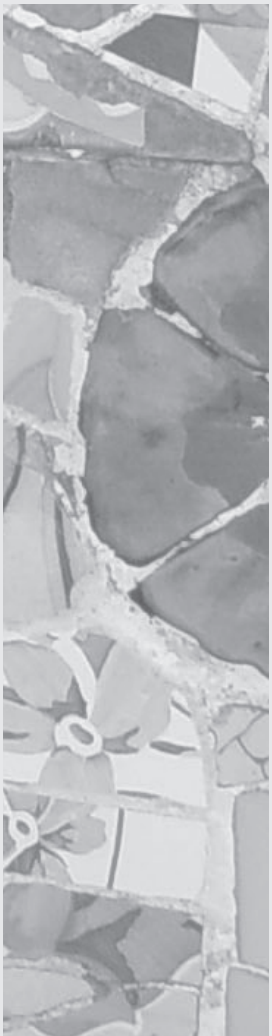
Chapter 9

Occurrence, course and risk factors of depression during antiviral treatment for chronic hepatitis C in patients with inherited bleeding disorders: a prospective study





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Abstract

Introduction

Treatment of hepatitis C virus (HCV) consists of pegylated interferon-□ and ribavirin for 24 or 48 weeks. An important side-effect of interferon-□ is depression. The occurrence, course and risk factors of depression during antiviral treatment were studied prospectively in HCV patients with inherited bleeding disorders.

Methods

The Beck Depression Inventory, indicating no, mild, moderate or severe depression, was administered to 47 patients before starting therapy, after 4, 12, 24, and 48 weeks of treatment, and 4 weeks after cessation of therapy.

Results

At baseline, five patients (11%) had mild depression. Depression worsened during treatment in three of these patients. In all five patients (mild) depression persisted 4 weeks after treatment. Of the remaining 42 patients, 23 (55%) developed depression during treatment (14 mild, eight moderate and one severe), mostly (78%) during the first 12 weeks. Four weeks after cessation of treatment, three of 23 patients still had mild depression. The only independent risk factor for development of depression was a history of depression or other psychiatric problems (odds ratio 9.7).

Conclusions

For patients with inherited bleeding disorders depression is a significant, mostly transient, problem during HCV treatment. We recommend close monitoring of patients, especially those with previous psychiatric problems, to ensure adequate detection and treatment of depression during antiviral therapy.

Introduction

Many haemophilia patients were infected with hepatitis C virus (HCV) after receiving contaminated clotting factor products before 1992 [1]. About 80% of infected patients develop chronic hepatitis C, resulting in end-stage liver disease (cirrhosis) in 10-20% of these patients, after a period of over 20 years [2,3]. The objective of antiviral treatment is eradication of HCV, to stop progression of liver damage. Today, optimal treatment consists of a combination of pegylated interferon (PegIFN) and ribavirin. Treatment duration is either 24 or 48 weeks, depending on HCV genotype and treatment-effect. Success rates vary from 50% in genotypes 1 and 4 to 80-90% in genotypes 2 and 3 [4,5]. Success rates and side-effects of antiviral treatment in patients with inherited bleeding disorders are comparable to those in the general population [3,6]. The most important side-effects of antiviral treatment are fatigue, flu-like symptoms, haematologic abnormalities and neuropsychiatric symptoms, like depression [7].

The mechanisms through which IFN-☐ induces depression are largely unknown. Interferon seems to have a direct effect on different cerebral processes. Down-regulation of the glucocorticoid receptor, which is an important component of the negative feedback system of the hypothalamic-pituitary-adrenal axis, and of the serotonin receptor 1A (5-HTR1A), is suggested to play an important role [8]. In addition, an increase in transcription and uptake activity of serotonin transporters may also induce depressive symptoms [9]. Changes in the levels of certain cytokines, most importantly increased levels of interleukin (IL)-6 and IL-8, are reported to be associated with increasing depression scores during IFN-☐ therapy [10]. The opioid system and the norepinephrine system may also be important in the development of depression during IFN-☐ treatment [11]. Ribavirin is reported to increase the risk of developing depressive symptoms, in addition to the effect of IFN-☐ [12,13].

Depression is reported in 22-41% of HCV patients on antiviral treatment, often associated with impaired quality of life, and may require dose reduction or even cessation of therapy [4,5,14-16]. Little is known about the occurrence of this major side-effect of antiviral treatment in patients with inherited bleeding disorders. Because they already suffer from a chronic illness, they might be more inclined to develop depression than other HCV patients. On the other hand, patients with inherited bleeding disorders might be better able to cope with side-effects of antiviral treatment, because of their previous experience with dealing with chronic and debilitating medical problems.

The aim of this study was to prospectively assess the occurrence, course, treatment and risk factors of depression during antiviral treatment for chronic hepatitis C in patients with inherited bleeding disorders.

Patients and methods

All patients with inherited bleeding disorders treated for chronic HCV during the period 2003 - 2007 at the Van Creveldkliniek, Department of Haematology of the University Medical Center Utrecht, were enrolled in this study. Chronic HCV infection was defined as the presence of HCV antibodies and HCV-RNA in plasma for more than six months. Patients were treated with a combination of PegIFN alpha-2b (1.5 µg/kg once a week) and ribavirin (800-1400 mg per day depending on body weight). In accordance with current guidelines, patients with genotypes 2 or 3 were treated for 24 weeks and patients with genotypes 1 or 4 for 48 weeks. In patients who had no response to antiviral therapy (less than 2 $\log_{10}$ reduction of viral load in week 12 or HCV RNA detectable in week 24), treatment was discontinued.

Data collection

Patients completed the Dutch version of the Beck Depression Inventory (BDI, version IA), before starting therapy (baseline), at weeks 4, 12, 24 and 48 (for genotypes 1 and 4 only) of treatment, and 4 weeks after cessation of therapy (week 28 or 52 after starting treatment). In patients who discontinued treatment prematurely, a final BDI measurement was also performed 4 weeks after cessation of therapy.

The BDI is a 21-item self-report depression inventory questionnaire, measuring characteristic attitudes and symptoms of depression during the previous week [17]. Each of the 21 items consists of four statements, describing symptom severity along an ordinal continuum from absent or mild to severe, and can be assigned 0-3 points. Patients are classified according to their total score: no depression (0-9 points), mild (10-16 points), moderate (17-29 points) or severe depression (30-63 points). Treatment with antidepressants is recommended for all patients with moderate or severe depression (17 points or more) [17].

Baseline characteristics (age, gender, type and severity of inherited bleeding disorder, co-infection with HIV or hepatitis B virus) and data on treatment duration, treatment efficacy, side-effects of treatment, use of antidepressant medication before and during treatment and history of depression or other psychiatric disorders were collected for all patients.

Inherited bleeding disorders in our study population included haemophilia A and B and Von Willebrand disease, which were classified according to their factor activity levels as mild (6-40% activity), moderate (1-5%) or severe (< 1%). Successful treatment (sustained virological response, SVR) was defined as absence of HCV-RNA in serum at the end of treatment and six months after completing therapy. Relapse was defined

as the absence of HCV-RNA in serum at the end of treatment, but a positive value six months later. Non-response was defined as a positive HCV-RNA value both at the end of treatment and six months later.

Patients were classified as having a history of depression or other psychiatric problems if they reported such a disorder, for which treatment was indicated. HCV genotype was determined before starting treatment. HCV genotype and HCV-RNA testing was performed as described previously, using the reverse hybridization line probe assay and the COBAS AMPLICOR HCV Test, respectively [18].

The study was approved by the medical ethics review board of the University Medical Center Utrecht. Written informed consent was obtained from all patients.

Data analysis

The maximum BDI score during treatment was determined for each patient. In patients without a BDI score at baseline or 4 weeks after cessation of antiviral treatment, the presence and severity of depression at that time point were assessed using available clinical information. Only when no BDI score was available and clinical assessment could not be made, depression was classified as unknown for that specific time point. A distinction was made between patients with depression (BDI score 10 points or more) and without depression at baseline. Three depression-related outcome classes were analysed: (1) any depression, (2) moderate or severe depression (representing an indication for antidepressant therapy) and (3) an increase by one or more categories of the BDI score from baseline levels during antiviral treatment.

Patients were further categorised for analysis according to treatment duration (24 weeks, 48 weeks or other duration), severity of their bleeding disorder (severe versus non-severe bleeding disorder) and effect of treatment (SVR versus relapse or no response). Median BDI scores were calculated using all available BDI scores for each time point. The Mann-Whitney U-test was used to compare median BDI scores according to treatment duration. A non-parametric statistical test was used because data were not normally distributed. A p-value < 0.05 was considered statistically significant.

Univariate and multivariate logistic regression analyses were used to examine the association between the development of depression, represented by an increase by one or more categories of the BDI score (no, mild, moderate or severe depression) during antiviral treatment compared with baseline levels, and a number of determinants. Determinants with an odds ratio (OR) significant at the 0.15 level in the univariate

analysis were included in the multivariate analysis. Data were analysed using SPSS 14.0 statistical software (SPSS Inc., Chicago, IL, USA).

Results

Forty-seven patients were included in the study. All patients were infected with hepatitis C through clotting factor concentrates. Baseline characteristics are summarised in Table 1. Thirty-three patients (70%) had severe haemophilia. The one patient with Von Willebrand disease had a mild bleeding pattern. HCV genotype 1 was most prevalent (62%), followed by genotypes 3 (23%), 2 (13%) and 4 (2%). Two patients (4%) were co-infected with hepatitis B virus. No patients were co-infected with HIV. Mean age at start of antiviral treatment was 37.7 years (range 17-65). Fifteen patients (32%) had a known history of depression or other psychiatric problems prior to antiviral treatment (eight had depression, four had other problems and three had both).

Table 1. Baseline characteristics of study population.

Total number of patients	47	
Male gender	46	(98%)
Haemophilia A	39	(83%)
Haemophilia B	7	(15%)
Von Willebrand disease type 2B	1	(2%)
Severe bleeding disorder	33	(70%)
Mean age at start treatment in years (range)	37.7	(17-65)
History of psychiatric problems	15	(32%)
Depression only	8	(17%)
Other psychiatric problems only	4	(9%)
Both depression and other problems	3	(6%)
Mild depression at start of treatment	5	(11%)
Treatment duration		
24 weeks	20	(43%)
48 weeks	24	(51%)
Successful antiviral treatment	31	(66%)

At baseline, five patients had mild depression according to the BDI questionnaire (BDI scores 10, 10, 12, 14 and 16, respectively). Forty-one patients (87%) had no depression and one patient had no depression assessment. Two patients without depression were using antidepressant medication at baseline because of past depressive symptoms. One patient had mild depression at baseline (BDI score 10 points) despite antidepressant medication.

Twenty patients (43%) were treated for 24 weeks, including six patients with genotype 1 who were non-responders. Twenty-four patients (51%) were treated for 48 weeks, and

in three patients (6%) therapy was discontinued at various other time points (19, 36 and 38 weeks, respectively) for reasons other than depression. Antiviral treatment was successful in the majority of patients (n = 31, 66%). Ten patients (21%) experienced a relapse and six patients (13%) were non-responders.

Development and course of depression

Data on the occurrence of depression during antiviral treatment are shown in Table 2. A large proportion of patients (60%, 95% confidence interval (CI) 46-74%) had depression during antiviral treatment. The presence of depression at baseline, during antiviral treatment and 4 weeks after cessation of antiviral therapy is shown in Figure 1. Depression worsened during treatment in three (60%) of five patients who had mild depression at baseline. Of the 42 patients without depression at baseline, 23 (55%) developed depression during antiviral treatment (14 mild, eight moderate and one severe). In the majority (78%), depression developed during the first 12 weeks. Four of seven patients with depression 4 weeks after cessation of antiviral treatment also had depression at baseline, whereas three developed depression during treatment (two moderate and one severe). Unfortunately, no depression assessment could be made 4 weeks after cessation of therapy for one patient with mild depression at baseline and during treatment, three other patients who developed depression during treatment (two mild and one moderate) and one patient without depression.

Table 2. Outcome parameters in all patients and in patients without depression at baseline.

	All patients (n = 47)		**Patients without depression at baseline** (n = 42)	
Depression at any point during antiviral treatment	28	(60%)	23	(55%)
Depression present before week 12	23/28	(82%)	18/23	(78%)
Increase of one or more depression categories during antiviral treatment	26	(55%)	23	(55%)
Indication for antidepressant treatment during antiviral therapy (moderate or severe depression)	12	(26%)	9	(21%)
Patients actually receiving antidepressant treatment during antiviral therapy	7/12	(58%)	6/9	(67%)

Regardless of the presence of depression at baseline, 55% of patients showed an increase by one or more categories of the BDI score (no, mild, moderate or severe depression). The median BDI score at baseline was 1.0 point (range 0-16 points), indicating a low baseline level of depressive symptoms. The median maximal BDI score during antiviral treatment was 13.0 points (range 1-35). Four weeks after cessation of antiviral treatment, the median BDI score was almost back to baseline (3.0 points, range 0-14).

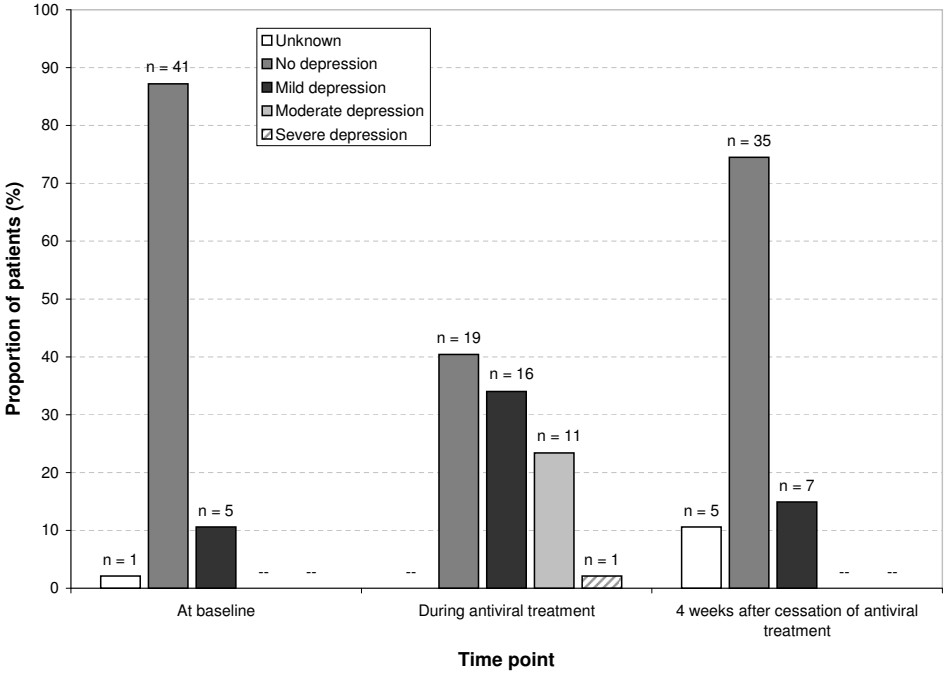

Figure 1. Proportion of patients without or with mild, moderate or severe depression at baseline, at any time point during antiviral treatment and 4 weeks after cessation of antiviral treatment (n = 47).

Figure 2 shows that the course of depression depended on treatment duration (24 or 48 weeks). The graph shows a clear increase in BDI score over time for both treatment durations. A trend towards a greater increase in depression score for the longer treatment duration was seen (median BDI score at end of treatment 9.0 versus 13.5 points, p-value of the Mann-Whitney U-test 0.10), which was already present at 24 weeks (median BDI score at 24 weeks 9.0 versus 12.0 points, p-value 0.10). After cessation of antiviral treatment, a very large reduction in BDI score was seen in both groups.

Antidepressant treatment

Only seven out of 12 patients who had an indication for antidepressant treatment were actually treated with antidepressant medication, including one patient who had already been receiving antidepressant therapy at start of antiviral treatment. Other patients were offered antidepressant medication but declined. In five out of seven patients, antidepressants were stopped within 4 weeks after cessation of antiviral treatment.

Antidepressant medication did not prevent worsening of depression during antiviral treatment in any of the three patients who were on antidepressant therapy at baseline (maximum BDI score during treatment 14, 15 and 18 points, respectively).

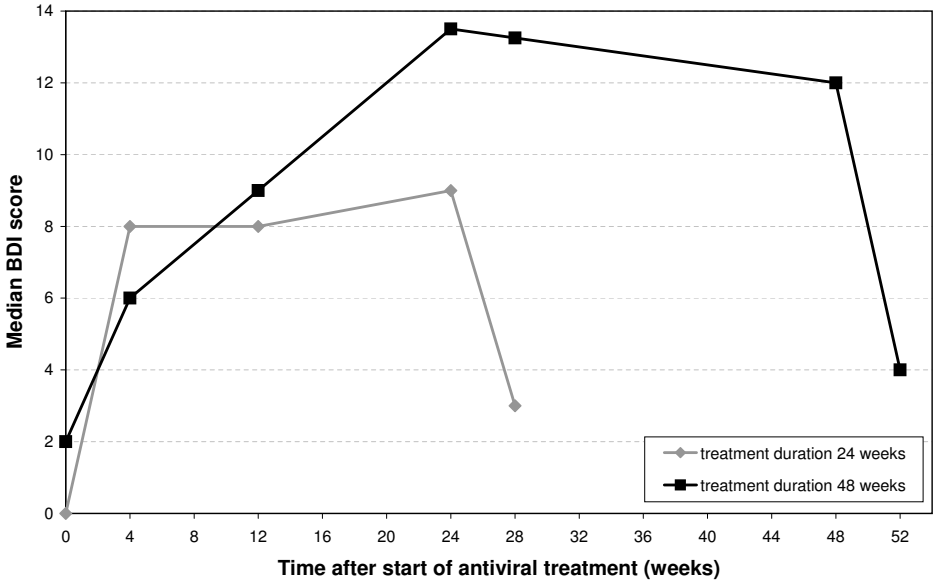

Figure 2. Median BDI scores over time according to treatment duration.
BDI = Beck Depression Inventory.

Four weeks after cessation of antiviral therapy, all three patients were still using antidepressant therapy, and one of them still had mild depression.

Risk factors

The results of the logistic regression analyses to determine risk factors for the development of depression during antiviral treatment are presented in Table 3. The only independent risk factor for developing depression during antiviral treatment was a past history of depression or other psychiatric problems (adjusted OR 9.7, 95% CI 1.8-51.9, p-value < 0.01).

Age was not a relevant factor in the development of depression in our study population. No association was found between the occurrence of depression during antiviral treatment and the presence of a severe bleeding disorder, treatment duration or treatment response. The BDI score at baseline was not associated with a worsening of depression by one or more categories during antiviral treatment either. Almost all patients with a history of depression or other psychiatric problems (14 of 15, 93%) had depression during antiviral treatment. Of patients without such a history, 14 of 32 (44%) had depression. In five out of 15 (33%) patients with a past history of psychiatric problems, antidepressant treatment was indicated.

Table 3. Univariate and multivariate logistic regression analyses of determinants possibly associated with an increase of one or more categories of the BDI score during antiviral treatment compared with baseline levels.

	Univariate analysis			**Multivariate analysis**		
	Crude OR (95% CI)		*p-value*	*Adjusted OR (95% CI)*		*p-value*
Age at start of antiviral treatment (continuous)	1.04	(0.99-1.10)	0.13	1.04	(0.99-1.10)	0.15
Severe bleeding disorder (yes versus no)	2.1	(0.6-7.3)	0.27	-		-
Treatment duration (48 versus 24 weeks)	1.4	(0.4-4.6)	0.58	-		-
Past history of depression or other psychiatric problems (yes versus no)	9.5	(1.8-49.3)	<0.01	9.7	(1.8-51.9)	<0.01
Successful antiviral treatment (yes versus no)	1.4	(0.4-4.7)	0.60	-		-
Baseline BDI score in points (continuous)	1.0	(0.9-1.2)	0.90	-		-

OR = odds ratio
CI = confidence interval
BDI = Beck Depression Inventory

Discussion

To our knowledge, this is the first prospective study reporting the occurrence, course, treatment and risk factors of depression during antiviral treatment for chronic HCV in patients with inherited bleeding disorders. Five of 47 patients (11%) had mild depression at baseline. Depression developed during antiviral treatment in 55% of the other 42 patients, generally during the first 12 weeks of therapy. In the majority of patients, depressive symptoms resolved within 4 weeks after cessation of treatment. Most patients were adequately treated with antidepressants. The only independent risk factor for depression was a past history of depression or other psychiatric problems. This implies that, in clinical practice, patients with such a history should be monitored even more closely for the development of depression during antiviral treatment than other patients.

The most important limitation of our study is the relatively small sample size. In a larger study population, it might have been possible to better identify characteristics that might put patients at high risk of developing depression. Another limitation of our study is that we used only the BDI questionnaire to evaluate depression severity. No evaluation was undertaken to make a clinical diagnosis of a psychiatric illness. The BDI has, however, been shown to be valid and reliable, with results corresponding to clinician ratings of depression in more than 90% of cases [19]. It is also reported to be

a reliable tool to assess depression during antiviral therapy in patients with chronic hepatitis C [20].

One drawback of the BDI-IA is that it was developed primarily to reflect symptoms found in severe depression and does not provide complete coverage of symptoms used in the DSM-IV criteria for diagnosing psychiatric disorders [21]. For example, items reflecting increase in appetite or sleep, and agitation are not included. Future studies should use the revised BDI-II [22], which better corresponds with criteria for diagnosing depressive disorders as defined in the DSM-IV. Furthermore, the BDI's reliance on physical symptoms such as fatigue might artificially inflate scores resulting from side-effects of antiviral treatment or symptoms of concomitant physical illness, rather than of depression [23]. This should be taken into account when interpreting our results, although the same problem may occur when using clinical criteria for diagnosing depression in patients with physical limitations.

In our study, the cumulative incidence of depression was 60% (95% CI 46-74%). This is higher than the 22-41% reported in other studies [4,14-16]. No overlap is seen between the 95% confidence intervals of the proportion of patients with depression in our study and those of Fried et al (18.2-25.8%) and Hunt et al (14.5-43.4%), and only a small overlap is seen with the study of Castéra et al (29.1-48.4%) [4,15,16]. This might reflect a higher risk of development of depression during antiviral treatment in patients with inherited bleeding disorders and hepatitis C. However, differences in study design, depression assessment, treatment duration, treatment strategies and the relatively small sample sizes temper the strength of any conclusions.

Because of the psychological burden of long hospital admissions during childhood and arthropathy, haemophilia patients, especially older patients with a severe bleeding disorder, might have higher depression scores at baseline or develop depression more easily than patients without these problems. Our data do not confirm these hypotheses. In our study, five out of 47 patients with inherited bleeding disorders had depression at baseline (11%). This is lower than the 28-30% prevalence of depressive disorders reported in HCV patients in other studies [16,24]. In the remaining 42 patients, the median baseline depression score was low (1.0 point), indicating absence of depressive symptoms. Moreover, in our study, patients with a severe bleeding disorder were not significantly more likely to develop depression during antiviral treatment than other patients (crude OR 2.1, CI 0.6-7.3, p-value 0.27). This is in accordance with the results of Posthouwer et al, who reported that the severity of haemophilia did not have an effect on the mental component summary scores of the RAND-36 quality of life questionnaire

[25]. It is not clear why the cumulative incidence of depression in our patients with inherited bleeding disorders is higher than in other HCV patients.

The association between the effect of antiviral treatment and the occurrence of depression is controversial. Raison and colleagues reported that, in a population of 102 HCV patients, individuals who experience a significant increase in depressive symptoms during PegIFN-□/ribavirin therapy may be less likely to successfully clear HCV from their bloodstream (adjusted OR 3.6, 95% CI 1.3-9.5, p-value 0.01), possibly, in part, because of an effect of depression on treatment adherence [26]. On the other hand, Loftis et al found that in a group of 39 HCV patients, IFN-□ response rates were significantly higher in those patients who developed depression during antiviral treatment than in those who did not (SVR rates 38.5% versus 11.5%, p-value of the $\square^2$-test 0.049). They hypothesise that optimal dosing of IFN-□ could be associated with both the development of depression and higher response rates [27]. In accordance with Castéra et al and Schäfer et al, in our study no association at all was found between effect of antiviral therapy and depression [15,28].

In the majority (78%) of patients, depression developed during the first 12 weeks of antiviral treatment, which is in accordance with other reports [15,29,30]. A possible explanation as to why there seems to be a trend towards a higher depression score associated with a longer treatment duration, already evident at 24 weeks into treatment, is that these patients anticipate yet another 24 weeks of therapy, possibly influencing depressive symptoms.

Most patients who developed depression during antiviral treatment were adequately treated with antidepressant medication, indicating sufficient awareness of this problem. Since physicians knew that patients were participating in this study, this may not be representative for all doctors treating similar patients. Physicians were not aware of patients' BDI scores.

Selective serotonin reuptake inhibitor (SSRI) antidepressants are most often used for treating depression in these patients [11,31], although recent publications suggest that these drugs might not be effective at all in depressive patients or effective only in patients with severe depression [32,33]. The debate about the best treatment strategy in depressive patients is ongoing. In our experience, SSRIs had a positive effect in most patients. Some patients, however, declined any form of antidepressant therapy. Although SSRIs are reported to be associated with increased bleeding risks (based on anti-platelet activity) [34], we did not observe an increased bleeding risk in our patients.

Preventive treatment with SSRI antidepressants during antiviral therapy is suggested in the literature [35,36]. Since most patients do not develop moderate or severe depression, this approach would lead to overtreatment, with an increased risk of side-effects. Prophylactic SSRI treatment could be considered in patients with a past history of depression or other psychiatric problems, but only one third of such patients required antidepressant treatment in our study. In order to prevent development of more serious depressive symptoms, we suggest a reassessment of antidepressant dosage for patients who are receiving this treatment at start of antiviral therapy.

Depressive symptoms disappeared within 4 weeks after cessation of antiviral therapy in almost all patients who developed depression during treatment. This indicates that depression is a transient problem, associated with the use of antiviral medication, and that in most patients cessation of antidepressant treatment is possible within a few weeks after antiviral therapy concludes. Antiviral treatment was never discontinued because of depressive symptoms of any degree.

Conclusion

Our findings, although based on small numbers, indicate that depression is a frequent, but transient, complication of HCV treatment in patients with inherited bleeding disorders. We recommend close monitoring of patients with bleeding disorders during antiviral treatment for HCV, especially those with a history of depression or other psychiatric disorders. Using a standardised questionnaire, like the BDI-II, as a screening tool at regular clinic visits enables the treating physician to detect the presence of depressive symptoms and start appropriate treatment when indicated. Further research in comparable patient populations is needed to confirm our results and further explore possible predictive factors for the development of depression. Studies on preventative interventions based on patients' individual risk profiles might enable better treatment and reduce patient burden.

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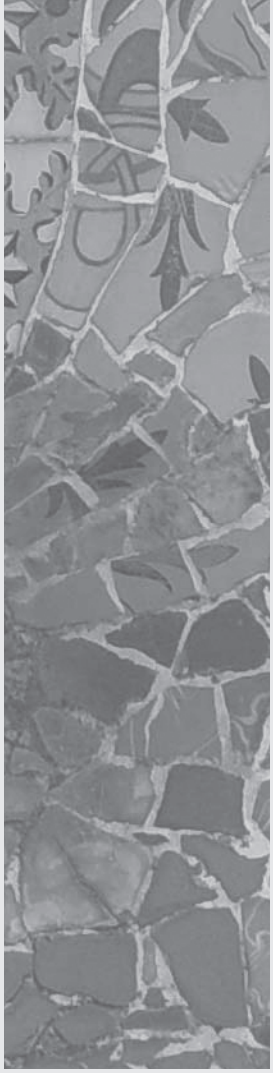
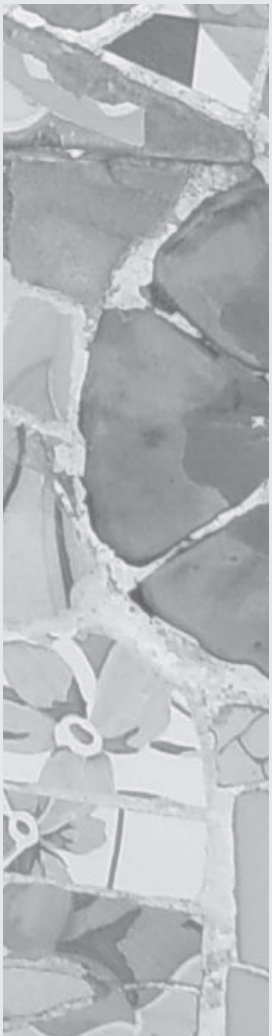


Chapter 10

Liver stiffness measurements to assess progression of fibrosis in HCV infected patients with inherited bleeding disorders



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Abstract

Introduction

Hepatitis C is a major co-morbidity in patients with inherited bleeding disorders, leading to progressive liver fibrosis and eventually cirrhosis. Liver stiffness measurement (LSM) is a non-invasive way of assessing the extent of liver fibrosis. This article describes our experience with serial LSM to prospectively assess progression of fibrosis in a cohort of patients with inherited bleeding disorders and chronic hepatitis C.

Methods

A total of 84 patients underwent serial LSM, with a median interval of 3.7 years. The change in LSM results over time was assessed.

Results

Overall, there was no significant difference between the median results of LSM 1 and LSM 2. The median result of LSM 2 was low (6.6 kPa), after a median duration of infection of 37 years. On the individual level, deterioration of LSM results of more than 2 kPa was seen in 13 patients (16%), 44 patients (52%) remained stable and 27 patients (32%) showed improvement of LSM results of more than 2 kPa. These results are comparable with those of paired liver biopsy studies.

Conclusions

LSM appears to be a good alternative for liver biopsies in patients with hepatitis C and inherited bleeding disorders, although the interpretation of the unexpected improvement we found in some of our patients is not straightforward. Liver stiffness measurements will be repeated in our patient population in a few years to be able to better assess the value of serial LSM.

Introduction

Hepatitis C virus (HCV) infection is an important co-morbidity in patients with inherited bleeding disorders. Some infected patients spontaneously clear the virus, but about 80% develop chronic hepatitis C, which will result in end-stage liver disease in 10-20%, after a period of over 20 years [1]. Before severe liver fibrosis and cirrhosis develop, patients can maintain adequate liver function, without major liver damage, for many years [2]. In the follow-up of patients with chronic HCV, it is important to regularly assess the extent of liver damage. In patients who show significant liver damage or progression of liver fibrosis, antiviral treatment is strongly recommended. In patients in whom the liver shows only little damage, remaining stable over time, however, antiviral treatment, with its many side-effects, could be postponed. Successful antiviral treatment has been shown to reduce liver damage, even in patients who have been infected with HCV for a long time and in patients who have significant fibrosis or even cirrhosis [3-5].

Liver biopsy is still the gold standard for the assessment of liver damage. Major disadvantages of this method include its invasive nature, a risk of sampling error and a large inter-observer variability [6,7]. Moreover, in patients with inherited bleeding disorders, liver biopsies carry a risk of severe bleeding complications. Prevention of bleeding requires expensive clotting factor substitution during and several days after the procedure. Therefore, a non-invasive, reliable alternative to assess liver damage and its progression is especially valuable in this group of patients.

Liver stiffness measurement (LSM) is a patient-friendly, non-invasive method of assessing liver fibrosis. Using transient elastography, LSM measures the velocity of propagation of a shock wave within the liver tissue, which is related to the extent of liver fibrosis or cirrhosis. LSM can be performed rapidly, has high patient acceptance and high intra- and inter-observer agreement [8,9]. LSM results are highly correlated with liver biopsy results [10,11]. In patients with inherited bleeding disorders and chronic HCV infection, single liver stiffness measurements are reported to be a valid instrument to assess liver fibrosis and select those patients who have an indication for antiviral treatment [10]. Little is known, however, about the use of serial LSM to evaluate progression of liver damage, either in 'regular' HCV patients or in HCV patients with inherited bleeding disorders.

This article describes our experience with serial liver stiffness measurements in patients with inherited bleeding disorders and chronic hepatitis C who did not undergo antiviral treatment, or in whom treatment was not successful.

Materials and methods

Patients

Liver stiffness measurements were performed in a cohort of HCV infected patients in 2005 (LSM 1) and in 2009 (LSM 2) at the Van Creveldkliniek, a large treatment centre for patients with inherited bleeding disorders in The Netherlands. This article describes all 84 patients with inherited bleeding disorders who were not, or not successfully, treated for HCV and of whom two successful liver stiffness measurements were available.
Our study population consisted of patients with haemophilia A, haemophilia B, Von Willebrand disease and factor II, VII and X deficiencies, which were classified according to their clotting factor activity levels as mild (6-40% activity), moderate (1-5%) or severe (< 1%).

Baseline characteristics, liver-related laboratory parameters, abdominal ultrasound examinations and LSM results were recorded for all patients. There was a maximum of 6 months' time between LSM and laboratory measurements and of 1 year between LSM and ultrasound examinations.

Duration of HCV infection was defined as the time from first exposure to inadequately or non-virus inactivated clotting factor products to the moment of LSM 2.

This study was approved by the Medical Ethics Review Board of the University Medical Center Utrecht. Written informed consent was obtained from all patients.

Liver stiffness measurements

Liver stiffness measurements were obtained by performing transient elastography with a Fibroscan® device (EchoSens, Paris, France), as described previously [9]. In short, the patient lies in dorsal decubitus position with his right arm in maximal abduction, and a transducer probe is placed on the right lobe of the liver in an intercostal space. An elastic shear wave is sent through the liver tissue and its velocity is measured by pulse-echo ultrasound acquisitions. With increasing liver fibrosis, the liver tissue is stiffer and the shear wave moves faster. The median result of at least 10 successful measurements, with a success rate (the number of successful measurements divided by the total number of acquisitions) of at least 60%, is considered representative of liver stiffness [11,12].

LSM results are expressed in kilopascals (kPa). Higher liver stiffness values indicate more liver fibrosis [9]. Different stages of liver fibrosis are assigned for increasing liver stiffness values, according to the cut-off points described by Castéra et al: F0-F1 (no-minimal fibrosis) for LSM result < 7.1 kPa, F2 (moderate fibrosis) for 7.1-9.4 kPa, F3

(severe fibrosis) for 9.5-12.4 kPa and F4 (cirrhosis) for LSM result ≥ 12.5 kPa. Liver fibrosis stage F2 or higher represents an indication for antiviral treatment [12]. Measurements at LSM 2 were performed by a different operator and with a later version of the Fibroscan® device than at LSM 1.

Statistical analyses

For continuous variables medians, ranges and interquartile ranges (IQR) were calculated. Categorical variables were reported as proportions. The change in LSM results between LSM 1 and LSM 2 was calculated and expressed in kPa (positive values indicating progression of liver fibrosis, while negative values indicate reduction of fibrosis). Since there are no published reports on how large a change in fibrosis between two liver stiffness measurements has to be to be considered clinically relevant, cut-off points of 2 kPa and 3 kPa were used in the analyses, based on the differences in kPa between the different fibrosis stages in the Castéra classification [12]. To avoid too much enumeration of details, only the more conservative cut-off of 2 kPa was used in many of the descriptive results. For all patients, changes in stage of fibrosis between LSM 1 and LSM 2 were recorded. Wilcoxon's Signed Rank Test for paired samples was used to assess changes in liver fibrosis and in laboratory parameters between LSM 1 and LSM 2. P-values <0.05 were considered statistically significant. Data were analysed using SPSS 15.0 statistical software (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics of the 84 patients included in the study are reported in Table 1. The large majority of patients (95%) were male. Most patients (93%) had haemophilia. There were three patients with Von Willebrand disease (one type 1, two type 2B), one with factor II deficiency, one with factor VII deficiency and one with factor X deficiency. In sixty-one patients (73%), the bleeding disorder was severe. HCV genotype 1 was present in the majority of patients (89%), partly because this is the most common genotype in HCV infected Dutch haemophilia patients, but also because this genotype is difficult to treat and therefore overrepresented in patients who were treated unsuccessfully or in whom treatment was postponed. Fifty five patients never underwent antiviral treatment. Of the remaining 29 patients, 62% received state-of-the-art treatment with pegylated interferon plus ribavirin, seven of whom were treated between LSM 1 and LSM 2. Six patients were co-infected with HIV and two other patients with hepatitis B virus. All HIV-positive patients were on highly active antiretroviral treatment (HAART) and had undetectable HIV viral loads. Median duration of HCV infection at time of LSM 2 was 37.3 years (range 17.5-43.0 years) and median interval between LSM 1 and LSM 2 was 3.7 years (range 2.0-3.8 years).

Table 1. Patient characteristics at the time of LSM 2.

Total number of patients	84	
Male	80	(95%)
Median age in years (range)	45.5	(19-74)
Haemophilia A	69	(82%)
Haemophilia B	9	(11%)
Von Willebrand disease	3	(4%)
Other bleeding disorders	3	(4%)
Severe bleeding disorder	61	(73%)
HCV genotype		
1	75	(89%)
2	3	(4%)
3	2	(2%)
4	3	(4%)
unknown	1	(1%)
Median duration of HCV infection in years (IQR)	37.3	(31.0;42.4)
HCV treatment status		
Never treated	55	(66%)
Treated once	20	(24%)
Treated twice	6	(7%)
Treated three times	3	(4%)
Optimal HCV treatment (n = 29)		
IFN alone	5	(17%)
IFN + ribavirin	6	(21%)
Pegylated IFN + ribavirin	18	(62%)
HIV co-infection	6	(7%)
Hepatitis B co-infection	2	(2%)
Median interval between LSM 1 and LSM 2 in years (IQR)	3.7	(3.6;3.8)

HCV = hepatitis C virus
IQR = interquartile range
IFN = interferon
HIV = human immunodeficiency virus
LSM = liver stiffness measurement

Table 2 summarises the results of LSM 1 and LSM 2. The median results of LSM 1 and LSM 2 were 7.3 and 6.6 kPa, respectively. Wilcoxon's test showed a p-value of 0.16, indicating no significant difference overall. On the individual patient level, however, some patients showed deterioration of LSM results, and quite a large group showed improvement. A total of 13 patients (16%) showed an increase in liver stiffness (indicating progression of fibrosis) of 2 kPa or more, and 12 patients (14%) had an increase of 3 kPa or more. In 27 patients (32%), liver stiffness improved by more than 2 kPa, and in 19 patients (23%) by more than 3 kPa.

Table 2. Results of first and second LSM.

	LSM 1		LSM 2	
Result of LSM in kPa	7.3	(5.9;10.0)	6.6	(5.2;9.0)
Stage of liver fibrosis				
F0-F1 (no - mild fibrosis)	38	(45%)	51	(61%)
F2 (moderate fibrosis)	23	(27%)	14	(17%)
F3 (severe fibrosis)	10	(12%)	4	(5%)
F4 (cirrhosis)	13	(16%)	15	(18%)
Change in liver stiffness between LSM 1 and LSM 2 in kPa	-		-0.2	(-2.6;+0.9)
Increase in liver stiffness ≥ 2 kPa	-		13	(16%)
Increase in liver stiffness ≥ 3 kPa	-		12	(14%)
Increase in stage of liver fibrosis				
One stage	-		6	(7%)
Two stages	-		3	(4%)
Three stages	-		2	(2%)
Decrease in liver stiffness ≥ 2 kPa	-		27	(32%)
Decrease in liver stiffness ≥ 3 kPa	-		19	(23%)
Decrease in stage of liver fibrosis				
One stage	-		19	(23%)
Two stages	-		7	(8%)

Values are medians (interquartile range) or numbers (proportion).
LSM = liver stiffness measurement
Stages of liver fibrosis according to Castéra: F0-F1 (no-minimal fibrosis) LSM result < 7.1 kPa, F2 (moderate fibrosis) 7.1-9.4 kPa, F3 (severe fibrosis) 9.5-12.4 kPa, F4 (cirrhosis) ≥ 12.5 kPa [12].

LSM changes according to treatment history

Table 3 shows the changes in LSM results according to HCV treatment history. When using a cut-off of 2 kPa, 33 of the 55 patients who never underwent antiviral treatment remained stable (60%), whereas six (11%) showed progression and 16 (29%) showed reduction of fibrosis. In the 22 patients who underwent unsuccessful antiviral treatment before LSM 1, the proportion of stable patients was slightly lower (46%), and more patients showed progression of fibrosis (27%), while a similar proportion (27%) showed reduction of fibrosis. For a higher cut-off of 3 kPa, 73% of untreated patients remained stable, 9% showed progression and 18% showed reduction of fibrosis. These percentages were 55%, 27% and 18%, respectively, in patients who were unsuccessfully treated before LSM 1.

Seven patients (8%) underwent unsuccessful antiviral treatment between LSM 1 and LSM 2. The median change in LSM results was much larger in these patients than in the other groups. Five patients showed improvement in LSM results (median change in LSM results -4.2 kPa, range -3.0 to -10.7), one remained stable (13.8 versus 14.1

kPa, stable stage F4) and one showed progression of fibrosis (19.8 versus 26.6 kPa, stable stage F4). This last patient was co-infected with hepatitis B virus. The differences in changes in LSM results between the three treatment groups were not statistically significant.

Table 3. Changes in LSM results according to HCV treatment history.

	Never treated (n = 55)		**Unsuccessful HCV treatment before LSM 1** (n = 22)		**Unsuccessful HCV treatment between LSM 1 and LSM 2** (n = 7)	
Difference in LSM results between LSM 1 and LSM 2 (kPa)	-0.1	(-2.4;+0.9)	-0.2	(-2.1;+3.6)	-3.5	(-5.0;+0.3)
Cut-off of 2 kPa for clinically relevant change						
Progression of fibrosis	6	(11%)	6	(27%)	1	(14%)
Fibrosis stable	33	(60%)	10	(46%)	1	(14%)
Reduction of fibrosis	16	(29%)	6	(27%)	5	(71%)
Cut-off of 3 kPa for clinically relevant change						
Progression of fibrosis	5	(9%)	6	(27%)	1	(14%)
Fibrosis stable	40	(73%)	12	(55%)	1	(14%)
Reduction of fibrosis	10	(18%)	4	(18%)	5	(71%)

Values are medians (interquartile range) or numbers (proportion).
LSM = liver stiffness measurement
HCV = hepatitis C virus

Patient characteristics according to LSM results

Of the 27 patients who showed improvement of LSM results of 2 kPa or more, four had fibrosis stage F0-F1 both at LSM 1 and LSM 2, and three had stage F4 at both measurements. In these patients, the change in LSM result did not have any clinical consequences, because they remained on the same end of the fibrosis-spectrum. Five patients were treated with pegylated interferon and ribavirin between LSM 1 and LSM 2. Of the 44 patients who remained stable (difference between LSM 1 and LSM 2 results between -2.0 and +2.0 kPa), one patient, who remained stable F4, underwent antiviral treatment between LSM 1 and LSM 2. There were 13 patients who showed deterioration of their LSM results of 2 kPa or more. Six of these patients had fibrosis stage F4 both at LSM 1 and at LSM 2, while the other seven patients (8% of the total group) appeared to have a clinically relevant deterioration of fibrosis.

The association between alcohol use and LSM results was not very consistent. In the group with improvement of LSM results of more than 2 kPa, 7% (two patients) used more than 10 alcohol units per week at LSM 2 (both stable stage F0-F1), while in the rest of our study group this proportion was 23%. In three patients excessive alcohol use was associated with large increases in LSM results, and in two patients a reduction in alcohol use was associated with improvement in LSM results. In other patients with large changes in alcohol use, however, no association with LSM results was apparent.

Laboratory parameters proved of limited value: except for a significant increase in bilirubin values (median 9.0 μmol/l at LSM 1 and 14.5 μmol/l at LSM 2, p-value <0.01) and a significant decrease in albumin levels (medians 42.2 and 40.4 g/l, respectively, p-value <0.01), no overall differences in laboratory parameters (alanine aminotransferase (ALT), gamma-GT and platelet count) were observed between LSM 1 and LSM 2 in our patient group.

Ultrasound examinations appeared less sensitive than LSM. Overall, about half of patients had normal ultrasound results (45% at LSM 1 and 55% at LSM 2). In a large proportion of patients, however, no ultrasound examinations were available (38% at LSM 1 and 19% at LSM 2). Steatosis was present in 11% of patients at LSM 1 and 16% at LSM 2. There were no patients who had liver cirrhosis on ultrasound at time of LSM 1, whereas five patients had cirrhosis on ultrasound at time of LSM 2 (6%). These five patients all had stable fibrosis stage F4 on LSM.

Discussion

In this study of 84 patients with inherited bleeding disorders, who were not, or not successfully treated for their HCV infection, serial evaluation with LSM, with a median interval of 3.7 years, detected seven patients (8%) who had a clinically relevant deterioration of their LSM results. Moreover, in quite a large group of patients, unexpected improvement in LSM results was observed. In some patients this improvement may have clinical implications, because antiviral treatment would be recommended based on the presence of fibrosis stage F2 or higher at LSM 1, but would no longer be (strongly) indicated based on stage F0-F1 at LSM 2.

The median result of LSM 2 was 6.6 kPa, which is quite low considering the median infection duration of 37 years in our patient group. This confirms the very slow progression of liver damage in many HCV infected patients described before [1,2].

When considering differences in stage of fibrosis, one should take into account that in some cases a small change in LSM results can lead to a change in fibrosis stage. In our analyses we therefore used the difference in LSM results in kPa, rather than the difference in fibrosis stage. Since it is not known how large a change in LSM results has to be to be clinically relevant, we used two different cut-off values for our analyses.

The apparent inconsistencies we found in our study are not limited to LSM, but have been observed in studies reporting on serial biopsies in patients with chronic HCV as well. In these studies, with a median interval of 2-3 years, improvement of fibrosis was reported in 10-24% of untreated patients, while progression was present in 15-45%,

depending on the severity of disease at baseline [13-16]. The results of our study are similar: improvement of LSM results of 2 kPa or more was seen in 22 of 77 patients who were not treated between LSM 1 and LSM 2 (29%, 95% confidence interval (CI) 18-39%) and progression of fibrosis was present in 12 untreated patients (16%, 95% CI 7-24%). For the cut-off value of 3 kPa, improvement was seen in 18% (CI 10-27%), while progression was present in 14% (CI 6-22%).

LSM results can be influenced by several technique-specific factors, which could (partly) explain the variation in LSM results in our study. Reproducibility of transient elastography has been reported to be influenced by the presence of hepatic steatosis. LSM should therefore be used cautiously as a surrogate of liver biopsy in patients with steatosis [8]. Results in this area are, however, conflicting [17,18]. The same is true for histological activity [11,12,17-19]. Moreover, changes in LSM results were associated with a shift in alcohol consumption in some patients. Little is known about the effect of HIV co-infection and HAART on LSM. HAART has been reported to influence hepatic fibrosis [20,21]. Hepatitis B co-infection can also be associated with the extent of fibrosis. In our study, LSM 1 and LSM 2 were performed by different operators and on different versions of the Fibroscan® device. However, such changes are part of normal clinical practice and the aim of this study was to describe our experience with LSM in clinical practice. Moreover, since the inter-observer variability of LSM is small [8,22] and the inter-equipment reproducibility is excellent [22], this can, in our opinion, only explain a minor part of the observed variation in results between LSM 1 and LSM 2. It is possible that, in individual patients, LSM 1 and LSM 2 were performed at different measurement sites on the liver, which has been reported to influence inter-observer agreement [22]. This problem can, however, never be avoided when performing serial measurements.

Other possible explanations for the variation between first and second measurements, both in biopsies and LSM, could be that there is an uneven distribution of fibrosis in the liver, or that, depending on variations in for example viral load, use of medication or other factors, fibrosis formation is a dynamic process, with a balance between fibrosis progression and regeneration of liver tissue, which can shift over time. Regression of fibrosis is reported to occur when the disease causing factor is eliminated (for example after successful antiviral treatment or cessation of excessive alcohol use) [23], but may also occur when stressors are reduced or beneficial factors are introduced. Finally, regression of fibrosis could indeed (partly) reflect measurement variation, which would be a limitation of the performed investigation. LSM has been reported to have somewhat lower accuracy in the low fibrosis range [24]. However, measurement variation appears to be similar in biopsies and LSM, indicating that LSM can be used as a non-invasive

alternative for liver biopsies. Measurement variation would, in any case, influence results in both directions, and not just result in an overestimation of reduction of fibrosis.

Interestingly, an improvement in LSM results of more than 3 kPa was observed in five of seven patients who underwent unsuccessful antiviral treatment between LSM 1 and LSM 2. This indicates that antiviral therapy has a beneficial effect on the liver, regardless of whether this treatment was successful or not, as previously reported [3,5,25].

Conventional ultrasound examination only showed liver cirrhosis in five patients who were stable stage F4, indicating that this type of investigation can only be used to detect end-stage liver disease.

So far, serial LSM did not completely live up to the expectation of being the ideal tool for the follow-up of our patient population, mainly because it is difficult to explain the improvement in LSM results that was seen in quite a few patients. Moreover, it is as yet not clear how large a change in LSM results has to be to be considered clinically relevant, and how long the ideal interval between liver stiffness measurements should be. Three to four years might be too short an interval because of the slow fibrosis progression in many patients. Limitations of LSM, however, seem to be similar to those of paired liver biopsies. In addition, in patients with inherited bleeding disorders, the cost of LSM, which can be performed in the outpatient clinic without the use of any (extra) clotting factor concentrates, is significantly lower than the cost of performing liver biopsies.

Overall, non-invasive LSM for the assessment of fibrosis, with greatly reduced complication risks and costs, remains a very attractive alternative for serial liver biopsies in patients with HCV and inherited bleeding disorders. Based on the results of our study, we recommend repeating liver stiffness measurements after a few years in all patients with a change in LSM results of 2 kPa or more (either improvement or deterioration) to ensure adequate clinical follow-up, unless patients remain clearly on one end of the fibrosis spectrum (F0-F1 or F4), in which case there are no clinical consequences. Third liver stiffness measurements in the complete patient group might help us to better evaluate the longitudinal use of LSM and determine whether the observed improvements in LSM results in some of our patients reflect actual improvements, or rather measurement variations over time.

Conclusion

This article describes our experience with serial liver stiffness measurements to evaluate progression of liver fibrosis in patients with inherited bleeding disorders and chronic hepatitis C. Overall, our results indicate very slow fibrosis progression in this patient

group. On the individual level, no clear trend was observed in the direction of changes in LSM results. Unexpectedly, we found improvement of LSM results in quite a large group of patients, making interpretation of our results and their clinical implications less straightforward in some cases. Our results are, however, comparable with those seen in paired liver biopsy studies, indicating that LSM, which is safer and much less expensive, can be used as a reliable alternative to biopsies. We will continue performing liver stiffness measurements in this patient group to be able to better asses the value of longitudinal LSM.

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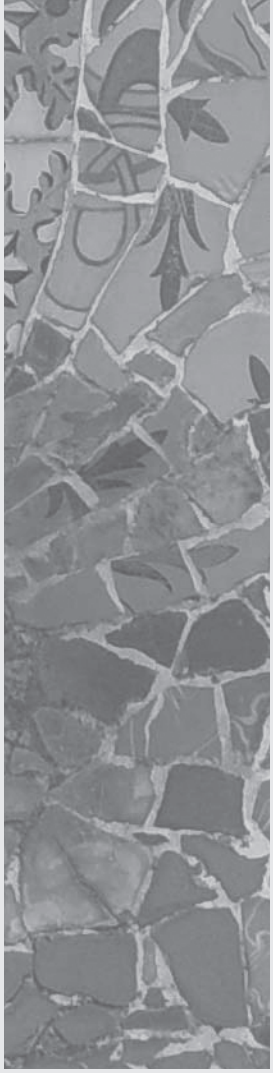
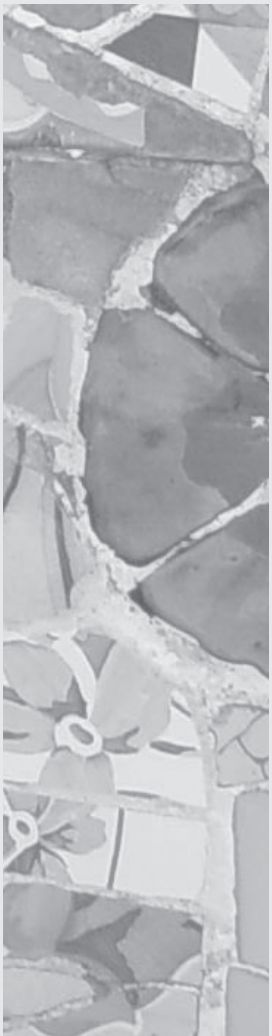
Chapter 11

Beneficial effect of successful HCV treatment in patients with inherited bleeding disorders, assessed by liver stiffness measurements





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Abstract

Introduction

Hepatitis C infection is a major co-morbidity in patients with inherited bleeding disorders. Successful antiviral treatment leads to a reduction in liver fibrosis, as shown by liver biopsies. Liver stiffness measurement (LSM) is a non-invasive method of assessing liver fibrosis.

The aim of this cohort study was to evaluate the long-term effect of successful antiviral treatment, using LSM, in HCV infected patients with inherited bleeding disorders.

Methods

Liver stiffness measurements were performed in 2005 (LSM 1) and 2009 (LSM 2) in 39 patients who were successfully treated for HCV. The change in liver fibrosis between LSM 1 and 2 was assessed.

Results

The median duration of HCV infection was 28.8 years. A total of 22 patients (56%) underwent successful antiviral treatment before LSM 1 (group 1) and 17 patients between LSM 1 and LSM 2 (group 2). The median time since antiviral treatment was 8.8 years in group 1 and 2.5 years in group 2. In group 1, the median results of LSM 1 and 2 were similar (6.0 versus 5.6 kPa, p-value 0.36), so overall, patients remained stable. In three patients in this group, all treated more than 15 years ago, an increase of liver stiffness was shown. Group 2 showed a significant improvement in median LSM results (10.3 versus 6.1 kPa, p-value < 0.01), with decrease of liver stiffness in 82%.

Conclusion

Even after a long HCV infection duration, successful antiviral treatment led to a significant improvement of fibrosis, measured by LSM, mainly in the first years after completing treatment.

Introduction

Hepatitis C virus (HCV) infection contributes significantly to co-morbidity in patients with inherited bleeding disorders. About 80% of infected patients develop chronic hepatitis C, resulting in end-stage liver disease in 10-20% of these patients, after a period of over 20 years [1]. Antiviral treatment with interferon became available in 1987. In 1995, ribavirin was added, increasing the success rate of treatment. Since 2000, standard treatment consists of pegylated interferon plus ribavirin. The objective of antiviral treatment is eradication of HCV, to stop progression of liver damage. Antiviral treatment has many side-effects, and, unfortunately, is not effective in all patients [2,3]. Successful antiviral treatment has, however, been shown to reduce the extent of liver fibrosis in patients with chronic HCV infection [4,5]. This improvement has been demonstrated using liver biopsies, still the gold standard for the assessment of liver damage. Disadvantages of liver biopsy include its invasive nature, a risk of sampling error and a large inter-observer variability [6-8].

In patients with inherited bleeding disorders, liver biopsies are associated with a risk of severe bleeding complications and a high cost of clotting factor substitution to prevent bleeding due to the procedure. Therefore, a non-invasive, reliable way of assessing liver damage and the effect of antiviral treatment is especially valuable in these patients.

Unfortunately, laboratory tests cannot be used to monitor liver fibrosis. Many haemophilia patients with chronic HCV infection have liver function abnormalities at laboratory analyses, but none of the available laboratory parameters can be used as a reliable marker for liver fibrosis.

Liver stiffness measurement (LSM) is an increasingly popular way of assessing liver fibrosis in patients with chronic HCV infection. Using transient elastography (TE), LSM evaluates the velocity of propagation of a shock wave within the liver tissue, which is related to the extent of liver fibrosis or cirrhosis. TE is a non-invasive and user-friendly technique, which can be performed rapidly, with immediate results and high patient acceptance [9]. It has high intra- and inter-observer agreement [10]. LSM results are highly correlated with liver biopsy results [11,12]. In patients with inherited bleeding disorders and chronic HCV infection, LSM has been shown to be a valid instrument to assess liver fibrosis and select those patients who have an indication for antiviral treatment [11]. LSM has also been used to determine the effect of antiviral treatment in patients with chronic HCV in several studies, with a maximum follow-up of two years after completing antiviral treatment [13-16].

The aim of the present descriptive study was to evaluate the long-term effect of successful antiviral treatment, using liver stiffness measurements, in HCV infected patients with inherited bleeding disorders.

Materials and methods

Patients

Liver stiffness measurements were performed in a group of HCV infected patients at the Van Creveldkliniek, a large treatment centre for patients with inherited bleeding disorders in The Netherlands, in 2005 (LSM 1) and in 2009 (LSM 2). This article describes all 39 patients with inherited bleeding disorders who were successfully treated for HCV and underwent two successful liver stiffness measurements.

Inherited bleeding disorders in our study population included haemophilia A, haemophilia B, Von Willebrand disease and factor VII deficiency, which were classified according to their clotting factor activity levels as mild (6-40% activity), moderate (1-5%) or severe (< 1%).
Baseline characteristics, HCV treatment characteristics and LSM results were recorded in all patients.

Successful antiviral treatment (sustained virological response, SVR) was defined as absence of HCV-RNA in serum six months after completing therapy.
Duration of HCV infection was defined as the time from first exposure to inadequately or non-virus inactivated clotting factor products to the end of successful antiviral treatment. The moment of first exposure to unsafe clotting factor products has been shown to be a reliable estimate of time of onset of HCV infection in patients with inherited bleeding disorders [17].

The study was approved by the Medical Ethics Review Board of the University Medical Center Utrecht. Written informed consent was obtained from all patients.

Liver stiffness measurements

Non-fasting liver stiffness measurements were obtained by performing transient elastography with a Fibroscan® device (EchoSens, Paris, France), as described previously [9,18]. LSM results are expressed in kilopascals (kPa). Higher liver stiffness values indicate more liver damage [9]. Different stages of liver fibrosis are assigned for increasing liver stiffness values, according to the cut-off points described by Castéra et al [19]: no-minimal fibrosis (F0-F1) when LSM result is < 7.1 kPa, moderate fibrosis (F2) for 7.1-9.4 kPa, severe fibrosis (F3) for 9.5-12.4 kPa and cirrhosis (F4) when LSM result is ≥ 12.5 kPa. Liver fibrosis stage F2 or higher represents an indication for antiviral treatment. The success rate is

calculated as the number of successful measurements divided by the total number of acquisitions. The median value of 10 measurements, with a success rate of at least 60%, is considered representative of liver stiffness [12,19]. Results are considered valid when the interquartile range (IQR) of the performed measurements divided by the median value (IQR/median ratio) is below 30% [10,20]. This was the case for all included LSM 2 results. Unfortunately, because this criterion was not yet widely investigated or recommended by the manufacturer in 2005, IQR data were not recorded at LSM 1. Measurements at LSM 2 were performed by a different operator and with a later version of the Fibroscan® device than at LSM 1, as is often the case in clinical practice.

Statistical analyses

For continuous variables medians, ranges and interquartile ranges were calculated. Categorical variables were reported as proportions. The change in liver stiffness between LSM 1 and LSM 2 was calculated and expressed in kPa. Positive values indicate progression, while negative values indicate reduction of liver fibrosis. There are no published criteria on how large a change in fibrosis between two LSM measurements has to be to be considered a clinically relevant change. In this study, the cut-off for clinical relevance was set at a change in LSM results of 2 kPa or more. Because in LSM differentiation between the intermediate stages of fibrosis is reported to be less accurate, fibrosis stages 2 and 3 were pooled in our analyses, thus creating three fibrosis groups: F0-F1, F2-F3 and F4. For all patients, increase or decrease in group of fibrosis was recorded.

Patients were divided into two groups: patients who underwent successful antiviral treatment before LSM 1 (group 1) and patients who were successfully treated between LSM 1 and LSM 2 (group 2). Because patients in group 1 had significantly lower LSM 1 results than patients in group 2, analyses were performed separately. Differences between these groups were assessed with Student's t-test and Chi-square test, when appropriate. Wilcoxon's Signed Rank Test was performed to assess whether or not there was a significant change in liver fibrosis between LSM 1 and LSM 2. P-values < 0.05 were considered statistically significant. Data were analysed using SPSS 15.0 statistical software (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics of the 39 patients who were included in this study are shown in Table 1. All but two patients were male. The median age at time of LSM 2 was 42 years (range 20-62 years). Thirty-seven patients (95%) had haemophilia, one patient had Von Willebrand disease and one patient had factor VII deficiency. HCV genotype 1 was most prevalent (15 patients, 39%). The proportion of patients with genotypes 2 or 3 was relatively large, because of the high success rate of antiviral treatment for these genotypes.

The median duration of HCV infection was 28.8 years (range 14-41 years). Four patients were co-infected with HIV. Three of these patients were on highly active antiretroviral therapy (HAART) and had undetectable HIV-RNA levels (below 50 copies). The fourth patient had a low HIV-RNA level (83 copies), without HAART.

Table 1. Baseline characteristics of our study population at time of LSM 2 overall and according to time of successful antiviral treatment.

	Total group		**Group 1**		**Group 2**	
Total number of patients	39		22		17	
Male gender	37	(95%)	21	(96%)	16	(94%)
Age in years (range)	42	(20-62)	44	(23-62)	39	(20-58)
Haemophilia A	29	(74%)	14	(64%)	15	(88%)
Haemophilia B	8	(21%)	7	(32%)	1	(6%)
Von Willebrand disease	1	(3%)	1	(5%)	-	
Factor VII deficiency	1	(3%)	-		1	(6%)
Severe bleeding disorder	30	(77%)	19	(86%)	11	(65%)
HCV genotype						
1	15	(39%)	3	(14%)	12	(71%)
2	9	(23%)	9	(41%)	-	
3	6	(15%)	2	(9%)	4	(24%)
4	1	(3%)	-		1	(6%)
5	1	(3%)	1	(5%)	-	
unknown	7	(18%)	7	(32%)	-	
Duration of HCV infection in years	28.8	(23.4-35.8)	27.8	(22.7-32.8)	33.2	(24.1-40.1)
HIV co-infection	4	(10%)	4	(18%)	-	
Hepatitis B co-infection	2	(5%)	1	(5%)	1	(6%)
Type of successful antiviral treatment						
IFN alone	8	(21%)	8	(36%)	-	
IFN + ribavirin	7	(18%)	7	(32%)	-	
Pegylated IFN + ribavirin	24	(62%)	7	(32%)	17	(100%)
Time since end of successful antiviral treatment in years	5.3	(2.5-10.6)	8.8	(5.8-14.1)	2.5	(1.8-2.8)
Interval between LSM 1 and LSM 2 in years	3.6	(3.5-3.7)	3.6	(3.6-3.7)	3.7	(3.5-3.7)
Body mass index in kg/m^2	25.0	(22.8-27.1)	24.9	(23.1-27.3)	25.0	(22.4-26.6)

Values are medians (interquartile range) or numbers (proportion), unless otherwise specified.
Group 1: successful antiviral treatment before LSM 1.
Group 2: successful antiviral treatment between LSM 1 and LSM 2.
HCV = hepatitis C virus
HIV = human immunodeficiency virus
IFN = interferon
LSM = liver stiffness measurement

A total of 22 patients (56%) underwent successful antiviral treatment before LSM 1 (group 1), while the remaining 17 patients were successfully treated between LSM 1 and LSM 2 (group 2). Antiviral treatment consisted of interferon alone in 21%, interferon plus ribavirin in 18% and pegylated interferon plus ribavirin in 62% of patients, includ-

ing all patients in group 2. The median time since the end of successful treatment was 5.3 years (range 0.3-16.8 years). The median interval between LSM 1 and LSM 2 was 3.6 years (range 1.8-4.0 years). Patients in group 2 were slightly younger than patients in group 1, and had more often haemophilia A and non-severe haemophilia, but these differences did not reach statistical significance. HCV genotype 1, however, was significantly more prevalent in group 2 than in group 1 (p-value 0.006). No patients in group 2 were infected with HIV.

The median results of LSM 1 and LSM 2 in kPa and changes in liver stiffness between LSM 1 and 2 overall, and for groups 1 and 2 separately, are shown in Figure 1 and Table 2. Figure 2 shows the difference between LSM 1 and LSM 2 (in kPa) for all patients individually, according to the time since completing successful antiviral treatment.

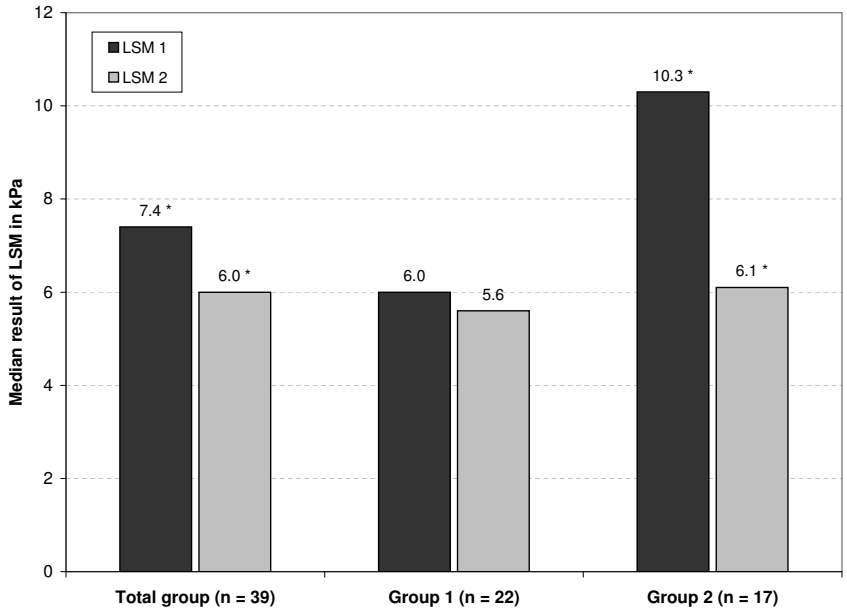

Figure 1. Median results of LSM 1 and LSM 2 in kPa overall and according to time of successful antiviral treatment (either before LSM 1 in group 1 or between LSM 1 and LSM 2 in group 2).
* statistically significant difference between LSM 1 and LSM 2.
LSM = liver stiffness measurement

The median time since the end of antiviral treatment in group 1 was 8.8 years (range 3.8-16.8 years). The median duration of HCV infection was 27.8 years (range 14-37 years). There were 10 patients (45%) who underwent successful antiviral treatment more than 10 years before LSM 2. The median time since the end of antiviral treatment

in group 2 was 2.5 years (range 0.3-3.0 years) and the median duration of HCV infection was 33.2 years (range 16-41 years).

Table 2. Changes between LSM 1 and LSM 2 overall, and according to time of successful antiviral treatment.

	Total group (n = 39)		**Group 1** (n = 22)		**Group 2** (n = 17)	
Change in liver stiffness between LSM 1 and LSM 2 in kPa	-2.2	(-4.1;+0.1)	-0.2	(-2.3;+0.4)	-4.8	(-6.4;-3.5)
Increase in liver stiffness ≥ 2 kPa	3	(8%)	3	(14%)	-	
Decrease in liver stiffness ≥ 2 kPa	20	(51%)	6	(27%)	14	(82%)
Liver fibrosis group at LSM 1						
No-mild fibrosis (F0-F1)	18	(46%)	16	(73%)	2	(12%)
Moderate-severe fibrosis (F2-F3)	17	(44%)	6	(27%)	11	(65%)
Cirrhosis (F4)	4	(10%)	-		4	(24%)
Liver fibrosis group at LSM 2						
No-mild fibrosis (F0-F1)	30	(77%)	18	(82%)	12	(71%)
Moderate-severe fibrosis (F2-F3)	9	(23%)	4	(18%)	5	(29%)
Cirrhosis (F4)	-		-		-	
Change in liver fibrosis group						
One group increase	3	(8%)	3	(14%)	-	
No change	18	(46%)	14	(64%)	4	(24%)
One group decrease	17	(44%)	5	(23%)	12	(71%)
Two groups decrease	1	(3%)	-		1	(6%)

Values are medians (interquartile range) or numbers (proportion). A negative change (decrease) in liver stiffness indicates regression of fibrosis.
Group 1: successful antiviral treatment before LSM 1.
Group 2: successful antiviral treatment between LSM 1 and LSM 2.
LSM = liver stiffness measurement

Overall, there was a significant improvement in LSM results (median 7.4 kPa (IQR 5.8-10.0) at LSM 1 versus 6.0 kPa (IQR 4.4-6.9) at LSM 2, p-value < 0.01). Subgroup analysis showed a significant improvement in group 2 (10.3 versus 6.1 kPa, p-value < 0.01), but not in group 1 (6.0 versus 5.6 kPa, p-value 0.36). This suggests that the improvement in LSM results occurs mainly in the first few years after successful antiviral treatment.

In patients who were treated a long time ago (group 1), no consistent trend in the change of fibrosis between LSM 1 and LSM 2 was seen. Of the 22 patients in this group, six (27%) had an improvement in LSM results of more than 2 kPa, while three patients (14%) had a deterioration of more than 2 kPa. Interestingly, these three were all patients who underwent successful HCV treatment more than 15 years before LSM 2. They were all treated with interferon only. The HCV genotype was unknown in these patients. In one 39-year old patient, without any co-infections, LSM results increased from 3.0 (stage F0-F1) to 9.5 kPa (borderline stage F3).

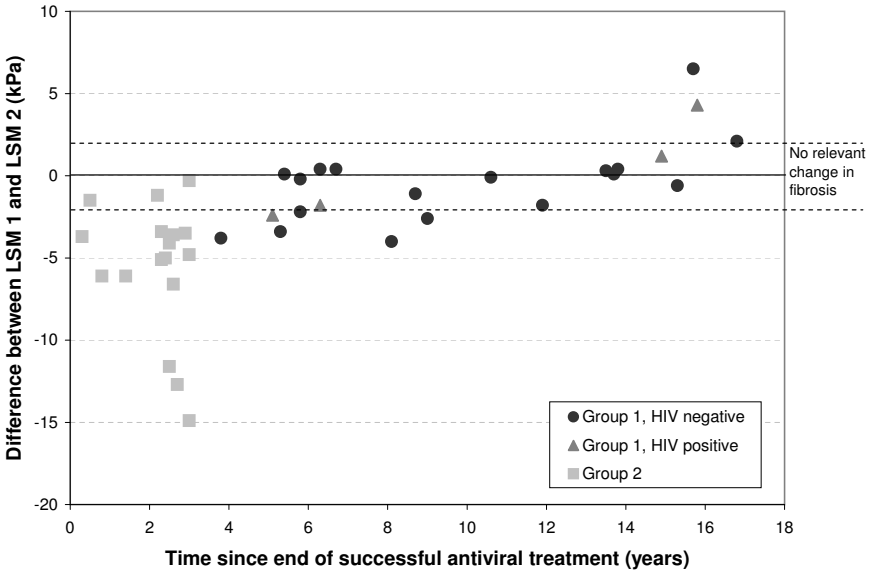

Figure 2. Change in LSM result in kPa according to time since completing successful antiviral treatment.
A negative change indicates improvement, while a positive change indicates progression of fibrosis. The dark dotted lines indicate a clinically relevant change in LSM results of 2 kPa or more. There was less improvement, and in some patients even deterioration of liver fibrosis, with increasing time since completing successful antiviral treatment. Significant deterioration was only seen in three patients, who were treated more than 15 years ago.
LSM = liver stiffness measurement

His gamma-GT and ALT levels and platelet count were normal. His most recent ultrasound examination showed mild steatosis, but no other abnormalities. In a 61-year old patient, who was on HAART for his HIV infection, LSM results increased from 5.9 (stage F0-F1) to 10.2 kPa (F3). At time of LSM 2, his gamma-GT and ALT levels and his platelet count were normal, but his bilirubin and alkaline phosphatase levels were elevated. A 36-year old HBV co-infected patient had LSM 1 result 6.7 kPa and LSM 2 result 8.8 kPa. His gamma-GT and platelet count were normal, while his ALT was slightly elevated. Ultrasound examination showed mild steatosis and a slightly enlarged spleen.

Of the 17 patients who were recently treated (group 2), 14 (82%) had improvement of their LSM results of more than 2 kPa. Only one patient had hardly any change in his LSM results (9.4 kPa versus 9.1 kPa). He was successfully treated in 2005, but had an average weekly alcohol intake of 30 units, which might explain his lack of improvement in LSM. The most recent ultrasound investigation of his upper abdomen showed cholelithiasis, but no other abnormalities. His bilirubin, gamma-GT levels and platelet count were normal, but his ALT and AST levels were slightly elevated.

Discussion

In this article, we describe our experience with LSM after successful antiviral treatment in patients with inherited bleeding disorders. To our knowledge, this is the first study using the non-invasive technique of LSM to assess the long-term effect of successful treatment in this patient group. In this study, patients who had been infected with HCV for several decades were assessed, and the time since reaching SVR was taken into account.

Our results indicate that, even after a long period of being infected with HCV, a significant improvement in LSM results occurs in the first few years after successful antiviral treatment. In group 2, even in patients with severe fibrosis or cirrhosis at LSM 1, large improvements were seen at LSM 2 (median 4.8 kPa), suggesting that successful antiviral treatment also reduces fibrosis when there already is considerable liver damage. In contrast, median change in LSM results in 84 HCV infected patients with inherited bleeding disorders from our clinic who did not undergo (successful) antiviral treatment was 0.2 kPa, after a median interval of 3.7 years, indicating stable fibrosis overall. In 16% of these patients LSM results increased with more than 2 kPa, indicating progression of fibrosis [18].

In most patients who were successfully treated long before LSM 1, fibrosis remained stable and within the normal range (stage F0-F1), indicating a lasting beneficial effect of treatment. These results could be used to help convince HCV patients with inherited bleeding disorders, whose long infection duration puts them at high risk of severe liver damage, but who are often reluctant to start HCV treatment, of the benefits of antiviral therapy. It should be noted though, that even after successful antiviral treatment, an increased risk of developing hepatocellular carcinoma remains in patients with severe fibrosis or cirrhosis (fibrosis stage F3-F4), and annual surveillance by abdominal ultrasound examination is recommended in these patients.

Unexpectedly, three patients treated more than 15 years ago showed an increase in LSM of more than 2 kPa. Because of small patient numbers, it is not clear to what extent individual patient factors contributed to this observation. We did not find any reports on the effect of antiviral treatment on liver fibrosis with a follow-up of more than 10 years. Reports on the effect of steatosis on LSM may, however, help explain the unexpected increase in LSM in two of our patients. Fraquelli et al reported that reproducibility of transient elastography is influenced by the presence of hepatic steatosis [10]. They recommend that TE should be used cautiously as a surrogate of liver biopsy for assessing liver fibrosis in patients with steatosis. The interaction of fat with low-frequency vibrations of TE may affect the signal-to-noise ratio, which is the relevant parameter

for assessing liver stiffness. Lupsor et al also reported that fibrosis and steatosis were independently associated with liver stiffness [21]. This suggests that the increase in LSM results in the two patients with steatosis in our study might not actually reflect progression of liver fibrosis, but rather a limitation of the use of LSM. In the study of Arena et al, however, LSM results were not influenced by the degree of steatosis [22]. In one patient, HBV co-infection might also have influenced his LSM result. A possible explanation for the increase in LSM results in the third patient could be cholestasis. His bilirubin and alkaline phosphatase levels were elevated. Millonig et al reported that extrahepatic cholestasis increases liver stiffness, irrespective of fibrosis. They observed a mean decrease of liver stiffness of 1.2 ± 0.56 kPa per 1 g/dl decrease in bilirubin after successful biliary drainage [23]. This patient was also co-infected with HIV. Di Martino et al showed that HIV co-infection does not compromise liver histological response to interferon therapy in patients with chronic hepatitis C over a two-year follow-up period [24]. However, the long-term effect of HIV infection and HAART on liver fibrosis after successful HCV treatment remains unknown. There were three other HIV infected patients in our study. They showed deterioration of fibrosis of 1.2 kPa (stable stage F0-F1) and improvement of 1.8 and 2.4 kPa (stage F2 became F0-F1 in both), respectively.

Since IQR data were not available for the LSM 1 measurements, IRQ/median ratios could not be calculated and used as a validity criterion. This could have resulted in a reduced reliability of some of the LSM 1 measurements and under- or overestimation of changes between LSM 1 and LSM 2. In our study, LSM 1 and LSM 2 were not performed by the same operator nor with the same version of the Fibroscan® device. However, since the inter-observer variability of LSM is reported to be small [10,25] and the inter-equipment reproducibility excellent [25], this will, in our opinion, not have had a large effect on our results. In any case, the way our study was performed reflects normal clinical practice and gave us an opportunity to look at LSM as it is actually used in our patients.

Recently, Ogawa et al and Vergniol et al published the first reports on the use of LSM to assess liver fibrosis before and after antiviral treatment in 145 and 416 HCV infected patients, respectively, who were followed for about 2 years after completing treatment. In accordance with the present study, both studies reported significant reduction of liver fibrosis during the first few years after antiviral treatment. Interestingly, this effect was independent of whether patients reached SVR or not [14,15].

The positive effect of successful antiviral treatment on the extent of liver fibrosis is in accordance with the excellent prognosis of patients with SVR found in other studies. Hardly any liver-related clinical events were reported in European sustained respond-

ers, after a mean follow-up of about 5 years [26-28], in HIV co-infected patients with SVR after a median follow-up of less than 2 years [29] or in patients with inherited bleeding disorders up to 15 years after successful treatment [30].

LSM results are influenced by hepatic inflammation and ALT flares in acute viral hepatitis [31]. In chronic hepatitis C, however, LSM results were not correlated with histological activity in multivariate analyses in the studies by Castéra et al and Ziol et al [12,19]. On the other hand, Arena et al and Lupsor et al did report an association between necro-inflammatory activity on biopsy and LSM results [21,22] and Coco et al showed that ALT levels were independently associated with liver stiffness [32]. Part of the (immediate) improvement of LSM results in group 2 could therefore reflect a decrease in inflammation and ALT levels, as a result of recent antiviral treatment, rather than reduction of fibrosis alone.

Because of its strong association with liver biopsy results, LSM could be the preferred technique to monitor change in liver fibrosis over time, especially in patients with bleeding disorders. Reported differences between LSM and biopsy results may very well be due to limitations of the biopsies, for example inadequate biopsy lengths or a large inter-observer variability [33]. The risk of sampling error also is an important issue in liver histological examination. LSM measurements are performed on a cylinder of hepatic tissue of about 1 cm diameter and 4 cm length, which is a volume at least 100 times larger than an average liver biopsy sample [34]. This greatly reduces the possibility of sampling error. In obese patients, however, LSM result can be difficult to obtain, because a fatty thoracic belt attenuates both elastic waves and ultrasound [35]. It is, of course, important to always look at LSM results in individual patients in their clinical context and take factors that can influence these results (like fasting or non-fasting status, BMI, liver parameters, co-morbidity, etcetera) into account.

Our study was limited by a small number of patients, with quite some variation in factors that could have influenced our outcome, like type and regimen of antiviral treatment. However, even in this small patient group, a striking effect of antiviral treatment was observed. Further studies in larger, more uniform cohorts (preferably using the same type of antiviral treatment), with a longer follow-up period, might give more insight in which factors are associated with reduction of liver damage after antiviral treatment. They will also show whether or not the extent of fibrosis could indeed increase again many years after completion of antiviral treatment, and perhaps clarify the effect of steatosis and cholestasis on LSM.

Conclusion

In patients with inherited bleeding disorders, with a median duration of HCV infection of almost 30 years, liver stiffness measurements indicated a significant improvement of liver fibrosis after successful antiviral treatment, especially during the first years after completing treatment. In most patients who were treated long before the first LSM, the extent of fibrosis remained limited and stable. This indicates that, even after long duration of HCV infection, antiviral treatment has a beneficial and sustained effect on the liver. This effect will most likely be similar in HCV infected patients without inherited bleeding disorders.

Liver stiffness measurements appear to be useful to assess changes in the extent of fibrosis over time, especially in patients in whom liver biopsies are contra-indicated. Further studies are needed to establish the effect of successful antiviral treatment on the very long term, and to determine factors associated with changes in fibrosis.

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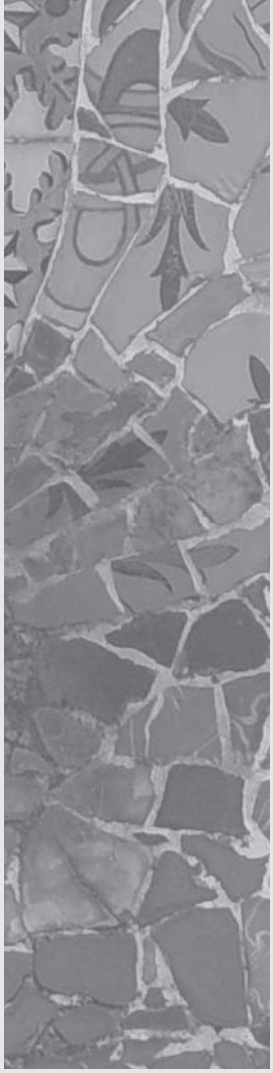
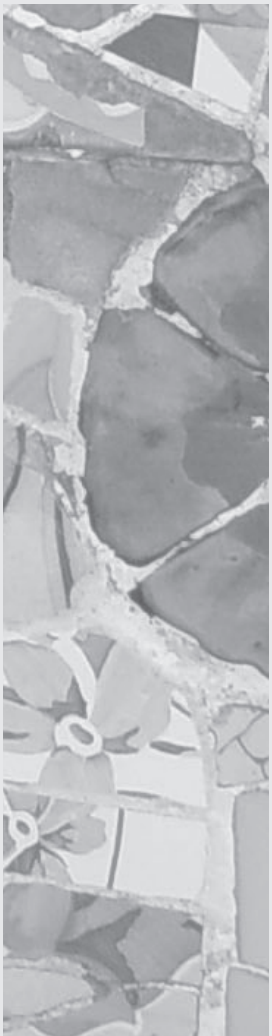


Chapter 12

Morbidity and mortality in ageing HIV infected haemophilia patients



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Haemophilia - in press

Abstract

Introduction

Over 25 years of follow-up are now available for HIV infected haemophilia patients. The aim of this study was to retrospectively asses the morbidity and mortality of HIV infection and the effects of HAART in these patients.

Methods

Data on HIV infection, its treatment and all types of co-morbidity were collected from medical records of all 60 HIV positive haemophilia patients who were treated at the Van Creveldkliniek since 1980 and compared with data from 152 HIV negative patients with severe haemophilia and the general age-matched male population.

Results

AIDS developed in 27 patients (45%), while 31 patients died (52%). Death was solely or partially AIDS-related in 71%. Development of AIDS and AIDS-related deaths declined strongly after the introduction of HAART. Only one major ischemic cardiovascular event occurred in our study population. Of the 27 patients who were still treated at our clinic in 2010, 25 (93%) were on HAART. They had more often hypertension and diabetes, but less often overweight and obesity and lower cholesterol levels than the general population. The occurrence of spontaneous intracranial bleeding was higher in HIV positive haemophilia patients on HAART than in HIV negative patients with severe haemophilia (16.6 versus 1.2 per 1000 patient years).

Conclusion

Since the introduction of HAART, the impact of HIV infection on morbidity and survival has decreased. The increased prevalences of hypertension and diabetes, however, warrant regular screening. HIV positive haemophilia patients on HAART appear to have an increased risk of spontaneous intracranial bleeding.

Introduction

Infection with HIV (human immunodeficiency virus) was an important and often devastating complication of haemophilia treatment in the 1980s. Fortunately, since 1985, all clotting factor products have been free of HIV. Of 335 Dutch haemophilia patients known at our haemophilia centre in 1995 who were at risk for HIV infection, 53 (15.8%) were actually infected [1]. Before the introduction of highly active antiretroviral therapy (HAART) in 1996, many of these patients died. HAART, today also called combined antiretroviral therapy (CART), nowadays consists of a combination of nucleoside reverse transcriptase inhibitors, protease inhibitors and/or non-nucleoside reverse transcriptase inhibitors. Because of HAART, life expectancy of HIV positive patients has improved dramatically, and HIV-related complications have become rare. With ageing, HIV infected haemophilia patients are also expected to increasingly experience age-related co-morbidity like cardiovascular disease and malignancies. Some reported long-term side-effects of HAART are dyslipidemia, insulin resistance/diabetes mellitus type 2 and an increased risk of myocardial infarction [2-7]. An increased bleeding tendency (mainly joint, muscle, and subcutaneous bleeding, but also spontaneous intracranial bleeding) has been reported in patients with inherited bleeding disorders using protease inhibitors [8-11].

Over 25 years of follow-up are now available for haemophilia patients who were infected with HIV in the 1980s. The aim of this study was to retrospectively asses the course and complications of HIV infection, the presence of co-morbidity and the effects of HAART in these patients. Data on the first 14 years of follow-up of a large proportion of our cohort were published by Roosendaal et al in 1998 [12].

Materials and methods

As part of a retrospective evaluation of co-morbidity in a large cohort of haemophilia patients [13], data on HIV infection, its treatment and all types of co-morbidity were collected of all HIV positive haemophilia patients who were treated at the Van Creveldkliniek, a large haemophilia treatment centre in the Netherlands, at any point between 1980 and 2010. Patients visit our clinic at least once a year and their medical records have been meticulously kept since 1972, enabling reliable retrospective data collection. Follow-up ended at either last clinical evaluation before 1 September 2010, transfer to another treatment centre, or death. For patients who were still alive and treated at our centre in 2010, recent height, weight, blood pressure, HIV RNA levels, CD4 counts and cholesterol and triglyceride levels were recorded as well.

The date of HIV seroconversion was estimated by calculating the mid-point in time between the last negative and first positive anti-HIV ELISA tests. For patients for whom

the date of seroconversion could not be calculated, the mean date of seroconversion of the total group was imputed.

AIDS (acquired immunodeficiency syndrome) was diagnosed according to the 1993 European definition [14]. HAART was defined as a combination of at least three antiretroviral drugs that typically includes a protease inhibitor (PI) or a non-nucleoside-analogue reverse-transcriptase inhibitor (NNRTI) plus two nucleoside-analogue reverse-transcriptase inhibitors (NRTIs).
Hypertension was defined as blood pressure over 140/90 mmHg and/or the use of antihypertensive medication.

The study was approved by the Medical Ethics Review Board of the University Medical Center Utrecht.

Statistical analyses

Kaplan-Meier survival analyses were performed to assess AIDS-free survival and overall survival. AIDS-free follow-up ended at the moment of diagnosis of the first AIDS-defining disease. Data were censored at the moment of death, transfer to another haemophilia treatment centre, or last clinical visit before 1 September 2010.

To assess differences in co-morbidity according to HIV status, cumulative incidences and prevalences of specific types of co-morbidity in the HIV positive patients with severe haemophilia (factor level < 1%) were compared to those of all 152 HIV negative patients with severe haemophilia born before 1 January 1971 from the same retrospective research database (further referred to as 'severe controls'). Because the mean age at the end of follow-up in this comparison cohort was higher than in the HIV positive patients, logistic and linear regression analyses were corrected for age. Age-adjusted logistic regression was also performed to assess the effect of HIV infection on survival.

For the HIV positive patients who were still alive and treated at our centre in 2010 and using HAART, data on blood pressure, cholesterol levels, diabetes mellitus and weight distribution were compared with data from the age-matched general male population (obtained from the Dutch Central Bureau of Statistics (www.cbs.nl), the Dutch Heart Foundation (Nederlandse Hartstichting, www.hartstichting.nl) and the Dutch National Institute for Healthcare and Environment (RIVM, www.rivm.nl)). Age-matched reference risks were obtained by weighing reference data from different age groups in the general population according to the age distribution of the patients in our study population.

To assess the effect of HIV and HAART on intracranial bleeding, the cumulative incidence of non-traumatic intracranial bleeding in HIV positive patients with severe haemophilia on HAART was compared with the cumulative incidences in these patients in the period before HAART and in the 152 HIV negative severe controls. The number of patient years on HAART for the HIV positive patients was calculated. The HAART-free follow-up years were those between HIV seroconversion and start of HAART or, in patients who never used HAART, end of overall follow-up. For the one patient for whom the exact date of start of HAART was unknown, because it was started in another centre, the mean date of start of HAART of the total group was imputed. For the HIV negative patients, the number of patient years was calculated as the time between birth and end of follow-up. 95% confidence intervals (CIs) were calculated for all results. A statistically significant difference (p-value < 0.05) was assumed when there was no overlap in 95% CIs. Data were analysed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

Results

Baseline characteristics of the 60 HIV infected patients who were treated at our centre are shown in Table 1. Nearly all patients (97%) had severe haemophilia. There was one patient with moderate and one with mild haemophilia. Thirty-one patients (52%) were deceased, while 27 patients (45%) were still alive and treated at our centre in 2010. Forty-one patients (68%) had chronic hepatitis C infection. Twenty-six of these patients underwent antiviral treatment (21 once and five twice), which was successful in 11 patients (42%). For 10 patients (17%), hepatitis C status was unknown, because they died before HCV testing became available. Dates of HIV seroconversion could be calculated for 55 patients (92%). The total number of HIV positive follow-up years was 1061, while the mean number of HIV positive follow-up years per patient was 17.7.

Table 1. Baseline characteristics of our study population.

Total number of patients	60	
Haemophilia A	51	(85%)
Severe haemophilia	58	(97%)
Current status		
Deceased	31	(52%)
Still treated at our centre	27	(45%)
Transferred to another treatment centre	2	(3%)
Chronic hepatitis C infection	41	(68%)
Mean month of HIV seroconversion (range)	July 1983	(March 1981-August 1985)
Mean age at HIV seroconversion in years (range)	22.2	(5-61)
Mean age at end of follow-up in years (range)	39.8	(14-66)
Total number of HIV positive follow-up years	1061	

HIV = human immunodeficiency virus

Development of AIDS

Figure 1 shows the Kaplan-Meier curve for the cumulative AIDS-free survival in our study cohort. Mean AIDS-free survival in the total cohort was 18.9 years (95% CI 16.4-21.4, range 3.0-27.7 years). Twenty-seven patients (45%) developed AIDS, at a mean age of 33.4 years (range 12-63 years), after a mean infection duration of 10.0 years (range 3-26 years). AIDS developed in 22 of 51 haemophilia A patients (43%, 95% CI 31-57%) and five of nine haemophilia B patients (56%, 95% CI 27-81%), showing no significant difference between these two groups.

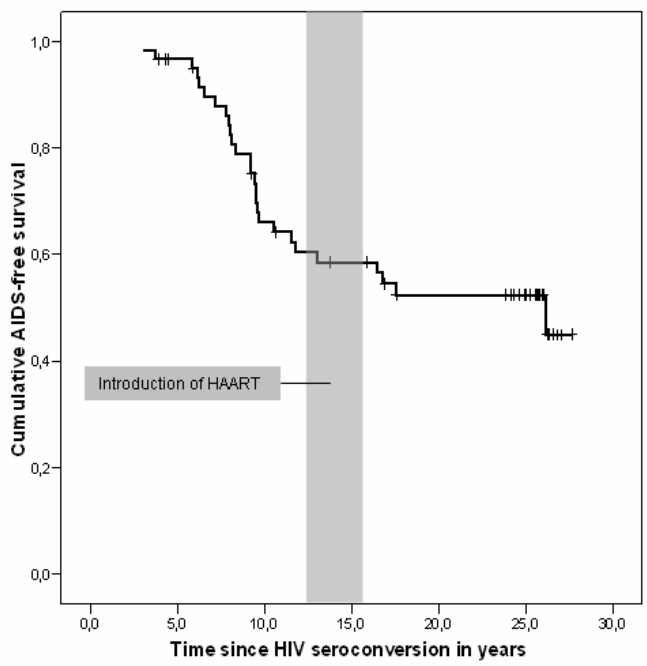

Figure 1. Cumulative AIDS-free survival in our cohort of 60 HIV positive haemophilia patients.
HIV = human immunodeficiency virus

Most common AIDS-defining diseases were candidiasis and pneumocystis jiroveci pneumonia (Table 2). Shortly after the introduction of HAART, a strong reduction in the progression to AIDS was seen. AIDS-defining conditions were diagnosed in only three patients on HAART: one case of candida oesophagitis (after three years on HAART), one case of HIV encephalopathy (after five years on HAART) and one patient who was diagnosed with a fatal plasmablastic Non-Hodgkin lymphoma a few months after starting HAART. He had refused treatment despite low CD4 counts for a long time. The first two patients were still alive in 2010. One additional patient who developed AIDS (mycobacterium avium infection in 1993) was also still alive in 2010. One other patient was lost to follow-up, while the remaining 22 patients who developed AIDS were deceased.

Table 2. AIDS-defining diseases in the 27 haemophilia patients who developed AIDS.

AIDS-defining disease	Patients not on HAART	Patients on HAART
Candidiasis	9	1
Pneumocystis jiroveci pneumonia	6	-
Mycobacterium avium infection	2	-
HIV encephalopathy	1	1
Toxoplasmosis encephalitis	1	-
Cryptosporidiosis	1	-
Recurrent pneumonia	1	-
Plasmablastic Non-Hodgkin lymphoma	-	1
Kaposi's sarcoma	1	-
HIV wasting syndrome	1	-
Unknown	1	-

AIDS = acquired immunodeficiency syndrome
HAART = highly active antiretroviral treatment
HIV = human immunodeficiency virus

Co-morbidity

Three ischemic cardiovascular events were reported. Unstable angina pectoris requiring bypass surgery occurred in a patient aged 48 years, who was on HAART and regular clotting factor prophylaxis, and who had both hypertension and diabetes mellitus type 2. Transient ischemic attacks were reported in two other patients. Acute thrombotic cardiovascular events such as myocardial infarction, ischemic stroke, deep vein thrombosis or pulmonary embolism were not observed at all. Atrial fibrillation was present in one patient. Intracranial bleeding occurred in 13 patients (22%, seven non-traumatic, four traumatic, two cause unknown). Two cases of traumatic intracranial bleeding were fatal.

Six patients had a total of seven malignancies: two basal cell carcinomas, one hepatocellular carcinoma (in a HCV co-infected patient), one Kaposi's sarcoma, one plasmablastic Non-Hodgkin lymphoma, one giant B-cell Non-Hodgkin lymphoma and one Hodgkin lymphoma. Three of these tumours were fatal.

At end of follow-up, the 58 HIV positive patients with severe haemophilia were significantly younger (39.6 years, range 14-66 years) than the 152 HIV negative severe controls from our comparison cohort (53.1 years, range 30-78 years). Angina pectoris and atrial fibrillation both occurred in 2% of the HIV positive patients, while the cumulative incidences were 5% and 3%, respectively, in the HIV negative patients. Intracranial bleeding occurred in 22% of HIV positive and 11% of HIV negative patients. When corrected for age, this difference was borderline significant (p-value 0.05). The cumulative incidences of malignancies were similar in both groups (9% versus 10%). Diabetes mellitus, on the other hand, occurred significantly more often in HIV positive than in HIV negative patients (12% versus 7%, adjusted p-value 0.006). All but one of the HIV positive patients were on HAART when

their diabetes was diagnosed. The prevalence of chronic hepatitis C infection was not associated with HIV status. Body mass indexes (BMI) could be calculated for 42 HIV positive (72%) and 134 HIV negative haemophilia patients (88%). Mean BMI was significantly lower in the HIV positive patients (22.1 versus 25.7 kg/m^2, adjusted p-value < 0.001), and the prevalences of overweight (BMI 25.1-30.0 kg/m^2) and obesity (BMI > 30.0 kg/m^2) were also much lower in these patients (10% and 2% versus 45% and 10%, respectively).

Mortality and causes of death

Thirty-one HIV positive patients (52%) were deceased at the end of follow-up. Causes of death are shown in Table 3. Death was reported to be solely AIDS related in 19 patients (61%) and caused by a combination of HIV and hepatitis C in three patients (10%). Mean age at death was 36.9 years (range 14-65 years). All but two AIDS-related deaths occurred in patients who were not on HAART. Only the two lymphoma patients were on HAART at time of diagnosis, but the second patient had started this treatment only a few months earlier. In one other patient on HAART, death was reported to be caused by a combination of HIV and hepatitis C. Median interval between HIV seroconversion and death was 11 years (range 4-26 years). No fatal non-virus related malignancies occurred in our cohort, nor were there any fatal ischemic cardiovascular events.

Table 3. Causes of death in HIV positive and HIV negative haemophilia patients.

Cause of death	HIV positive patients not on HAART		HIV positive patients on HAART		HIV negative patients with severe haemophilia	
All causes	26		5		28	
AIDS related	17	(65%)	2	(40%)	-	
- opportunistic infection	6		-		-	
- intracerebral toxoplasmosis	2		-		-	
- lymphoma	-		2		-	
- exact cause unspecified	9		-		-	
Hepatitis C related	2	(8%)	2	(40%)	4	(14%)
- liver failure	1		1		2	
- hepatocellular carcinoma	-		1		2	
- hepatorenal syndrome	1		-		-	
Combination of AIDS and hepatitis C	2	(8%)	1	(20%)	-	
Non-virus related malignancies	-		-		5	(18%)
Intracranial bleeding	2	(8%)	-		6	(21%)
Other types of bleeding	-		-		3	(11%)
Sepsis/infection	1	(4%)	-		3	(11%)
Other causes	-		-		5	(18%)
Unknown	2	(8%)	-		2	(7%)

HIV = human immunodeficiency virus
HAART = highly active antiretroviral treatment
AIDS = acquired immunodeficiency syndrome

Interestingly, seven of nine HIV infected haemophilia B patients (78%) were deceased, but only 24 of 51 HIV infected haemophilia A patients (47%). Death was solely or partially AIDS related in five haemophilia B patients (71%) and in 17 haemophilia A patients (71%). Median interval between HIV seroconversion and death was similar across haemophilia types (10 years in haemophilia B and 11 years in haemophilia A, p-value 0.21). In comparison, 28 of the 152 HIV negative severe controls (18%) were deceased at the end of follow-up. Main causes of death in these patients were intracranial bleeding, malignancies, hepatitis C, other bleedings and infections. Compared with the HIV negative patients, the age-adjusted odds ratio for dying was 4.1 in HIV positive patients (95% CI 1.9-8.7, p-value < 0.001). The cumulative survival since 1980 for both the HIV positive and HIV negative patients with severe haemophilia is shown in Figure 2.

Use of antiretroviral treatment

Fifty patients (83%) ever received antiretroviral treatment, 32 of whom were treated with HAART. Median month of start of HAART was January 1997 (range January 1996 - April 2008). Of the 27 patients who were still alive and treated at our centre in 2010, 25 (93%) were on HAART. One patient had low HIV RNA levels (230 copies per ml) and normal CD4 counts (561 cells/mm^3) without HAART, while the last patient refused to start HAART, in spite of increasing HIV RNA levels and decreasing CD4 counts since 2005 (at last measurement 4250 copies/ml and 350 cells/mm^3, respectively). Most commonly used types of HAART medication were tenofovir/emtricitabine (truvada, 12 patients), ritonavir (10), atazanavir (7), lopinavir/ritonavir (kaletra, 4), efavirenz (4) and lamivudine/zidovudine (combivir, 4). Of the 23 patients for whom current HAART regimens were known, 16 (70%) used at least one protease inhibitor.

HAART effects and cardiovascular disease risk factors

Table 4 shows antiretroviral treatment effects and several cardiovascular disease risk factors for the 25 patients who were on HAART and were still treated at our centre in 2010. Mean age at the end of follow-up was 44 years (range 32-66 years). Two patients had detectable HIV RNA levels (64 and 158 copies/ml, respectively) and eight had CD4 counts below normal, while only one patient had a CD4 count below 300 cells/mm^3 (295 cells). The prevalence of hypertension was higher in our patients than in the general age-matched male population (64 versus 33%). Overall, cholesterol levels were lower than in the general population, but the prevalence of hypertriglyceridemia was high (60%). The prevalence of diabetes mellitus type 2 was increased compared with the general population (24 versus 4%), while the prevalences of overweight and obesity were decreased (24% and 4% versus 36% and 12%, respectively).

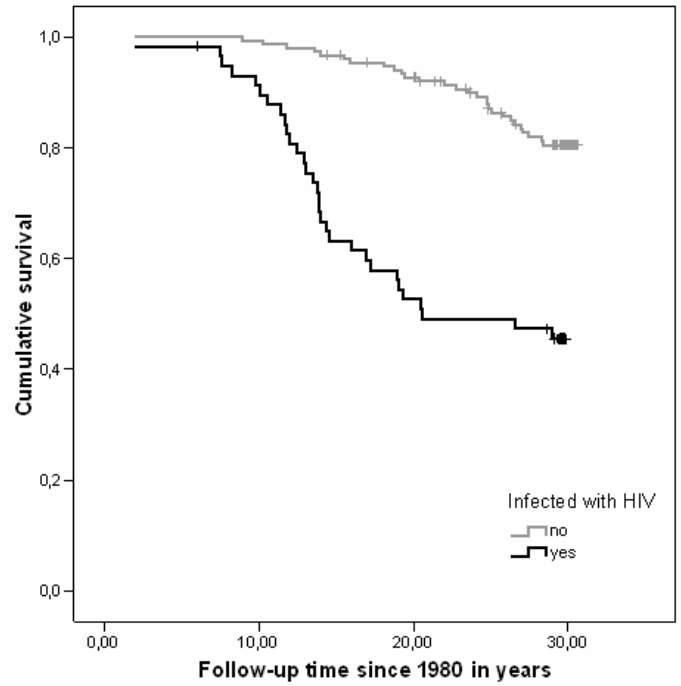

Figure 2. Cumulative survival since 1980 of 58 HIV positive and 152 HIV negative patients with severe haemophilia.
HIV = human immunodeficiency virus

Table 4. Antiretroviral treatment effects and cardiovascular disease risk factors in 25 HIV positive haemophilia patients on HAART who were still treated in our centre in 2010, and comparison with the age-matched general male population.

	HIV positive patients on HAART (n = 25)		**Age-matched general male population**
HAART including at least one protease inhibitor	70%	(18-49)	-
Detectable HIV RNA levels	8%	(1-26)	-
CD4 count below normal (560 cells/mm^3)	32%	(15-54)	-
CD4 count < 300 cells/mm^3	4%	(0-20)	-
Hypertension	64%	(43-82)	33%
Total cholesterol level ≥ 5.0 mmol/l	32%	(15-54)	70%
HDL cholesterol level < 1.0 mmol/l	68%	(47-85)	8%
LDL cholesterol level > 3.5 mmol/l	12%	(3-31)	n.a.
Triglyceride level > 2.0 mmol/l	60%	(39-79)	n.a.
Diabetes mellitus type 2	24%	(9-45)	6%
BMI 25.1-30.0 kg/m^2 (overweight)	24%	(9-45)	36%
BMI > 30.0 kg/m^2 (obesity)	4%	(0-20)	12%

Values are proportions (95% confidence interval).
HIV = human immunodeficiency virus
HAART = highly active antiretroviral treatment
BMI = body mass index
n.a. = not available

HAART and intracranial bleeding

Of 30 HIV positive patients with severe haemophilia on HAART, five patients suffered from non-traumatic intracranial bleeding (17%, 95% CI 6-35%). These bleeds occurred during a total of 301 patient years on HAART (16.6 bleeds per 1000 patient years, 95% CI 5.4-38.3) (Table 5). In four of these patients, HAART included at least one protease inhibitor (ritonavir only, ritonavir and saquinavir, ritonavir and lopinavir, and amprenavir only, respectively). Two patients were on low-frequency regular prophylactic clotting factor treatment (once or twice per week) when the bleeding occurred. One of these two patients had thrombocytopenia, while in the other four patients platelet counts were normal. Intracranial bleeding occurred 1-12 years (mean 7 years) after start of HAART, at a mean age of 43.6 years (range 34-65 years). None of these events were fatal.

Table 5. Spontaneous intracranial bleeding in HIV positive patients with and without HAART and a comparison group of HIV negative patients with severe haemophilia.

	HIV positive patients with severe haemophilia not on HAART	HIV positive patients with severe haemophilia on HAART	HIV negative patients with severe haemophilia
Number of patients	58	30	152
Number of patient years	716	301	8068
Number of spontaneous intracranial bleeds	2	5	10
Spontaneous intracranial bleeds per 1000 patient years (95% CI)	2.8 (0.3-10.1)	16.6 (5.4-38.3)	1.2 (0.6-2.3)

HIV = human immunodeficiency virus
HAART = highly active antiretroviral treatment
CI = confidence interval

Two cases of non-traumatic intracranial bleeding occurred in the 58 HIV positive patients with severe haemophilia in 716 HAART-free follow-up years (2.8 bleeds per 1000 patient years, 95% CI 0.3-10.1). In comparison, 10 non-traumatic intracranial bleeds occurred in nine out of the 152 HIV negative severe controls (6%, 95% CI 3-11%), during a total of 8068 patient years (1.2 bleeds per 1000 patient years, 95% CI 0.6-2.3), showing a significantly decreased risk in this group compared with the HIV positive patients on HAART. The mean age at intracranial bleeding in the HIV negative patients was 53.8 years (range 7-70 years). Five of these bleeds (50%) were fatal.

Overall, non-traumatic intracranial bleeding occurred in five of 49 HIV positive patients with severe haemophilia A (10%, 95% CI 3-22) and two of nine HIV positive patients with severe haemophilia B (22%, 95% CI 3-60) and in eight of 136 HIV negative severe controls with haemophilia A (6%, 95% CI 3-11) and one of 16 HIV negative severe con-

trols with haemophilia B (6%, 95% CI 0-30), indicating similar cumulative incidences across haemophilia types in these relatively small groups.

Discussion

This cohort of HIV infected haemophilia patients, with a well-defined moment of seroconversion and mode of HIV transmission, gave us an opportunity to study the natural history of HIV infection, the effects of HAART, and the occurrence of different types of co-morbidity in this specific sub-population of haemophilia patients over a follow-up period of over 25 years. Although based on relatively small numbers, we feel our results are representative for those in other haemophilia treatment centres and provide a good overview of the problems that occurred, and are still occurring, in these patients.

Before the introduction of HAART, the impact of AIDS on survival was large: 23 patients died before 1997, in 19 (83%) of whom death was reported to be solely or partly AIDS related. After the introduction of HAART, stabilisation occurred in AIDS-related mortality: eight patients have died since 1997, in three (38%) of whom death was solely or partly AIDS related. Only one of these three patients, who had a giant B cell lymphoma, had been on long-term HAART. The incidence of Non-Hodgkin lymphoma has been reported to be substantially reduced in patients who are on HAART compared with the pre-HAART era, but was still reported to be 2-4 per 1000 person years in non-haemophilic HIV positive patients [15,16], indicating that this remains an important complication of HIV infection. In our study, liver disease was reported to be the cause of death (in combination with AIDS) in one patient (4%) before 1997 and in four patients (50%) after 1997, confirming the findings of others that liver disease is an increasingly important cause of death in the current haemophilia population, both in HIV positive and HIV negative patients [17-19]. As expected, overall survival was significantly lower in HIV positive patients than in our comparison group of HIV negative severe controls.

The proportions of patients in our study who developed AIDS (45%) and who were deceased (52%) were slightly lower than those reported in other studies in HIV infected haemophilia patients with long-term follow-up (AIDS development in 48-69% and death in 62-67% of patients during follow-up periods of 20-23 years) [20-23]. As expected, the proportion of patients who developed AIDS did not increase since Roosendaal's earlier report on this cohort, while the proportion of deceased patients did [12]. The relatively large proportion of HIV infected haemophilia B patients who were deceased (78% (95% CI 40-97%), versus 47% (33-62%) in haemophilia A patients) could, at first sight, corroborate the more unfavourable prognosis of HIV infection in haemophilia B reported by others [24-26]. This is hypothesised to be related to the type of clotting factor product that was contaminated with the HIV virus (carrying for example different

strains of HIV or different viral loads) [12,26]. In our study cohort, however, the proportions of deceased patients in whom death was solely or partially AIDS related were the same in patients with haemophilia A and B (71% each), suggesting no difference in HIV prognosis in this small cohort.

Factors influencing progression to AIDS, such as age at seroconversion and baseline CD4 counts, were extensively studied by others [27-29]. Because of small patient numbers, we did not perform any specific analyses on these factors in our study cohort. Co-infection with HCV has been described to have a negative effect on prognosis and treatment response in HIV infected patients [30,31]. Because HCV status was unknown for patients who developed AIDS before the introduction of HCV tests, the effect of HCV infection on AIDS-free survival could not be reliably assessed.

Kaposi's sarcoma was present in one patient in our study. These tumours are thought to be primarily associated with human herpesvirus 8 and mainly occur in patients who acquired HIV through sexual contact [32]. They are rare in HIV infected haemophilia patients [33,34]. We have, however, no reason to believe that our patient acquired HIV any other way than through the use of contaminated clotting factor concentrates.

Nowadays, almost all surviving HIV positive haemophilia patients are on HAART and HIV infection has become another chronic condition. The risk of myocardial infarction is reported to be increased for specific types of HAART medication [4,6,35], and also to increase with longer treatment duration [36]. So far, no myocardial infarctions were reported in our study population, but one patient had unstable angina pectoris requiring bypass surgery. A decreased risk of ischemic cardiovascular disease has been reported in haemophilia patients, especially those with severe haemophilia, which could have attenuated any increased risk caused by use of HAART [13,37,38]. The relatively young age of our patients at the end of follow-up will also have lowered the risk of cardiovascular events. Overall, the prevalences of overweight and obesity were significantly lower in HIV positive patients than in HIV negative severe controls, while the prevalence of diabetes was higher. Diabetes occurred mainly in HIV positive patients using HAART. Because the prevalences of many other cardiovascular disease risk factors, such as smoking habits, hypertension and hypercholesterolemia, could not be reliably assessed from our retrospective database, no overall comparison could be made of these risk factors between HIV positive and HIV negative haemophilia patients.

Haemophilia patients using HAART did have more hypertension and diabetes mellitus than the age-matched general male population, but less overweight and obesity and lower overall HDL and total cholesterol levels. The prevalence of increased triglyc-

erides, however, was high (60%). The high prevalence of hypertension and relatively low cholesterol levels are in concordance with earlier reports in haemophilia cohorts (including both HIV positive and HIV negative patients) [39,40]. Lower prevalences of overweight and obesity have also been reported before in patients with severe haemophilia [41]. The increased prevalence of diabetes, however, has not been reported in other haemophilia cohorts and could be associated with the use of HAART, as could the high triglyceride levels.

Our data suggest an increased risk of spontaneous intracranial bleeding in HIV positive haemophilia patients using HAART. Non-traumatic intracranial bleeding occurred significantly more often and at a younger age in these patients than in HIV negative severe controls. In four of five patients on HAART who experienced spontaneous intracranial bleeding, at least one protease inhibitor was used. Unfortunately, complete data on other severe bleeding complications or unusual types of bleeding were not available from our retrospective database. According to the treating physicians, however, no other major bleeding complications were reported in our patients. Our results are in accordance with reports by others of an increased risk of intracranial bleeding associated with protease inhibitor treatment in haemophilia patients [8-10]. The increase in bleeding tendency in these studies, however, seemed to mainly occur within several months after starting the PI treatment [10,42,43]. The exact cause of the increased bleeding tendency associated with protease inhibitor treatment remains unknown. It has been suggested that inhibition of cytochrome P450 by certain PIs interferes with platelet function, thus increasing bleeding risk, especially in patients with pre-existing bleeding disorders, but the evidence in this area is not consistent [10]. The haemophilia patient with intracranial bleeding reported by Kodoth et al did have low platelet counts [42], and Graff et al reported decreased platelet aggregation in five non-haemophilic patients after administration of the PI tipranavir [44], but extensive investigation in six haemophilia patients with increased bleeding tendencies reported by Yee et al and Stanworth et al, including full coagulation factor assays and platelet aggregation studies, did not show any abnormalities [9,45]. Because of the benefits of HAART containing PI, we would not necessarily recommend switching to a regimen without PIs in haemophilia patients, but treating physicians should be aware of a possible increased risk of severe bleeding complications, especially spontaneous intracranial bleeding, in these patients.

To assess any independent effect of HIV on intracranial bleeding, we also looked at the occurrence of spontaneous intracranial bleeding in the same HIV positive haemophilia patients before starting HAART and found a lower cumulative incidence. It should be noted though, that the mean age of these patients during the HAART-free follow-up years was lower than during the follow-up years on HAART, which could have influ-

enced our results. Koumbarelis et al showed that mortality by cerebral haemorrhage was five times higher in HIV positive than in HIV negative haemophilia patients (8 in 1431 versus 8 in 7210 patient years), but in their study no data on non-fatal intracranial bleeding were available [46]. Nuss et al reported that HIV infection was a significant risk factor for intracranial bleeding in white haemophilia patients in the period 1993-1997 [47], but their findings could not be confirmed by Zanon et al over the period 1987-2008 [48]. These studies, however, did not analyse traumatic and spontaneous intracranial bleeding separately.

Conclusion

Since the introduction of HAART, the impact of HIV infection on morbidity and survival of haemophilia patients has decreased significantly. Although the occurrence of ischemic cardiovascular events was not increased, HIV positive haemophilia patients on HAART should be screened for cardiovascular risk factors such as hypertension, hypertriglyceridemia and diabetes mellitus and treated accordingly. Haemophilia doctors should be aware that HIV positive haemophilia patients on HAART (especially those using protease inhibitors) may have an increased risk of spontaneous intracranial bleeding.

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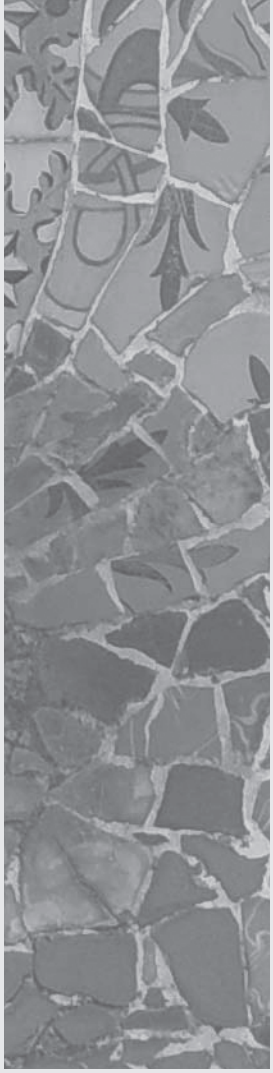
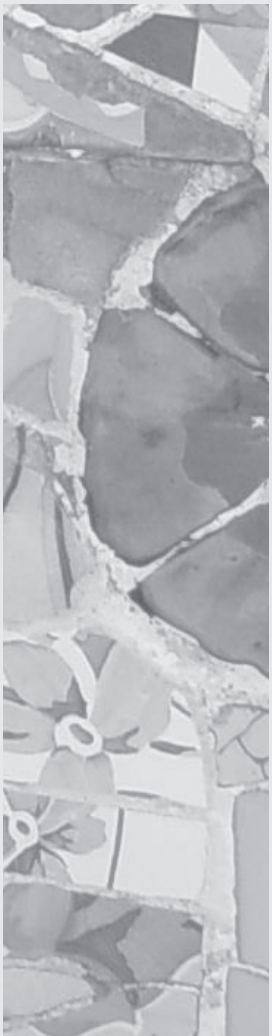
Chapter 13

General discussion





The aim of this thesis was to provide an overview of the occurrence and impact of various types of co-morbidity in adult haemophilia patients. Knowledge of this subject is important for optimal care and treatment of haemophilia patients and could also provide insight in basic pathological mechanisms. Because at the Van Creveldkliniek 50% of all Dutch haemophilia patients are treated and because of our collaboration with several other haemophilia treatment centres, we were able to study relatively large cohorts. This enabled us to reliably assess the burden of co-morbidity in haemophilia patients during the past decades.

With increasing life expectancy, more age-related co-morbidity does indeed occur. With progression of time since infection, more hepatitis C related problems are seen as well, making regular assessment of the extent of liver damage and adequate selection of patients for antiviral treatment important issues. Fortunately, since the introduction of highly active antiretroviral treatment (HAART), the current living haemophilia population no longer has to deal with the devastating effects of the HIV epidemic which occurred in the 1980s and 1990s.

In the end, information on the occurrence, course and impact of different types of co-morbidity is expected to help improve awareness, treatment and overall patient care and well being.

Part I. Age-related co-morbidity

Cardiovascular disease and its risk factors

The results of both the retrospective evaluation in a birth cohort of 408 haemophilia patients from our own centre and the analysis of the baseline data of the 709 patients from the international cohort of our prospective CVD study suggest a reduced risk of acute ischemic cardiovascular events in patients with severe haemophilia. Both these analyses were, however, performed on retrospective data. The advantage of the prospective CVD study cohort is that it consists of living patients only, thus removing the effect of competing risks of dying from infectious diseases like HIV or HCV or from severe bleeding complications. Moreover, these 'surviving' haemophilia patients are the population the haemophilia treatment centres are dealing with on a daily basis, and therefore the most interesting population to study. The fact that the decreased cumulative incidence of acute ischemic CVD was only present in patients with severe haemophilia and not in those with non-severe disease, suggests that the effect is dose-dependent and only very low clotting factor levels have a protective effect on CVD risk.

A reduced mortality from cardiovascular disease has been reported before in haemophilia patients [1-3], but ours are the first large studies focusing on the occurrence of non-fatal cardiovascular disease. We found a reduced cumulative incidence of myocardial infarction in patients with severe haemophilia, while the cumulative incidence of angina pectoris was not reduced. This suggests that very low clotting factor levels are mainly protective against the acute formation of arterial thrombi, and less so against the gradual development of atherosclerosis. This was confirmed by other studies, in which no differences were found in coronary artery calcification scores or carotid and femoral intima-media thickness between haemophilia patients and non-haemophilic males [4-7], and also in a haemophilia mouse model [8].

An explanation for the lower occurrence of acute ischemic cardiovascular events could perhaps be that haemophilia patients more often develop a mural thrombus on atherosclerotic plaque rupture than a (fatal) occlusive thrombus, or that plaque vulnerability is lower in haemophilia patients, resulting in a lower rate of plaque rupture or a higher prevalence of 'silent' plaque rupture without any consequences [7,9]. Since factor VIII and IX have been reported to be important in continued thrombin generation and thrombus survival, one could imagine that the course of plaque rupture could be relatively benign in patients with low levels of these factors [10]. The lack of fatal myocardial infarctions and the occurrence of only one case of fatal ischemic stroke in our retrospective cohort would confirm this relatively benign course when ischemic cardiovascular events do occur. Further research in this area, for example using magnetic resonance imaging to visualise plaque composition, or the use of haemophilia mouse models, could shed more light on this interesting subject.

If patients with severe haemophilia have a reduced tendency to form occlusive thrombi, the lifetime risk of acute cardiovascular events could be significantly reduced, or the occurrence of such events could merely be delayed until vascular occlusion due to advancing atherosclerosis, which appears to occur at a normal rate, is (nearly) complete. If the latter is the case, lifetime risks of CVD events may be less reduced than age-specific cumulative incidences. Long-term follow-up of our prospective cohort will help us to clarify this issue.

Any (protective) effect of low clotting factor levels in haemophilia patients will most likely be influenced by the use of clotting factor concentrates. In theory, the aim of prophylactic treatment is to keep trough levels of factor VIII or IX above 1%, to effectively turn patients with severe haemophilia into patients with moderate disease [11]. Mean clotting factor levels in haemophilia patients on prophylactic treatment will, however, remain much lower than in non-haemophilic males. If, on the other hand, peak clotting

factor levels are very high, they could be a risk factor for the occurrence of ischemic cardiovascular events. High factor VIII levels are reported to be associated with both arterial and venous thrombosis in the general population [12,13] and several case reports have been published on the occurrence of myocardial infarction in haemophilia patients shortly after administration of clotting factor concentrates [14,15]. Patients with severe haemophilia who are on prophylactic treatment may, therefore, have a higher risk of developing ischemic cardiovascular disease than patients with severe haemophilia who are not treated prophylactically, especially shortly after clotting factor administration. In the prospective analyses of our CVD study, we will be able to correct for treatment type (prophylaxis versus on demand) and, in case of a cardiovascular event, evaluate the administration of clotting factor concentrates shortly before the event occurred.

CVD risk profiles

QRISK®2 cardiovascular disease risk profiles were more unfavourable in our cohort of haemophilia patients aged 30 years or older than in the age-matched general male population. This effect was similar across haemophilia severities. It is thus unlikely that differences in risk factors could explain a lower occurrence of CVD in patients with severe haemophilia than in the general population.

The increased prevalence of hypertension we found in our cohort of haemophilia patients confirmed several earlier reports [7,16-18], while various other studies did not find any differences between haemophilia patients and the general population [19-21]. The mechanism behind the increased prevalence is largely unknown. Based on our results, including the fact that hypertension was already present in 24% of patients aged 30-40 years, we recommend incorporating regular blood pressure measurements in the standard care of all haemophilia patients aged 30 years or older. Adequate treatment of hypertension will reduce the risk of both ischemic CVD and intracranial bleeding [22].

The lower cholesterol levels we found in our haemophilia patients were also reported by others [7,17,23]. In our patients, they could partially be explained by a high prevalence of hepatitis C, which is reported to influence cholesterol levels [24,25]. In HCV negative patients, however, cholesterol levels were still significantly lower than in the age-matched general male population. The cause of this additional reduction in cholesterol levels is unknown. Increased total cholesterol levels were, however, still present in 44% of our study population, indicating that this CVD risk factor remains quite prevalent in haemophilia patients. In our CVD study, the increase in QRISK®2 risk became apparent after the age of 40 years. Based on these results, we would recommend regular screening for CVD risk factors, such as hypercholesterolemia, diabetes, overweight and smoking,

in all haemophilia patients aged 40 years or older, and initiation of the appropriate treatment or implementation of lifestyle changes when risk factors are present.

Future analyses

When follow-up data become available for our CVD study cohort, more reliable prospective data analysis on the occurrence of ischemic cardiovascular events will be possible. The use of the QRISK®2 cardiovascular disease risk score will enable us to compare the observed occurrence of cardiovascular events with the expected occurrence based on CVD risk profiles, thus correcting for the effect of many known risk factors. The first results are expected in about five years' time and will show us whether (very) low clotting factor levels really are protective against acute ischemic cardiovascular disease. If the occurrence of CVD events indeed turns out to be lower in our haemophilia patients than in the general population, this would be the case in spite of more unfavourable risk profiles, which would imply a relatively large protective effect of low clotting factor levels on CVD occurrence.

A possible pitfall in our prospective analyses is that, in some patients, treatment for cardiovascular risk factors will have been started after the baseline data on which the QRISK®2 risk profiles were based were collected, resulting in a lower actual CVD risk than the predicted risk. It should be taken into account that treatment for CVD risk factors reflects normal clinical practice and also occurs in the general population, and we will thus be able to reliably compare our data with general population data. The close surveillance of our patients could, however, result in more accurate or more timely diagnosis and treatment of risk factors than in non-haemophilic males. During our follow-up period, data on the use of, for example, antihypertensive medication and statins and changes in weight and blood pressure will be collected, so the final analyses can be corrected for these variables. The most interesting outcome of our prospective study will, in any case, be the occurrence of CVD events in regular clinical practice, so including any and all treatment and preventive measures patients receive.

Potential implications

If very low levels of factor VIII or IX indeed turn out to be protective against ischemic cardiovascular disease, one could imagine this to have treatment implications. In haemophilia patients, an optimal balance would have to be sought between the amounts of clotting factor needed to prevent bleeding, while maintaining levels low enough to benefit from the protective effect on CVD occurrence. This would mean individually tailoring treatment regimens to parameters such as bleeding pattern, treatment response and factor half life.

In patients without haemophilia, inhibiting factor VIII or IX could, theoretically, contribute to CVD prevention. If, however, the protective effect is only present in patients with very low clotting factor levels (as appears to be the case based on our study results), such inhibition would lead to severe bleeding complications and would therefore not be feasible. The finding of Sramek et al that mortality from ischemic heart disease was also lower in carriers of haemophilia would, however, imply that factor levels do not have to be dangerously low to have a beneficial effect on CVD occurrence and perhaps warrant further research in this area [26]. Inhibition of factor VIII or IX could also have a beneficial effect when an ischemic cardiovascular event occurs. A recent review by Roser-Jones et al lists several compounds inhibiting factor IX, which have been shown to reduce thrombus formation in coronary or intracerebral arteries in animal studies, with limited bleeding risk [27]. Extensive human studies using such inhibitors have, however, not been performed yet. Similar effects might be expected for inhibitors of factor VIII.

Other types of age-related co-morbidity

The results of our retrospective study showed similar occurrences of non-virus related co-morbidity in our patients and the general male population. No significant difference was seen in the overall cumulative incidences of malignancies, but the proportion of hepatocellular carcinoma (HCC) within the malignancy cases was higher in haemophilia patients than in the general population. All HCC cases occurred in hepatitis C infected patients (see the section on virus-related co-morbidity below). With a similar overall occurrence of malignancies, a reduction in the occurrence of other types of malignancies than HCC would be expected, but patient numbers in our study were not large enough to detect any such effect. A decreased mortality from non-virus related malignancies in haemophilia patients was reported in a recent Italian study of nearly 2500 patients, but the occurrence of non-fatal malignancies in their cohort was not compared with that in the general population [28]. A lower mortality in the presence of a similar occurrence of malignancies in haemophilia patients and the general population could confirm the hypothesis of Miesbach et al that low clotting factor levels may have a protective effect against cancer spread and dissemination [29]. This would also be in accordance with the reported protective effect of anticoagulant agents against growth and metastatic spread of various types of cancer [30]. Larger, preferably prospective studies will have to be performed to further assess this issue. Our results on the occurrence of malignancies in haemophilia patients confirm the findings of others [1,2,31], except for the increased incidence of Hodgkin lymphoma reported by Darby et al [1], which could not be corroborated by our data.

The occurrences of other types of age-related co-morbidity, such as diabetes mellitus, cataract and renal insufficiency, were similar in our patients and the general population (except for an increased prevalence of diabetes in HIV infected haemophilia patients using HAART, as described below). Since the mechanisms behind these conditions were not hypothesised to be affected by clotting factor levels, these results confirmed our expectations.

Part II. Virus-related co-morbidity

Course and complications of hepatitis C infection

Hepatitis C infection is a major co-morbidity in adult haemophilia patients. In The Netherlands, 68% of all patients who were treated with clotting factor products before 1992 were infected (50% of patients with mild haemophilia and 82% of patients with severe disease) [32]. Chronic HCV infection can be stable for a long time, but long-term inflammation of the liver will lead to progressive fibrous scar formation (fibrosis), liver cirrhosis, liver failure and an increased risk of developing hepatocellular carcinoma. The formation of fibrous scar tissue in the liver after continuous or repeated hepatic injury, as in chronic hepatitis C, leads to the development of portal-central septae and distortion of the vascular structure, resulting in shunting (the formation of varices between the arterial and venous blood flow) within the liver. Cirrhosis is caused by the 'arterialisation' of the liver because of these shunts. Liver cirrhosis progresses from compensated cirrhosis, with still adequate liver function, to decompensated cirrhosis, with gradual loss of liver function, leading to liver failure and resulting in death. Because of a large regenerative capacity of the liver, fibrosis is (partially) reversible in many cases, but once vascular shunts have formed, reversibility is limited [33].

There are several links between the coagulation cascade and liver fibrinogenesis. The suggestion has been raised that defects in the coagulation cascade, as seen in for example haemophilia patients, could result in slower fibrosis progression and a more favourable prognosis [34]. A possible mechanism could be that cirrhosis is associated with and may result from extensive thrombosis of the intrahepatic vasculature, which is hypothesised to occur less often in patients with inherited coagulation disorders, but determining whether thrombosis is the cause or a consequence of the cirrhosis is difficult in these cases [35,36].

In our international cohort of 700 patients with inherited bleeding disorders and chronic HCV, we found cumulative incidences of liver cirrhosis, end-stage liver disease (ESLD), HCC and liver-related death of 20%, 13%, 3% and 9%, respectively, after a mean infection duration of 31 years, indicating that these adverse liver-related events

are not uncommon in patients with inherited bleeding disorders. The fact that 21 of the 90 ESLD cases (23%) occurred during the past six years shows that this is an increasing problem in this population. The lack of appropriate control populations, in which infection duration can be adequately assessed and the number of confounding factors is limited, makes reliable comparison of our results with those in HCV infected patients without inherited bleeding disorders very difficult, if not impossible.

Determinants of progression to ESLD in our international cohort were higher age at infection, HIV co-infection and history of alcohol abuse, while successful antiviral treatment had a large beneficial effect. These results are in accordance with other reports [37-40]. Faster progression of fibrosis with increasing age has also been reported [41,42], but could not be confirmed in our study. Because of the lack of complete and time-specific data on other factors that are reported to influence fibrosis progression, such as AST levels, steatosis, insulin resistance and smoking [43-46], the association between these factors and ESLD occurrence could not be assessed. Co-infection with HIV accelerates the progression of chronic HCV to liver failure and death, possibly through suppression of the immune response against HCV [37,47-49]. The reports of the effect of HAART on fibrosis progression are, however, conflicting [50-53].

The cumulative incidence of hepatocellular carcinoma was 3% in our study cohort of 700 patients with inherited bleeding disorders and chronic HCV, after 31 years of follow-up. The fact that 41% of all HCC cases were diagnosed during the past six years confirms the report by Tagliaferri et al that HCC is an increasing problem in this patient population [28]. The incidence of HCC in patients with liver cirrhosis is reported to be 1-4% per year [54,55]. The significant proportion (11%) of living patients with chronic HCV who were diagnosed with cirrhosis in our cohort study indicates a large number of patients who are at risk for HCC development. Screening for HCC is recommended for all HCV infected patients who have advanced liver fibrosis or cirrhosis (fibrosis stage F3/F4 on biopsy or liver stiffness measurement), also after successful antiviral treatment [56,57]. In our international HCV cohort, only one case of HCC was seen after successful treatment in a patient who was a known alcohol abuser and had long-term cirrhosis before starting antiviral treatment. Further follow-up of our international cohort of HCV infected patients, including longitudinal LSM results, could shed more light on whether (and how often) HCC indeed develops after successful antiviral treatment and whether adhering to the current screening recommendations is warranted. The recommended screening interval for HCC using abdominal ultrasound examination is 6-12 months. Whether or not to combine this examination with analysis of alpha-fetoprotein (AFP) levels is currently under debate [56].

Antiviral treatment

Treatment of HCV in patients with inherited bleeding disorders should be performed according to the updated guidelines published in 2011 [57]. Combination treatment with pegylated interferon (PegIFN) and ribavirin for 24-48 weeks, depending on HCV genotype, should be offered to all treatment naïve patients with chronic HCV infection and to patients who have failed to respond to previous treatment with standard interferon with or without ribavirin. According to the guidelines of the European Association for the Study if the Liver (EASL), antiviral treatment is strongly recommended in patients who have extensive or progressive liver fibrosis [58]. Rapid virological response after 4 weeks of treatment (RVR) is the most important predictor of sustained virological response across genotypes in patients with chronic hepatitis C virus infection [59]. This variable could be used for prediction and management of response-guided combination antiviral therapies. Factors associated with a reduced chance of achieving SVR are a high pre-treatment HCV viral load, failure to achieve RVR or early virological response at 12 weeks (EVR), HCV genotype 1, the presence of cirrhosis and older age at infection [60]. A total of 361 of the 700 patients with inherited bleeding disorders and chronic HCV participating in our international HCV study underwent antiviral treatment, which was successful in 53%. In this cohort, determinants of successful treatment with PegIFN plus ribavirin were absence of HCV genotype 1 and of HIV co-infection, and treatment naivety, which is in accordance with other reports [61,62].

Polymorphisms in the gene IL28B are strongly associated with the first phase viral decline, and thus with SVR, during treatment with PegIFN plus ribavirin, irrespective of HCV genotype [63-65]. Because testing for the IL28B polymorphism is not yet routinely available, this parameter could not be included in our international HCV study. In future, however, treatment response could be predicted by genotyping the IL28 locus, and treatment regimens individually tailored according to the presence or absence of polymorphisms in this gene.

Side-effects of antiviral treatment

The burden of 24 to 48 weeks of antiviral treatment on patients is generally high. Important side-effects of antiviral therapy in our cohort of 47 patients with inherited bleeding disorders undergoing treatment with PegIFN plus ribavirin were fatigue, headache, pruritus/skin rash, concentration problems, decreased appetite, irritability, hair loss, fever, sleeping problems, anaemia, leucopenia and thrombocytopenia. Significant weight loss occurred in the majority of patients. Depression as assessed by the BDI depression inventory was present in 55% of patients who did not have depression before starting antiviral treatment. Patients should be closely monitored for depressive symptoms and treated accordingly with selective serotonin reuptake inhibitors. The only

independent risk factor for development of depression was a history of depression or other psychiatric problems. In patients with such a history, prophylactic antidepressant treatment during antiviral therapy should be considered. Health-related quality of life (HRQoL) was significantly reduced in all RAND-36 domains during antiviral treatment.

Forty-five percent of patients required dose-reduction of PegIFN, ribavirin or both, mainly because of weight loss or anaemia, and in two patients treatment was stopped prematurely because of side-effects. Close monitoring of patients and collaboration between all healthcare providers at our haemophilia treatment centre, however, ensured that the vast majority of patients were able to complete a full course of treatment. Fortunately, physical, psychological and haematological side-effects were mostly transient. It is, however, important for patients, physicians and employers to realise that the end of antiviral treatment does not necessarily mean the end of treatment-related problems. Patients are recommended to slowly build up their normal daily routines during the first weeks to months after cessation of treatment, to ensure optimal recuperation.

In our small study cohort, no association was found between side-effects and treatment effect. We could not confirm the hypotheses of others that the presence of severe side-effects either influences compliance to antiviral treatment and thus reduces the chance of successful treatment [66] or could reflect a larger impact of antiviral treatment on the body, thus increasing the chance of achieving SVR [67,68]. We had hoped to be able to deduce certain interventions from our data to lighten the burden of antiviral treatment in our patients, but unfortunately no associations were found between HRQoL and treatable side-effects such as weight loss or anaemia. There was, however, a clear association between depression and several HRQoL domains, suggesting that timely treatment of depressive symptoms could somewhat ease treatment burden. Treatment of other (severe) side-effects is, of course, also recommended. In case of anaemia, the use of epoetin could be considered to avoid dose reduction [69], but an increase in the occurrence and severity of thrombocytopenia has been described in patients using epoetin in addition to antiviral treatment [70]. In patients with thrombocytopenia due to severe liver disease, administration of the thrombopoietin-receptor agonist eltrombopag has been reported to significantly increase platelet counts [71]. Weight loss during antiviral treatment could be reduced by administration of food supplements or use of energy-enriched food, thus avoiding dose reduction of weight-based therapies.

Another possible strategy is adjusting the dosage of ribavirin to its plasma concentrations in individual patients [72], thus avoiding blood levels that are either too low (with a lower chance of success) or too high (increasing the risk of side-effects).

New treatment modalities

New HCV treatment strategies with better success rates are constantly being developed. Protease inhibitors such as telaprevir and boceprevir are now entering clinical practice. Combination treatment with pegylated interferon, ribavirin and telaprevir, with a treatment duration of 24 weeks for all HCV genotypes, has been shown to increase success rates, especially in patients with genotype 1. A large advantage of this strategy, besides the higher success rate, is the much shorter treatment duration in patients with HCV genotypes 1 or 4, while the down side appears to be an increased occurrence of severe skin problems such as rash and eczema during treatment [73,74]. Boceprevir is an inhibitor of a specific protease complex of HCV genotype 1, which does not have any clinically significant effect against other HCV genotypes. Combination treatment with PegIFN, ribavirin and boceprevir has been reported to significantly increase response rates to antiviral treatment, both in previously treated and untreated patients with HCV genotype 1. Frequently reported side-effects of boceprevir are anaemia and dysgeusia (distortion of the sense of taste) [75-77]. Triple therapy containing PegIFN, ribavirin and boceprevir is now available in our hospital for patients infected with HCV genotype 1 who have advanced fibrosis or cirrhosis. Development of more specific protease inhibitors and combining protease inhibitors with (new) nucleoside polymerase inhibitors could further improve antiviral treatment [78,79]. Further research will have to determine the effect and side-effects of such treatment strategies, both in 'regular' HCV patients and in HCV patients with inherited bleeding disorders.

An emerging question is whether we should monitor patients with chronic HCV to assess the extent and progression of fibrosis and recommend antiviral treatment based on the results of this monitoring, or recommend treatment to all patients regardless of the extent of fibrosis, especially once better treatment modalities become available. Because antiviral treatment has so many side-effects, and patients are reluctant to start, it is, in any case, important to be able to select those patients in whom this treatment is strongly indicated. At the moment, antiviral treatment is generally recommended for all patients with HCV genotypes 2 or 3, because of the high success rates in these subgroups. In patients with other genotypes, treatment is often postponed in the absence of clinical symptoms of liver disease. Unfortunately, most patients with inherited bleeding disorders are infected with HCV genotype 1. To be able to determine in which of these patients antiviral treatment is strongly recommended, it is important to be able to reliably assess the extent of fibrosis. The fact that HCC screening is indicated in all patients with advanced fibrosis or cirrhosis, even after successful antiviral treatment, also emphasises the need for continuous and reliable monitoring of the extent of liver damage.

Liver stiffness measurement

Correctly assessing the extent and progression of liver fibrosis in HCV infected haemophilia patients is a challenge. Liver biopsies are relatively contra-indicated because of possible complications and are expensive because of the need for clotting factor correction during and after the procedure. Moreover, they are not an ideal gold standard, because of a large risk of sampling error and high intra- and inter-observer variability [80-82]. Liver stiffness measurement (LSM) using transient elastography (TE) is a non-invasive alternative, which assesses the extent of liver damage by measuring the elasticity or stiffness of the liver with a Fibroscan® device [83-85]. Liver stiffness measurements are patient-friendly and can be performed quickly, in an outpatient setting, without the need for clotting factor correction. LSM results are reported to be strongly correlated with both liver biopsy results [85,86] and clinical outcomes [87,88]. According to a meta-analysis of 50 studies, LSM was excellent at distinguishing cirrhosis from non-cirrhosis, but slightly less accurate for diagnosing clinically significant fibrosis [89]. Degos et al reported that LSM was sensitive enough to diagnose stage F2 (moderate fibrosis) for determining treatment indication and stage F4 (cirrhosis) for prognostic purpose [90].

Several studies on the use of LSM in HCV infected patients with inherited bleeding disorders have been published during the past few years, but so far they have only described the cross-sectional use of LSM [91-93]. We performed serial LSM, with a median interval of 3.6 years, in 123 patients with chronic HCV and inherited bleeding disorders, 39 of whom underwent successful antiviral treatment. Our results show that LSM results strongly decrease after successful treatment, especially during the first few years after cessation of treatment, and that successful treatment has a lasting beneficial effect on the liver. To our knowledge, this was the first and thus far only study to assess the long-term effect of successful antiviral treatment on the liver using LSM. The improvement that was seen in LSM results shortly after successful antiviral treatment will probably, at least partially, reflect a reduction in inflammation of the liver, while the long-term effect represents reduction and later stabilisation of the extent of fibrosis after eradication of the hepatitis C virus. The lack of long-term adverse liver-related outcomes we found in our international cohort of HCV infected patients with inherited bleeding disorders after successful antiviral treatment is in accordance with the low median LSM result in our group of patients with longitudinal LSM assessment.

The large improvement in LSM results we observed emphasises the beneficial effect of successful antiviral treatment on the liver. These results could be used to help convince patients of the benefits of starting antiviral treatment, despite the many side-effects. Our study also showed that LSM is a useful tool for the assessment and follow-up of the extent of liver fibrosis after successful antiviral treatment.

Our attempt to use serial LSM as a follow-up tool in patients with inherited bleeding disorders who were not (successfully) treated for their chronic HCV infection met with some difficulties. Unexpected and unexplained improvement of LSM results was seen in some of the untreated patients. Similar improvements were also reported in several studies using liver biopsies for follow-up [94-96] and also in untreated hepatitis B patients who underwent serial LSM with an interval of 3 years [97]. These improvements could have been the result of variation in the extent of damage across the liver, natural changes in the liver parenchyma caused by for example fluctuations in HCV viral load or use of specific medication, or limitations of both LSM and liver biopsy. Whatever the cause, these unexpected findings make interpretation of the results of serial liver stiffness measurements difficult. The fact that unexpected improvements are not uniquely seen in LSM, but also in serial liver biopsies, does suggest that LSM could be used as a reliable substitute for biopsies. Results of third liver stiffness measurements are being collected in our patients, to assess the direction and effect of fluctuations in LSM results over time. These third measurements will hopefully also help us determine the ideal interval between serial liver stiffness measurements to monitor fibrosis progression. Lack of a specific cut-off point above which a change in LSM results has to be considered clinically relevant also complicates interpretation of serial LSM. It might be appropriate to use a 30% change in LSM result as the cut-off point for clinical relevance, since the manufacturer considers IQR fluctuations of up to 30% of the median result acceptable measurement variation [98]. Results indicating liver cirrhosis (stage F4) should of course always be taken seriously, as should large changes in LSM results. In patients with such results, who did not undergo optimal antiviral treatment, this treatment should be strongly recommended. In patients with stable LSM results over time, treatment could be postponed until new and better treatment modalities are available.

There are several factors influencing LSM results, such as inflammation and steatosis, which should be taken into account in future studies on this subject [99-102]. Zelber-Sagi et al suggested more reliable LSM results could be obtained by performing liver stiffness measurements at different sites and using the average result for treatment decisions and/or longitudinal evaluation [103]. A limitation of LSM is that it is difficult to acquire reliable results in obese patients with the original Fibroscan® M probe. A new XL probe has now been designed specifically for obese patients. Diagnostic accuracy of both probes is reported to be similar, but a subgroup of obese patients remains in which even the XL probe did not yield reliable results [104].

Other non-invasive methods to assess liver damage

Several non-invasive methods for assessing the extent and progression of fibrosis using only laboratory parameters have been developed. Indirect fibrosis tests include

measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), platelet count, AST/platelet ratio index (APRI) and several serum proteins. Direct fibrosis tests measure serum levels of extracellular matrix proteins reflecting the balance between fibrogenesis and fibrinolysis. FibroTest® combines total bilirubin, haptoglobin, gamma glutamyl transpeptidase (gamma-GT), alpha2-macroglobulin, apolipoprotein A, age and gender and is reported to be quite accurate in diagnosing cirrhosis, but less so in distinguishing intermediate fibrosis stages [105,106]. The Enhanced Liver Fibrosis (ELF) test combines measurement of the extracellular matrix proteins hyaluronic acid (HA), aminoterminal propeptide of type III procollagen (PIIINP) and tissue inhibitor of metalloproteinase 1 (TIMP-1) in serum. The ELF-score is calculated by combining these measurements in an algorithm [107,108]. The accuracy of ELF was similar to that of TE in a small cohort of German patients [107], and ELF has been reported to predict liver-related morbidity and mortality at least as well as liver biopsies [108]. A study evaluating the ELF-score, comparing its results with LSM results, other laboratory parameters and abdominal ultrasound examination results in HCV infected haemophilia patients, has recently been started in our treatment centre. A limitation of both FibroTest® and ELF is that measurement of their components needs to be validated and can often only be performed in specialised reference laboratories.

Magnetic resonance elastography uses MRI technology to image propagating waves in the liver and create so called elastograms, depicting tissue stiffness. It has the advantage of sampling the entire volume of the liver, apparently without being limited by obesity or narrow intercostals spaces, but the disadvantage of high costs and limited availability [109,110]. New non-invasive imaging modalities combining direct visualisation of the liver parenchyma with liver stiffness measurement include Acoustic Radiation Force Impulse imaging (ARFI, also using shear wave elastography) and Real-time Tissue Elastography (RTE, using strain tissue elastography). In a recent study, both transient elastography with Fibroscan® and ARFI had high diagnostic accuracy in the prediction of cirrhosis, but TE performed better when diagnosing significant fibrosis. The performance of RTE was inferior to that of the other two modalities [111].

Genetic factors influencing fibrosis development and progression can also be used as non-invasive markers of fibrosis. A cirrhosis risk score using seven single nucleotide polymorphisms (SNPs) to predict fibrosis progression and development of cirrhosis was validated as a predictor of fibrosis progression and cirrhosis, but it was not predictive of clinical outcome [112,113].

Several studies have reported improved diagnostic accuracy when combining LSM with other non-invasive tools than for LSM alone [114-117], especially for the detection of

significant fibrosis rather than cirrhosis. A combination of LSM, AST/ALT ratio and Model for End-Stage Liver Disease (MELD) score had a predictive accuracy of > 93% for clinical outcome [87]. Castéra et al proposed an algorithm in which Fibroscan® and FibroTest® are both performed to assess the extent of fibrosis and liver biopsies are only indicated when results of these two tests are discordant or in case of LSM failure, thus reducing the need for liver biopsies in a large proportion of patients [118]. The feasibility of this method in patients with inherited bleeding disorders, in whom liver biopsies are preferably avoided altogether, is probably limited. Further research will have to determine which is the most efficient, reliable and cost-effective way of combining available non-invasive imaging and laboratory modalities.

Course and complications of HIV infection

In The Netherlands, a relatively small proportion of haemophilia patients was infected with HIV in the 1980s (16% of the patients in our centre were infected, compared with 30 to 53% in various other European countries) [31,119-122]. Nevertheless, HIV and AIDS have had a very large impact on the haemophilia population. Of the 60 HIV infected haemophilia patients known at the Van Creveldkliniek, 27 (45%) developed AIDS, mostly during the early 1990s, and 31 (52%) died. Death was reported to be solely or partly AIDS-related in 71% of these patients. After the introduction of HAART in 1996, development of AIDS and AIDS-related deaths strongly declined. Nowadays, most HIV positive patients are on HAART, and the few patients who are not usually spontaneously maintain adequate CD4 counts and low HIV RNA levels. The down side of the use of HAART are reports of dyslipidemia, insulin resistance/diabetes mellitus and an increased risk of myocardial infarction [123-128]. In our small cohort of HIV infected haemophilia patients on HAART no myocardial infarctions occurred at all, but one case of unstable angina pectoris was reported. Any increased risk of myocardial infarction in these patients might be (partly) counterbalanced by a protective effect of very low clotting factor levels against ischemic cardiovascular disease (see the section on cardiovascular disease above). Total and HDL cholesterol levels were lower in our living HIV positive haemophilia patients who were on HAART than in the general age-matched male population, which was in accordance with the lower cholesterol levels we found in haemophilia patients in our prospective CVD study cohort. We did find an increased prevalence of diabetes mellitus in the HIV positive patients on HAART compared with the general population and high triglyceride levels, which may reflect actual side-effects of the HAART medication. Regular follow-up and treatment of these problems are warranted. Although based on small patient numbers, we also observed a significantly higher cumulative incidence of spontaneous intracranial bleeding in HIV positive haemophilia patients on HAART than in HIV negative patients with severe haemophilia. Several case reports have been published on the occurrence of intracranial

bleeding in haemophilia patients who were using HAART regimens containing protease inhibitors [129-134], but no systematic assessments were made. A possible cause of the increased bleeding tendency could be an effect of protease inhibitors on platelet function, the consequences of which are obviously most pronounced in patients with inherited bleeding disorders [132]. Physicians treating haemophilia patients should be aware of a possible increased risk of spontaneous intracranial bleeding in patients using HAART. Whether haemophilia patients should preferably be switched to HAART regimens without protease inhibitors is still under debate. Because the design of our study did not allow us to assess whether there was an increase in other severe bleeding complications in HIV positive haemophilia patients on HAART, this should be the subject of further study in larger cohorts.

Concluding remarks

The impact of haemophilia is changing over time. Fortunately, most young haemophilia patients will not have to deal with the debilitating effects of severe arthropathy, and new infections with hepatitis C or HIV no longer occur. The down side of the increased life expectancy for these patients is the occurrence of more age-related co-morbidity. The evidence that severe haemophilia could have a protective effect on the occurrence of ischemic cardiovascular disease is increasing, but the results of our large prospective CVD study will have to be awaited before we can draw firm conclusions on this subject. The impact of chronic hepatitis C infection is becoming more apparent, now that patients have been infected for over four decades. Antiviral treatment has many side-effects, but, when successful, strongly reduces morbidity and mortality in HCV infected patients. Liver stiffness measurements can be used to monitor the extent of liver fibrosis over time and guide treatment decisions, but there is still some debate about the correct interpretation of its results and optimal cut-off values. Very high LSM results, or large changes in these results, should always prompt further investigation or initiation of treatment. New treatment modalities will hopefully increase the proportion of patients in whom treatment is successful.

The impact of HIV infection has been reduced by the introduction of HAART, but this treatment can have severe side-effects. Haemophilia treaters should be aware of a higher prevalence of diabetes and hypertriglyceridemia in patients using HAART, and a possible increased risk of spontaneous intracranial bleeding in patients who are on HAART regimens containing protease inhibitors.

Research in this very interesting population of ageing haemophilia patients will have to continue to keep gaining more insight in the occurrence and burden of co-morbidity. Over time, the focus will shift from virus-related co-morbidity to age-related conditions.

Fortunately, many haemophilia patients are very dedicated to both their treatment and their haemophilia treatment centre. Time and again they have shown themselves willing to participate in research projects. Hopefully, they will continue to do so in the future. Without them, studies on co-morbidity would be both impossible and pointless.

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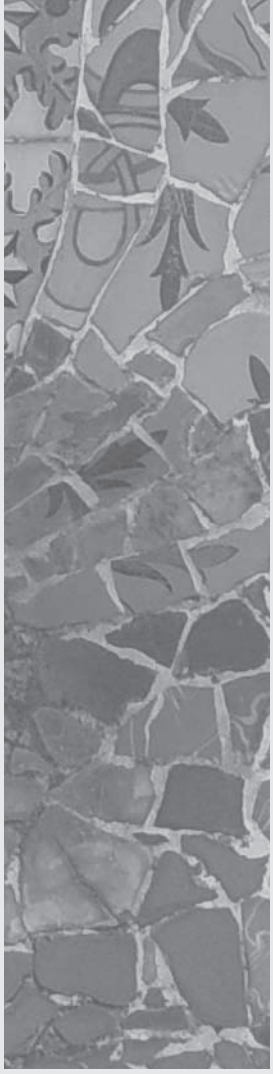
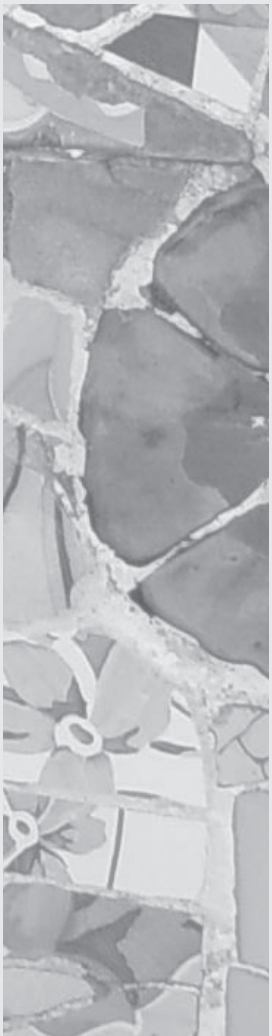
Summary





This thesis describes various aspects of age-related and virus-related co-morbidity in adult haemophilia patients.

Part I. Age-related co-morbidity

Life-expectancy of haemophilia patients has increased significantly due to the availability of treatment with clotting factor concentrates. As a consequence, patients are increasingly confronted with age-related health problems. **Chapter 2** provides a literature overview of what was known in 2009 about various types of haemophilia-related (such as arthropathy and inhibitor development), virus-related (hepatitis C (HCV) and HIV) and age-related co-morbidity in ageing haemophilia patients. The mortality from ischemic cardiovascular disease appeared to be lower in haemophilia patients than in the general population. A possible explanation for this could be that haemophilia patients have a decreased tendency to form occlusive arterial thrombi. Reports on the occurrence of cardiovascular disease (CVD) in haemophilia patients were, however, mainly based on mortality studies, while data on non-fatal cardiovascular events were lacking. Reports on the prevalences of CVD risk factors were conflicting. There might be an increased prevalence of hypertension in haemophilia patients, while the prevalences of other risk factors such as smoking, hypercholesterolemia, obesity and diabetes mellitus appeared to be similar to those in the general population. These results are, however, based on relatively small studies, some of which had methodological flaws. In contrast to ischemic cardiovascular events, the occurrence of intracranial bleeding is reported to be increased in haemophilia patients. Except for HCV-related hepatocellular carcinoma, mortality rates for malignancies are reported to be similar in haemophilia patients and the general population.

Chapter 3 describes the results of a retrospective study assessing medical records of 408 haemophilia patients born before 1971, who were treated between 1985 and 2010 at the Van Creveldkliniek at the University Medical Center Utrecht. The aim of this study was to compare the occurrence of both fatal and non-fatal co-morbidity in these haemophilia patients with the general age-matched male population. Hepatitis C infection was present in 56% of patients and HIV infection in 12%. Of the 408 patients, 78 (19%) were deceased in 2010. Main causes of death were malignancies, AIDS, hepatitis C and intracranial bleeding. As expected, intracranial bleeding occurred significantly more often in haemophilia patients than in the general population. A total of 11 myocardial infarctions were reported in 10 patients, none of which were fatal. The cumulative incidence of myocardial infarction was significantly lower in patients with severe haemophilia than in the general population (0.5 versus 4.8%). This cumulative incidence was, however, not reduced in patients with non-severe haemophilia (4.4%). The cumulative incidence of ischemic stroke was also lowest in patients with severe haemophilia, suggesting that very low levels of clotting factor VIII or IX have a protective effect against the

development of ischemic CVD. The cumulative incidence of angina pectoris was, on the other hand, similar in haemophilia patients and the general population, suggesting that low clotting factor levels are mainly protective against the formation of occlusive thrombi, and less so against the development of atherosclerosis. Hepatocellular carcinoma (HCC) occurred significantly more often in haemophilia patients than in the general population, and was only reported in patients with chronic HCV infection. The cumulative incidences of other types of malignancies and the prevalences of other types of co-morbidity such as diabetes mellitus, obesity, renal failure, cataract and prostate problems were similar in haemophilia patients and the general population.

Because prospective data collection yields the most reliable results, a prospective study on CVD occurrence in haemophilia patients was started in 2009 in the Van Creveldkliniek, in collaboration with haemophilia treatment centres in Sheffield, London, Glasgow, Cardiff and Groningen. Between 2009 and 2011, 709 haemophilia patients aged 30 years or older were included in this CVD study. They will be followed for 10 years to assess CVD occurrence. In **chapter 4**, the history of CVD events in these patients is described. Like in the retrospective study, the lowest number of ischemic cardiovascular events was reported in patients with severe haemophilia. The difference in the occurrence of CVD events between haemophilia patients and the general population was, however, not statistically significant in this study and only a trend towards a lower cumulative incidence in patients with severe haemophilia was shown. The occurrence of angina pectoris was similar in haemophilia patients and the general population in this study as well. The main difference with the study described in chapter 3 was that the CVD study included living patients only, thus avoiding any competing risks of dying at a relatively young age from, for example, AIDS or severe bleeding complications. The results of the CVD study therefore better reflect the occurrence of cardiovascular events in the population of living haemophilia patients who are currently treated at the haemophilia treatment centres. These results are, however, still based on retrospective data.

In **chapter 5**, the prevalences of CVD risk factors in the 709 patients participating in the prospective CVD study were assessed cross-sectionally at the time of inclusion in the study. The QRISK®2 score was used to calculate CVD risk profiles. This score estimates the risk of developing a heart attack or stroke within a period of 10 years using a number of CVD risk factors, and also provides a comparison risk for the general population. The prevalence of hypertension was significantly higher in haemophilia patients than in the age-matched general male population (49% versus 40%). The prevalences of obesity and hypercholesterolemia were lower in haemophilia patients than in the general population (15% versus 20% and 44% versus 68%, respectively), while those of

diabetes mellitus and smoking were similar. Because the QRISK®2 score had not been validated in Dutch patients, its characteristics were compared with those of the wider validated, but much more limited SCORE algorithm. The correlation between the two algorithms was very high and the results were comparable. The 10-year QRISK®2 risk was significantly higher in the haemophilia patients than in the general population (8.9 versus 6.7%), indicating more unfavourable CVD risk profiles in haemophilia patients. The increased CVD risk became apparent after the age of 40 years. Screening for and treatment of CVD risk factors should be an integral part of haemophilia care. In the prospective part of the study, the observed occurrence of CVD events after 10 years will be compared with the expected occurrence based on the QRISK®2 score to determine whether haemophilia indeed has a protective effect on CVD occurrence.

In **chapter 6**, the increased prevalence of hypertension in the haemophilia patients participating in the CVD study is described in more detail. Hypertension occurred significantly more often in patients with severe haemophilia than in patients with non-severe disease. The hypothesis that hypertension in these patients could be related to recurrent renal bleeding and/or the effect of HCV or HIV or their treatments on the kidneys could not be confirmed in our study, since there was no association between hypertension and renal function (represented by creatinin levels) and only a trend towards an association between hypertension and a reported history of renal bleeding. There was, however, an association between hypertension and age and overweight/obesity, but this is not specific for haemophilia patients. The cause of the increased prevalence of hypertension we found in our haemophilia patients therefore remains largely unknown. A possible explanation could be the close surveillance of haemophilia patients at their haemophilia treatment centres, resulting in early detection and treatment of hypertension. Further studies are needed to determine whether there are any other haemophilia-related factors influencing the development of hypertension.

Part II. Virus-related co-morbidity

Chronic hepatitis C infection is an important co-morbidity in adult haemophilia patients, since many patients were infected due to the use of contaminated clotting factor products. To ensure optimal treatment and follow-up, knowledge on the course of HCV infection in these patients is essential. **Chapter 7** describes over 30 years of follow-up of a group of 863 HCV infected patients from the Van Creveldkliniek and haemophilia treatment centres in Sheffield and London. Of all infected patients, 19% spontaneously cleared the virus, while 81% developed chronic HCV infection. Of the chronically infected patients, 361 (52%) underwent antiviral treatment, which was successful in 53%. End-stage liver disease (ESLD, including decompensated cirrhosis and hepatocellular

carcinoma) was diagnosed in 13% of patients who had chronic HCV infection. ESLD was hardly present at all in patients who spontaneously cleared the virus or underwent successful antiviral treatment. Risk factors for the development of ESLD were higher age at HCV infection, co-infection with HIV and alcohol abuse. Hepatocellular carcinoma was present in 22 patients (3%) and appeared to be an increasing problem, since many cases were diagnosed within the past six years. This emphasises the importance of careful follow-up and timely antiviral treatment to avoid severe complications. Antiviral treatment, however, also has its down side.

In **chapter 8**, side-effects of antiviral treatment with pegylated interferon and ribavirin in 47 patients with inherited bleeding disorders and chronic HCV are described. Antiviral treatment was successful in 66% of these patients. Important side-effects were fatigue, headache, pruritus and skin rash, concentration problems, decreased appetite, fever, irritability, hair loss, depression, weight loss and anaemia. Most side-effects disappeared within a few weeks after cessation of antiviral treatment, but fatigue, concentration problems and sleeping problems were still present in more than 30% of patients one month after the end of treatment. In 45% of patients, dose reduction of antiviral treatment was indicated, mainly because of anaemia and/or weight loss. Premature cessation of treatment due to side-effects, however, occurred in only two patients. Health-related quality of life decreased significantly during antiviral treatment, but increased again within a few weeks after cessation of treatment. No association was found between the severity of side-effects and treatment effect.

In **chapter 9**, the occurrence of depression during antiviral treatment in these patients is described in more detail. Depression occurred in 55% of patients who did not have significant depressive symptoms at baseline. A major risk factor for the development of depression was a history of depression or other psychiatric disorders. Patients who have such a history should be monitored very closely during antiviral treatment and treatment with antidepressants should be started early, perhaps even before starting antiviral therapy. The association we found between the occurrence of depression and a reduction in quality of life during antiviral treatment emphasises the importance of early recognition and treatment of depression, to maintain quality of life at as high a level as possible.

Assessing the extent of liver damage in patients with chronic HCV to guide treatment decisions and determine prognosis is very important. Liver stiffness measurement (LSM) using a Fibroscan® device is a non-invasive method to assess the extent of fibrosis. It could be a valuable alternative to liver biopsies, avoiding the complication risk and high cost of treatment with clotting factor concentrates associated with biopsies. Because LSM is a relatively new method, little was known on how to use it and what its

reliability is, especially in patients with inherited bleeding disorders. In **chapter 10**, we describe our experience with LSM in 84 patients with inherited bleeding disorders and chronic HCV who were not, or not successfully treated for their HCV infection. These patients underwent liver stiffness measurements in 2005 (LSM 1) and 2009 (LSM 2). The median interval between the two measurements was 3.7 years. Median results of LSM 1 and LSM 2 were similar and quite low (7.3 kPa at LSM 1 and 6.6 kPa at LSM 2). On an individual level, progression of fibrosis (increase in LSM results of more than 2 kPa) was seen in 16% of patients, while 52% remained stable. In 32% of patients, on the other hand, unexpected improvement of LSM results was seen, which could not be explained in a number of these patients. Similar improvements are, however, also reported in studies with serial liver biopsies. A careful conclusion of this study is that LSM could be used as an alternative to liver biopsies, especially in patients with inherited bleeding disorders, but the correct interpretation of the results can be challenging in individual patients. Results of third liver stiffness measurements will be collected for our patients to further investigate the longitudinal use of LSM.

In **chapter 11**, LSM was used to assess the effect of successful antiviral treatment on the liver in 39 patients with inherited bleeding disorders. Some of these patients underwent successful antiviral treatment before the first liver stiffness measurement (LSM 1), while in the remainder successful treatment was administered between LSM 1 and LSM 2. In the first group, the results of LSM 1 and LSM 2 were stable and low. In the second group, a large improvement in LSM results was seen between LSM 1 and LSM 2, shortly after successful treatment. These results indicate that successful antiviral treatment has a beneficial and lasting effect on the extent of liver damage and could be used to convince patients of the benefits of antiviral treatment.

In **chapter 12**, over 25 years of follow-up are described of the 60 HIV positive haemophilia patients who were treated at the Van Creveldkliniek. In 2010, 27 of these patients (45%) had developed AIDS and 31 (52%) were deceased. Death was AIDS-related in 71% of these patients. After the introduction of highly active antiretroviral treatment (HAART) in the 1990s, AIDS developed in only a few patients, highlighting the beneficial effect of this treatment. HAART can, however, also have side-effects. Of the 27 HIV positive patients who were still alive and treated at the Van Creveldkliniek in 2010, 25 (93%) used HAART. The prevalences of hypertension and diabetes mellitus were higher in these patients than in the general age-matched male population, while those of overweight/obesity and hypercholesterolemia were lower. Hypertriglyceridemia was present in 60%. An increased prevalence of hypertension, lower cholesterol levels and lower weight are also seen in HIV negative haemophilia patients, but diabetes and hypertriglyceridemia are probably side-effects of HAART. The occurrence of spontaneous

intracranial bleeding was significantly higher in haemophilia patients on HAART than in HIV negative patients with severe haemophilia, indicating that an increased risk of intracranial bleeding might also be an important side-effect of HAART in this patient group, with potentially severe consequences. An increased risk of ischemic cardiovascular events has been reported as a severe side-effect of HAART, but in our small study cohort of HIV positive haemophilia patients using HAART such events were very rare. This could be (partly) due to the possible protective effect of low clotting factor levels on CVD occurrence.

Conclusions

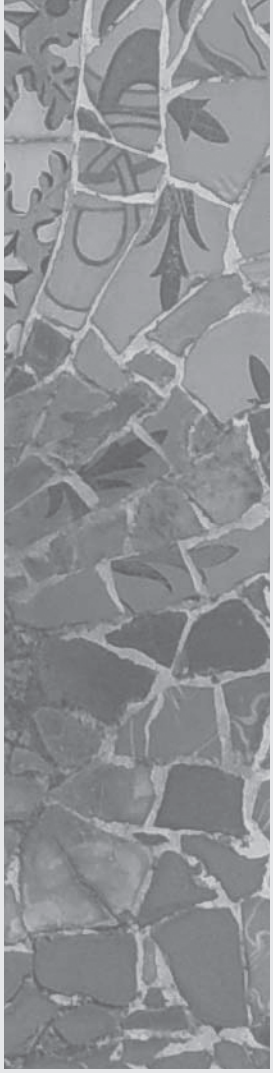
The problems haemophilia patients are facing have changed during the past decades. Young haemophilia patients are no longer confronted with severe arthropathy and new infections with hepatitis C or HIV do not occur. Due to the availability of clotting factor concentrates, life expectancy of haemophilia patients has increased significantly. As a result, the prevalence of age-related co-morbidity is increasing as well. Severe haemophilia appears to have a protective effect against the occurrence of ischemic cardiovascular disease. The results of our prospective CVD study will have to confirm this. Hypertension appears to be more prevalent in haemophilia patients than in the general population and the overall CVD risk profiles are more unfavourable. If the occurrence of ischemic cardiovascular events is indeed lower in haemophilia patients, in spite of their more unfavourable risk profiles, this would suggest a large protective effect of (very) low levels of clotting factor VIII or IX on CVD risk. Screening for CVD risk factors should be an integral part of care in all haemophilia treatment centres.

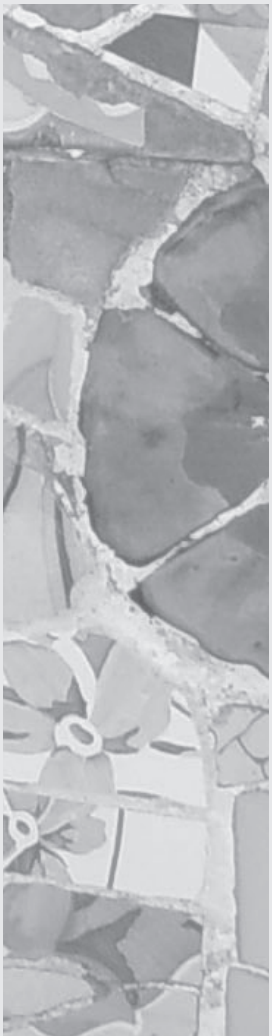
The impact of chronic HCV infection has become clearer during the past years, in which the number of patients developing complications appeared to be increasing. Antiviral treatment has many side-effects, but, when successful, has a strong and lasting beneficial effect on the extent of liver damage. Liver stiffness measurements using Fibroscan® can be used in patients with inherited bleeding disorders to monitor fibrosis progression and guide treatment decisions. The interpretation of LSM results can, however, be difficult in some cases. The new treatment regimens which have recently become available will, hopefully, further increase the success rate of antiviral therapy.

The impact of HIV infection has decreased strongly after the introduction of HAART, but HAART can have significant side-effects, such as the development of diabetes mellitus and possibly and increased risk of spontaneous intracranial bleeding.

Further studies will have to shed more light on the occurrence and impact of various types of co-morbidity in this very interesting patient population.

Samenvatting





Achtergrond

In het menselijk lichaam is de bloedstolling geregeld via de zogenoemde 'stollingscascade'. Hierin werken een groot aantal stollingsfactoren en andere stoffen samen om te zorgen voor adequate bloedstolling bij bijvoorbeeld een verwonding, en het uitblijven van bloedstolling in de normale situatie. Het evenwicht tussen bloeden en bloedstolling is nauwkeurig geregeld.

Hemofilie is een erfelijke stollingsstoornis, waarbij er een afwijking is in de aanmaak van stollingsfactor VIII (bij hemofilie A) of stollingsfactor IX (bij hemofilie B). Door een DNA-afwijking in respectievelijk het factor 8 of factor 9 gen, wordt er te weinig of helemaal niets van deze stollingsfactoren aangemaakt. Het factor 8 en het factor 9 gen liggen beiden op het X-chromosoom. Het X-chromosoom is één van de twee geslachtschromosomen die bij mensen voorkomen. Vrouwen hebben twee X-chromosomen en mannen hebben één X- en één Y-chromosoom. Een afwijking op één van de twee X-chromosomen, bijvoorbeeld in het factor 8 of factor 9 gen, zal bij vrouwen in principe niet tot problemen leiden, omdat op het andere X-chromosoom een normaal gen ligt. Dit normale gen kan zorgen voor voldoende stollingsfactorproductie. Mannen hebben echter maar één X-chromosoom. Bij hen zal een afwijking in het factor 8 of 9 gen tot verschijnselen leiden, omdat deze niet gecompenseerd kan worden door een tweede gen. Dit is de reden dat vrijwel alle hemofiliepatiënten van het mannelijk geslacht zijn.

Er wordt onderscheid gemaakt tussen ernstige, matig ernstige en milde hemofilie. Bij ernstige hemofilie wordt er (vrijwel) geen stollingsfactor VIII of IX gemaakt (minder dan 1% van de normale hoeveelheid). Bij matig ernstige en milde hemofilie is de hoeveelheid stollingsfactor respectievelijk 1-5% en 6-40% van normaal. Een hoeveelheid stollingsfactor van meer dan 40% is voldoende voor een normale bloedstolling. Het gevolg van de verminderde hoeveelheid stollingsfactor is dat hemofiliepatiënten een verhoogde bloedingsneiging hebben. Bij patiënten met ernstige hemofilie worden spontane bloedingen gezien, vooral in spieren en gewrichten, maar ook op andere plekken in het lichaam. Als er regelmatig bloedingen in gewrichten optreden kan er blijvende gewrichtsschade ontstaan, die vaak leidt tot ernstige invaliditeit. Bij matig ernstige en milde hemofilie komen minder spontane bloedingen voor, maar is er wel een verhoogde kans op (ernstige) bloedingen bij bijvoorbeeld (kleine) verwondingen en operaties.

Tot halverwege de jaren zestig van de vorige eeuw bestond de behandeling van bloedingen bij hemofiliepatiënten uit het fixeren van het aangedane lichaamsdeel met bijvoorbeeld gips of spalken, in combinatie met langdurige bedrust. Indien nodig werden bloedtransfusies gegeven. Later werd behandeling met stollingsfactorconcentraten mogelijk. Deze concentraten kunnen zowel 'profylactisch' (een aantal keren per week, om bloedingen te voorkomen) als 'on demand' (op het moment van een (begin-

nende) bloeding) toegediend worden. De gebruikte stollingsfactorconcentraten werden oorspronkelijk gemaakt uit bloed van bloeddonoren. Tegenwoordig kunnen ze ook kunstmatig gemaakt worden.

Sinds de invoering van betere behandelmethodes is de levensverwachting van hemofiliepatiënten sterk toegenomen. Dit heeft tot gevolg dat deze patiënten steeds vaker te maken krijgen met 'gewone' leeftijdsgerelateerde problemen, zoals bijvoorbeeld hart- en vaatziekten en kanker.

Helaas bleek in het verleden dat een deel van de stollingsfactorconcentraten die gebruikt werden voor de behandeling van hemofiliepatiënten besmet waren met het hepatitis C virus (HCV). In een deel van de gevallen waren ze ook besmet met HIV (het humaan immunodeficiëntie virus dat AIDS veroorzaakt). Een groot deel van de Nederlandse hemofiliepatiënten is zo geïnfecteerd geraakt met deze virussen. Gelukkig zijn de stollingsfactorproducten sinds 1985 vrij van HIV en sinds 1992 ook van hepatitis C. Sindsdien zijn er geen nieuwe infecties meer opgetreden. Vóór het beschikbaar komen van de huidige HIV-behandeling in de vorm van 'higly active antiretroviral treatment' (HAART) in 1996, is een groot aantal met HIV geïnfecteerde patiënten overleden aan AIDS. De patiënten die deze periode overleefd hebben worden tegenwoordig behandeld met HAART, waardoor ze een relatief normaal leven kunnen leiden. De HAART-medicatie heeft echter ook een aantal bijwerkingen.

Ongeveer 20% van de patiënten die geïnfecteerd zijn geraakt met HCV bleek in staat te zijn om het virus zelf op te ruimen (dit noemen we 'spontane klaring' van het virus). De meeste geïnfecteerde patiënten hebben echter een chronische hepatitis C infectie ontwikkeld. Zo'n infectie kan tientallen jaren aanwezig zijn in de lever zonder dat er klachten zijn. Geleidelijk ontstaat er bij de meeste patiënten littekenvorming (fibrose) in het leverweefsel, leidend tot ernstige leverschade (cirrose) en een verminderde werking van de lever. Dit kan leiden tot leverfalen en een hoge kans om leverkanker te ontwikkelen. De snelheid waarmee dergelijke complicaties ontstaan verschilt per patiënt. De behandeling van een chronische hepatitis C infectie bestond tot voor kort uit een combinatie van twee medicijnen (gepegyleerd interferon (PegIFN) en ribavirine), gedurende een periode van 24 of 48 weken. Inmiddels is er een derde medicijn, een zogenoemde 'proteaseremmer' aan de behandeling toegevoegd. Deze nieuwe behandeling is echter nog niet meegenomen in de studies in dit proefschrift. Het doel van antivirale behandeling is het opruimen van het hepatitis C virus, om het ontstaan of verergeren van leverschade te voorkomen en de lever de kans te geven zich (gedeeltelijk) te herstellen. Deze behandeling heeft veel bijwerkingen en is helaas niet bij iedereen succesvol. Dit maakt dat veel patiënten twijfelen over of ze wel aan deze behandeling moeten

beginnen. Om het juiste moment voor het starten van behandeling te bepalen is het belangrijk bij een individuele patiënt vast te stellen hoeveel leverschade er is en of deze schade toeneemt. De standaard methode hiervoor is de leverbiopsie. Hierbij wordt met een naald een klein stukje van de lever weggenomen dat onderzocht kan worden onder een microscoop. Deze methode heeft een aantal beperkingen en vanwege de kans op ernstige (bloedings)complicaties worden biopsieën bij hemofiliepatiënten liever niet gedaan.

Een alternatief voor leverbiopsie is het meten van de stijfheid van de lever (de zogenoemde 'liver stiffness measurement' of LSM) met behulp van een Fibroscan® apparaat. Dit is een soort echo-apparaat, dat ter hoogte van de lever op de huid gezet wordt en de stijfheid of elasticiteit van de lever meet met behulp van bewegingsgolven. Een hogere uitslag (uitgedrukt in kilopascals of kPa) wijst op een hogere stijfheid van de lever en meer leverschade. Dit onderzoek is snel, makkelijk en niet invasief en lijkt betrouwbare resultaten te geven. Omdat LSM nog niet zo lang beschikbaar is, was er weinig bekend over het beste gebruik hiervan op de lange termijn.

Gezondheidsproblemen die niet gerelateerd zijn aan de belangrijkste ziekte die iemand heeft, in dit geval hemofilie, noemen we co-morbiditeit. Dit proefschrift beschrijft een aantal studies naar verschillende vormen van leeftijdsgerelateerde en virusgerelateerde co-morbiditeit bij volwassen hemofiliepatiënten.

Deel I. Leeftijdsgerelateerde co-morbiditeit

Zoals hierboven beschreven hebben hemofiliepatiënten een betere levensverwachting dan vroeger, zeker als ze niet geïnfecteerd geraakt zijn met HIV of hepatitis C. Hierdoor komen leeftijdsgerelateerde gezondheidsproblemen steeds vaker voor bij deze patiënten. Omdat patiënten vroeger over het algemeen niet oud genoeg werden om dergelijke problemen te krijgen, is er in het verleden nauwelijks onderzoek gedaan om te kijken of er verschillen zijn in het vóórkomen van dit soort aandoeningen tussen hemofiliepatiënten en mensen die geen hemofilie hebben. Inmiddels is dergelijk onderzoek wel mogelijk.

Hoofdstuk 2 geeft een overzicht van wat er in 2009 bekend was in de medische literatuur over verschillende vormen van hemofiliegerelateerde (zoals bijvoorbeeld gewrichtsklachten en antistoffen tegen factor VIII of IX), virusgerelateerde (HCV en HIV) en leeftijdsgerelateerde co-morbiditeit bij ouder wordende hemofiliepatiënten. In dit overzichtsartikel wordt onder andere beschreven dat hemofiliepatiënten minder vaak lijken te overlijden aan hart- en vaatziekten, zoals een hartinfarct of een herseninfarct, dan mannen zonder hemofilie. Een mogelijke verklaring hiervoor is dat, bij lage

hoeveelheden van stollingsfactor VIII of IX in het lichaam, de kans kleiner is dat er een bloedstolsel ontstaat dat een bloedvat rond het hart of in de hersenen volledig af kan sluiten. Hersenbloedingen, daarentegen, komen vaker voor bij hemofiliepatiënten dan bij mensen zonder hemofilie. In de studies die zijn gedaan werd echter vooral gebruik gemaakt van sterftecijfers. Er was erg weinig bekend over het vóórkomen van niet dodelijke hart- en vaatziekten. Over de aanwezigheid van risicofactoren voor hart- en vaatziekten bij hemofiliepatiënten waren de meningen verdeeld. Een verhoogde bloeddruk komt mogelijk vaker voor dan bij mannen zonder hemofilie. Voor andere risicofactoren als roken, een verhoogd cholesterolgehalte, overgewicht en suikerziekte lijken er geen duidelijke verschillen te zijn. Deze resultaten zijn echter gebaseerd op vrij kleine studies, die soms ook methodologische beperkingen hadden.

Hoofdstuk 3 beschrijft de resultaten van een statusonderzoek, waarbij medische gegevens bekeken zijn van 408 hemofiliepatiënten die geboren zijn voor 1971 en die tussen 1985 en 2010 onder behandeling waren bij de Van Creveldkliniek in het UMC Utrecht. Dit is het grootste behandelcentrum voor hemofiliepatiënten in Nederland. Het doel van dit onderzoek was te inventariseren hoe vaak fatale en niet fatale co-morbiditeit voorkomt in deze groep hemofiliepatiënten en dit voorkomen te vergelijken met dat in de algemene bevolking. Hepatitis C infectie was aanwezig bij 56% van de patiënten en HIV infectie bij 12%. Van de 408 patiënten waren er 78 (19%) overleden in 2010. De belangrijkste doodsoorzaken waren kanker, AIDS, hepatitis C en hersenbloedingen. Zoals verwacht kwamen hersenbloedingen significant vaker voor bij de hemofiliepatiënten dan bij mannen zonder hemofilie. In totaal kwamen er 11 hartinfarcten voor bij 10 patiënten, waarvan er niet een fataal was. Hartinfarcten kwamen significant minder vaak voor bij patiënten met ernstige hemofilie dan in de algemene bevolking (0,5 versus 4,8%), terwijl de frequentie van voorkomen niet verlaagd was bij patiënten met niet ernstige (dus milde of matig ernstige) hemofilie (4,4%). Het vóórkomen van herseninfarcten was ook het laagst bij patiënten met ernstige hemofilie. Dit suggereert dat zeer lage hoeveelheden van stollingsfactor VIII of IX een beschermend effect hebben tegen het optreden van dergelijke hart- en vaatziekten. Angina pectoris ('pijn op de borst') kwam ongeveer even vaak voor bij hemofiliepatiënten als in de algemene bevolking. Dit suggereert dat lage hoeveelheden stollingsfactor inderdaad vooral beschermen tegen de uiteindelijke vorming van een afsluitend bloedstolsel (resulterend in een hart- of herseninfarct) en niet zo zeer tegen aderverkalking (resulterend in pijn op de borst). Leverkanker kwam vaker voor bij de hemofiliepatiënten dan in de algemene bevolking en werd uitsluitend gezien bij patiënten met een chronische hepatitis C infectie. Het vóórkomen van andere vormen van kanker en ook van andere vormen van co-morbiditeit zoals suikerziekte, overgewicht, nierfalen, staar en prostaatklachten was bij hemofiliepatiënten vergelijkbaar met de algemene bevolking. De resultaten van deze

studie zijn gebaseerd op retrospectief (terugkijkend) onderzoek in patiëntendossiers. De meest betrouwbare resultaten komen echter voort uit prospectieve (toekomstgerichte) studies, waarin de verzameling van gegevens gestructureerder kan plaatsvinden en patiënten gevolgd worden in de tijd.

In 2009 is er bij de Van Creveldkliniek, in samenwerking met hemofiliebehandelcentra in Sheffield, Londen, Glasgow, Cardiff en Groningen, een prospectief onderzoek gestart naar het vóórkomen van hart- en vaatziekten bij hemofiliepatiënten. Tussen 2009 en 2011 zijn 709 hemofiliepatiënten van 30 jaar of ouder geïncludeerd in deze CVD ('cardiovascular disease') studie. Zij zullen gedurende een periode van 10 jaar gevolgd worden om te inventariseren hoe vaak hart- en vaatziekten voorkomen in deze groep.
In **hoofdstuk 4** is de voorgeschiedenis van deze patiënten in kaart gebracht. Ook in deze studie werd het laagste aantal hartinfarcten en herseninfarcten gezien bij patiënten met ernstige hemofilie. In dit geval was het verschil tussen deze hemofiliepatiënten en de algemene bevolking in het voorkomen van hartinfarcten echter niet statistisch significant. Het gaat hier slechts om een 'trend' in de richting van een verminderd voorkomen. Het vóórkomen van pijn op de borst was ook in deze studie bij hemofiliepatiënten vergelijkbaar met de algemene bevolking. Het verschil met de studie in hoofdstuk 3 is dat het hier een groep levende hemofiliepatiënten betreft, terwijl in de eerdere studie gegevens gebruikt zijn van zowel levende als overleden patiënten. In tegenstelling tot in de eerdere studie, worden de resultaten nu niet beïnvloed door een hoog risico om op jonge leeftijd te overlijden aan bijvoorbeeld AIDS of ernstige bloedingen. Hierdoor zijn de resultaten beter van toepassing op de patiënten die nu in de hemofilieklinieken behandeld worden. Het gaat echter nog steeds om retrospectieve gegevens.

In **hoofdstuk 5** is gekeken naar de aanwezigheid van risicofactoren voor hart- en vaatziekten bij de 709 deelnemers aan de prospectieve CVD studie op het moment van inclusie in deze studie. Met behulp van de zogenoemde QRISK®2 score zijn cardiovasculaire risicoprofielen berekend. Zo'n risicoprofiel zegt iets over de kans hart- en vaatziekten te ontwikkelen op basis van de aan- of afwezigheid van risicofactoren. De QRISK®2 score berekent de kans om binnen een periode van 10 jaar een hart- of herseninfarct te ontwikkelen aan de hand van een groot aantal risicofactoren en geeft daarbij ook een vergelijkingsrisico voor de algemene bevolking. Verhoogde bloeddruk (hypertensie) bleek significant vaker voor te komen bij de hemofiliepatiënten dan in de algemene bevolking (49% versus 40%). Obesitas (ernstig overgewicht) en een verhoogd cholesterolgehalte kwamen daarentegen minder vaak voor (respectievelijk 15% versus 20% en 44% versus 68%). Er waren geen duidelijke verschillen in het vóórkomen van suikerziekte en roken. Het 10-jaars QRISK®2 risico was significant hoger in de hemofiliepatiënten dan in de algemene bevolking (8,9 versus 6,7%), wat wijst op een

ongunstiger risicoprofiel voor hart- en vaatziekten in de hemofiliepatiënten. Het verhoogde risico werd duidelijk vanaf de leeftijd van 40 jaar. Controle en behandeling van risicofactoren voor hart- en vaatziekten bij hemofiliepatiënten zou een vast onderdeel moeten zijn van het programma in de hemofiliebehandelcentra. In het prospectieve gedeelte van de studie zal het aantal hart- en vaatziekten dat na 10 jaar gerapporteerd wordt vergeleken worden met het 10-jaars QRISK®2 risico om te bepalen of hemofilie, ondanks het ongunstige risicoprofiel, inderdaad een beschermend effect heeft.

In **hoofdstuk 6** wordt het vaker voorkomen van hypertensie bij hemofiliepatiënten, zoals gevonden werd in de CVD studie, verder uitgewerkt. Een verhoogde bloeddruk bleek vaker voor te komen bij patiënten met ernstige dan bij patiënten met niet-ernstige hemofilie. Het vermoeden was dat de verhoogde bloeddruk veroorzaakt zou kunnen worden doordat hemofiliepatiënten relatief vaak nierbloedingen hebben. De nieren spelen namelijk een belangrijke rol bij de regulatie van de bloeddruk. In onze studie werd echter geen verband gevonden tussen verhoogde bloeddruk en de nierfunctie en er was slechts een zwak verband tussen verhoogde bloeddruk en nierbloedingen in de voorgeschiedenis. Wel was er een verband tussen hoge bloeddruk en leeftijd en overgewicht, maar dit is niet specifiek voor hemofiliepatiënten. Onze studie heeft dus niet goed kunnen aantonen waarom hemofiliepatiënten een hogere bloeddruk hebben dan mensen zonder hemofilie. Een mogelijke verklaring zou kunnen zijn dat hemofiliepatiënten beter gecontroleerd worden dan mensen zonder hemofilie en dat daarom een verhoogde bloeddruk eerder vastgesteld wordt. Verder onderzoek is nodig om hierover meer duidelijkheid te krijgen.

Deel II. Virusgerelateerde co-morbiditeit

Zoals hierboven beschreven is chronische hepatitis C infectie een belangrijk probleem bij volwassen hemofiliepatiënten. Om deze patiënten goed te kunnen begeleiden en behandelen is inzicht in het beloop van deze HCV infectie belangrijk.

In **hoofdstuk 7** wordt meer dan 30 jaar follow-up beschreven van een groep van 863 patiënten met erfelijke stollingsstoornissen die geïnfecteerd zijn geraakt met HCV. Dit zijn patiënten uit de Van Creveldkliniek en hemofiliebehandelcentra in Sheffield en Londen. Van alle geïnfecteerde patiënten was 19% in staat om het virus spontaan te klaren en ontwikkelde 81% een chronische HCV infectie. Van de patiënten met een chronische infectie hadden er 361 (52%) een antivirale behandeling gehad. Deze behandeling was succesvol bij 53% van deze patiënten. Ernstige leverziekte (‘end-stage liver disease’ of ESLD, hieronder vallen leverfalen en leverkanker) was aanwezig bij 13% van de patiënten die een chronische HCV infectie hadden. ESLD werd nauwelijks gezien bij patiënten die het hepatitis C virus spontaan geklaard hadden of patiënten die een succesvolle

antivirale behandeling hadden gehad. Risicofactoren voor het ontwikkelen van ESLD waren een hogere leeftijd op het moment van besmetting met HCV, co-infectie met HIV en alcoholmisbruik. Leverkanker kwam voor bij 22 patiënten en werd met name de laatste jaren veel vastgesteld, dus het lijkt erop dat dit een toenemend probleem is. Dit onderstreept nog eens het belang van goede follow-up en tijdige behandeling van HCV om dergelijke complicaties te voorkomen. Deze behandeling heeft echter ook nadelen.

In **hoofdstuk 8** worden de bijwerkingen van de antivirale behandeling met PegIFN en ribavirine beschreven in een groep van 47 patiënten met erfelijke stollingsstoornissen en chronische HCV. De behandeling was succesvol bij 66% van deze patiënten. Belangrijke bijwerkingen waren vermoeidheid, hoofdpijn, jeuk en huiduitslag, concentratieproblemen, verminderde eetlust, koorts, geïrriteerdheid, haarverlies, depressie, gewichtsverlies en bloedarmoede. De meeste bijwerkingen verdwenen binnen een paar weken na het stoppen met de behandeling. Vermoeidheid, concentratieproblemen en slaapproblemen waren echter na een maand nog aanwezig bij meer dan 30% van de patiënten. Bij 45% van de patiënten moest de dosering van de behandeling aangepast worden, voornamelijk omdat er sprake was van bloedarmoede en/of gewichtsverlies. De behandeling hoefde slechts bij twee patiënten voortijdig gestopt te worden wegens ernstige bijwerkingen. De kwaliteit van leven nam sterk af tijdens de behandeling, maar herstelde weer binnen enkele weken na stoppen. Er leek geen verband te zijn tussen de ernst van de bijwerkingen en het succes van de behandeling.

In **hoofdstuk 9** wordt het vóórkomen van depressie tijdens de antivirale behandeling bij deze patiënten in meer detail beschreven. Depressie kwam voor bij 55% van de patiënten die bij het starten van de behandeling geen depressie hadden. Een belangrijke risicofactor voor het ontwikkelen van depressie was het eerder gehad hebben van een depressie of andere psychiatrische problemen. Patiënten met een dergelijke voorgeschiedenis moeten extra goed in de gaten worden gehouden tijdens de antivirale behandeling en behandeling met antidepressiva moet tijdig worden gestart, eventueel al voor er met de antivirale behandeling begonnen wordt. Het verband dat gevonden werd tussen de aanwezigheid van depressie en een afname in de kwaliteit van leven tijdens de antivirale behandeling benadrukt dat tijdige herkenning en behandeling van depressie belangrijk is om de kwaliteit van leven zo hoog mogelijk te houden.

Zoals eerder besproken is het vaststellen van de mate van leverschade erg belangrijk bij patiënten met chronische HCV en is 'liver stiffness measurement' met behulp van een Fibroscan® apparaat een nieuwe, niet-invasieve manier om dit te doen. Omdat het om een nieuwe methode gaat, was niet goed bekend hoe je LSM het beste kunt gebruiken,

bijvoorbeeld hoeveel tijd er tussen metingen zou moeten zijn, wanneer je spreekt van een relevante verandering in resultaten en hoe betrouwbaar de metingen zijn.
In **hoofdstuk 10** beschrijven we onze ervaring met LSM bij 84 patiënten met erfelijke stollingstoornissen en chronische HCV die geen, of geen succesvolle antivirale behandeling gehad hebben. Deze patiënten hebben liver stiffness metingen gehad in 2005 (LSM 1) en 2009 (LSM 2). De gemiddelde tijd tussen de metingen was 3.7 jaar. De gemiddelde uitslagen van LSM 1 en LSM 2 waren vergelijkbaar en relatief laag (7.3 kPa bij LSM 1 en 6.6 kPa bij LSM 2). Op individueel niveau werd een verslechtering van het resultaat gezien bij 16% en bleef 52% van de patiënten stabiel. Er werd echter bij 32% van de patiënten een onverwachte verbetering van de resultaten gezien, die bij een deel van deze patiënten niet goed te verklaren was. Een vergelijkbare verbetering wordt echter ook gezien in studies die kijken naar herhaling van leverbiopten. Een voorzichtige conclusie is dat LSM, zeker bij hemofiliepatiënten, gebruikt kan worden als alternatief voor leverbiopten, maar dat er aan de juiste interpretatie van de resultaten nog wel wat haken en ogen zitten. Op dit moment worden er bij deze patiëntengroep derde metingen gedaan, om het verdere verloop over de tijd te kunnen beoordelen.

In **hoofdstuk 11** is LSM gebruikt om bij 39 patiënten te kijken naar het effect van succesvolle antivirale behandeling op de lever. Een deel van deze patiënten had een succesvolle behandeling gehad vóór de eerste meting (LSM 1) en een ander deel was succesvol behandeld tussen LSM 1 en LSM 2. In de eerste groep waren de resultaten van LSM 1 en LSM 2 stabiel en laag. In de tweede groep werd tussen LSM 1 en LSM 2, dus kort na de succesvolle behandeling, een sterke verbetering gezien in LSM resultaten. Deze resultaten geven aan dat succesvolle behandeling een gunstig en blijvend effect heeft op de mate van leverschade. Deze gegevens zouden gebruikt kunnen worden om patiënten te overtuigen van het nut van antivirale behandeling.

In **hoofdstuk 12** wordt ruim 25 jaar follow-up beschreven van de 60 met HIV besmette hemofiliepatiënten die behandeld zijn bij de Van Creveldkliniek. In 2010 hadden 27 van deze patiënten (45%) AIDS ontwikkeld en waren er 31 (52%) overleden. Het overlijden was aan AIDS gerelateerd in 71% van de patiënten in deze laatste groep. Na de introductie van HAART in het midden van de jaren ’90 zijn er nog maar weinig nieuwe patiënten bij gekomen die AIDS ontwikkeld hebben. Dit onderstreept het gunstige effect van de HAART-medicatie. HAART kan echter ook bijwerkingen hebben. Van de 27 patiënten die in 2010 nog leefden en bij de Van Creveldkliniek onder behandeling waren gebruikten er 25 (93%) HAART. Deze patiënten hadden vaker een verhoogde bloeddruk en suikerziekte dan de algemene Nederlandse bevolking, maar ze hadden minder vaak overgewicht en ze hadden lagere cholesterolwaardes. Verhoogde triglyceridewaardes in het bloed werden gezien bij 60%. De verhoogde bloeddruk, lage cholesterolwaardes

en het verminderd voorkomen van overgewicht worden ook gezien in studies bij hemofiliepatiënten die niet met HIV besmet zijn. Het vaker voorkomen van suikerziekte en de hoge triglyceridewaardes zijn daarentegen waarschijnlijk bijwerkingen van de HAART-medicatie. Opvallend was dat spontane hersenbloedingen significant vaker gezien werden bij HIV positieve hemofiliepatiënten die HAART gebruikten dan bij HIV negatieve patiënten met ernstige hemofilie. Mogelijk is dit ook een bijwerking van de HAART-medicatie, die met name bij hemofiliepatiënten ernstige gevolgen kan hebben. Hart- en vaatziekten zijn in eerdere studies beschreven als een belangrijke bijwerking van HAART. In onze kleine groep van HIV geïnfecteerde hemofiliepatiënten kwamen ze echter nauwelijks voor. Dit zou te maken kunnen hebben met het beschermende effect dat hemofilie heeft tegen hart- en vaatziekten.

Conclusies

De problemen waar hemofiliepatiënten mee te maken krijgen zijn de afgelopen decennia sterk veranderd. Jonge hemofiliepatiënten zullen veel minder last hebben van ernstige gewrichtsproblemen en er zijn gelukkig geen nieuwe besmettingen meer met hepatitis C of HIV. Door de goede behandeling met stollingsfactorconcentraten is de levensverwachting van hemofiliepatiënten sterk toegenomen. Hierdoor krijgen deze patiënten steeds meer te maken met leeftijdsgerelateerde gezondheidsproblemen. Het lijkt erop dat ernstige hemofilie een beschermend effect heeft tegen het ontstaan van hart- en vaatziekten. De resultaten van het prospectieve gedeelte van onze CVD studie zullen hierover meer duidelijkheid moeten geven. Een verhoogde bloeddruk, een belangrijke risicofactor voor het ontstaan van hart- en vaatziekten, lijkt vaker voor te komen bij hemofiliepatiënten dan bij mensen zonder hemofilie. Het algemene risicoprofiel voor hart- en vaatziekten lijkt bij deze patiënten bovendien ongunstiger te zijn. Als hart- en vaatziekten inderdaad minder vaak voorkomen bij hemofiliepatiënten dan in de algemene bevolking, ondanks het ongunstigere risicoprofiel, dan wijst dit op een sterk beschermend effect van zeer lage hoeveelheden van stollingsfactor VIII of IX. Onderzoek naar risicofactoren voor hart- en vaatziekten zou een standaard onderdeel moeten zijn van het programma in hemofiliebehandelcentra.

De impact van chronische hepatitis C infectie lijkt de laatste jaren toe te nemen, gezien de steeds groter wordende groep patiënten met ernstige complicaties. Antivirale behandeling tegen hepatitis C heeft veel bijwerkingen, maar geeft, als de behandeling succesvol is, een sterke en blijvende vermindering van het ontstaan van ernstige leverproblemen. Liver stiffness metingen met de Fibroscan® kunnen bij hemofiliepatiënten gebruikt worden om veranderingen in de mate van leverschade in de loop van de tijd te bepalen en te helpen bij het vaststellen wanneer antivirale behandeling sterk moet worden geadviseerd. De juiste interpretatie van de LSM resultaten en het bepalen van de

juiste afkapwaardes blijven echter moeilijk. Bij een hele hoge LSM uitslag of bij sterke achteruitgang is er in ieder geval reden voor verder onderzoek en/of het starten met antivirale behandeling. Nieuwere behandelmethodes die recent beschikbaar gekomen zijn zullen er hopelijk voor zorgen dat de antivirale behandeling bij een groter gedeelte van de patiënten succesvol zal zijn.

De impact van HIV infectie is na het beschikbaar komen van HAART sterk afgenomen, maar HAART kan ernstige bijwerkingen hebben, zoals bijvoorbeeld het ontstaan van suikerziekte en mogelijk een verhoogd risico op hersenbloedingen.

De komende jaren zal het onderzoek bij deze zeer interessante groep van ouder wordende hemofiliepatiënten door moeten blijven gaan om meer inzicht te krijgen in de verschillende vormen van co-morbiditeit. In de loop van de tijd zal de focus verschuiven van virusgerelateerde problemen naar leeftijdsgerelateerde aandoeningen. Gelukkig zijn veel hemofiliepatiënten erg betrokken bij hun hemofiliebehandelcentrum en telkens bereid om aan wetenschappelijk onderzoek mee te werken. Hopelijk blijft dit zo in de toekomst, want zonder hen is onderzoek naar co-morbiditeit niet alleen onmogelijk maar ook volledig zinloos.

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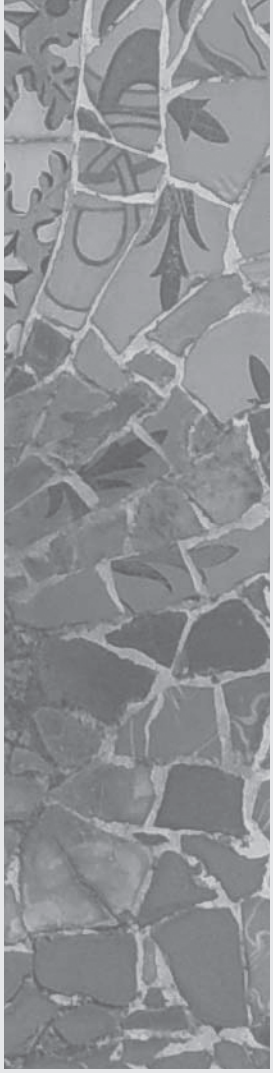

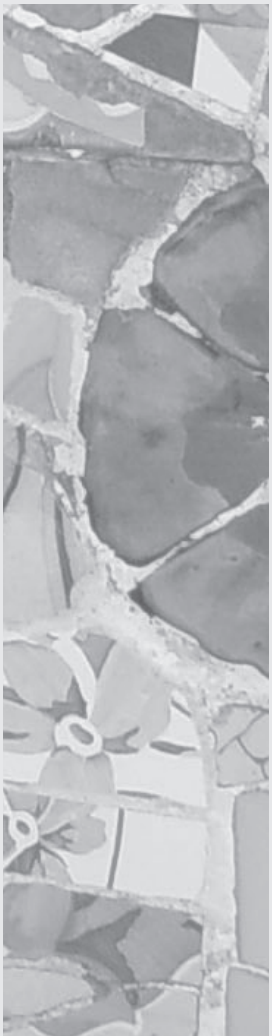

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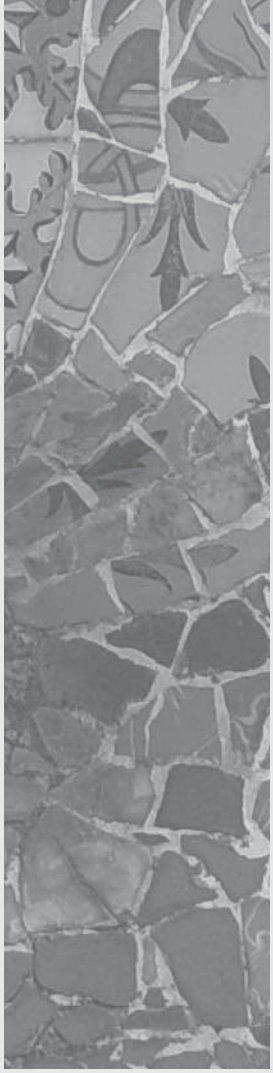

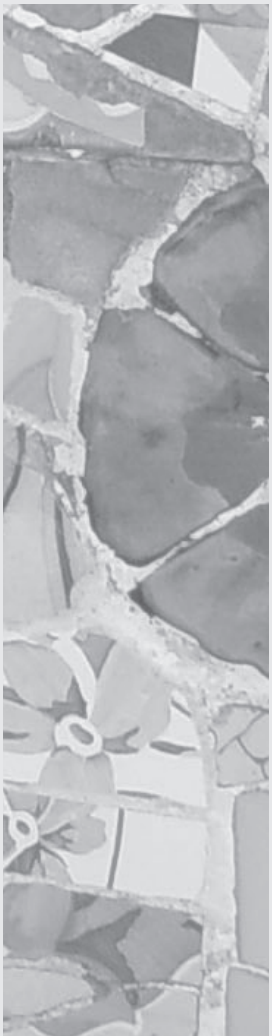

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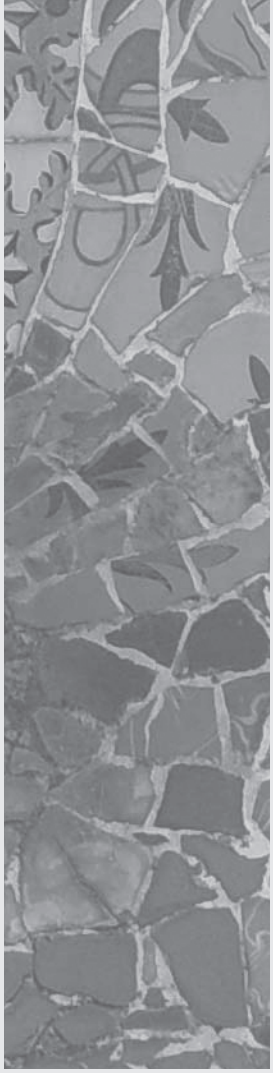

Dankwoord

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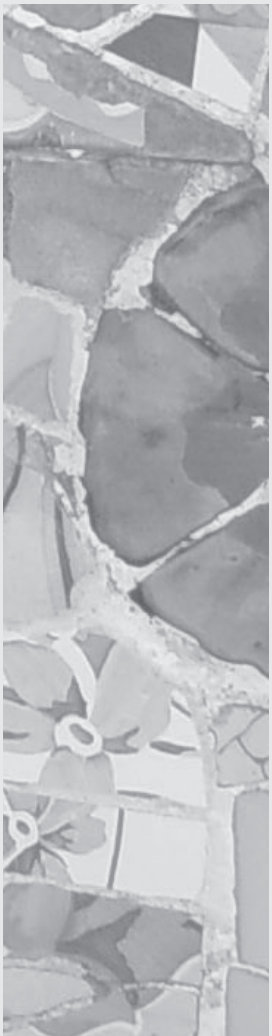

Het is klaar! Nu alleen nog het allerlaatste en waarschijnlijk meest gelezen onderdeel van dit proefschrift: het dankwoord.
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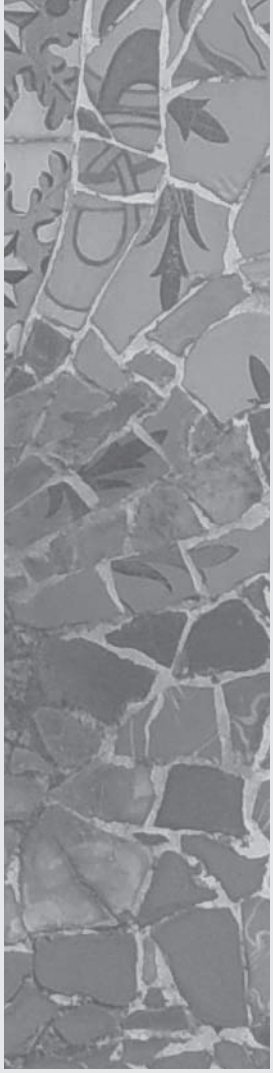
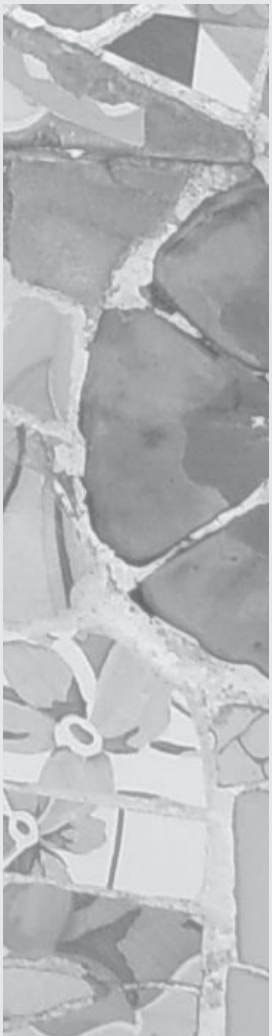
Curriculum vitae





Dietje Fransen van de Putte was born on April 17th 1980 in Delft, The Netherlands. She moved to Elsloo in the province of Limburg with her parents when she was a few months old. In 1998, she obtained her gymnasium diploma cum laude at the Scholengemeenschap St. Michiel in Geleen. She then studied Spanish for nine months at the Academia Mester in Salamanca, Spain. In 1999, she started medical school at Utrecht University. During the last year of this study, she did a senior internship at the Department of Clinical Genetics at the UMC Utrecht under supervision of professor F.A. Beemer and she worked on a research project on the Joubert syndrome in The Netherlands under supervision of doctor H.Y. Kroes. She obtained her medical degree in 2005. In January 2006, she started working at the Department of Clinical Genetics at the Leiden University Medical Center, where she started her official clinical genetics training in July 2008 under supervision of professor M.H. Breuning and doctor S.G. Kant. She hopes to complete this training in 2014. Since June 2007, she also worked at the Van Creveldkliniek, UMC Utrecht, on the research projects that resulted in this thesis, under supervision of doctor E.P. Mauser-Bunschoten, doctor K. Fischer and professor D.H. Biesma. In 2010, she obtained her master's degree in Clinical Epidemiology at Utrecht University.

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