
COMPLICATIONS OF HAEMOPHILIA THERAPY

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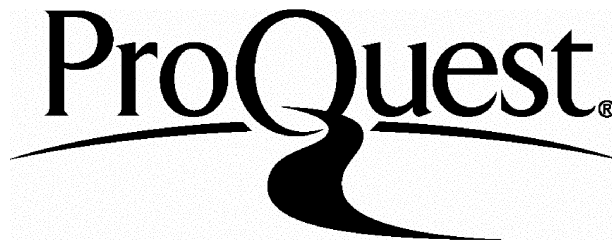
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ABSTRACT

The aim of this thesis is to evaluate and investigate the magnitude of the two major complications of haemophilia treatment: the development of inhibitory antibodies to exogenous clotting factor proteins and blood borne viral infections and their sequelae experienced in a large population of people with haemophilia.

All retrospective and prospective studies presented in this thesis have been conducted on a population of 580 haemophilia patients registered through out the years from 1964 to the present time at the Katharine Dormandy Haemophilia Centre and Haemostasis Unit (KDHC&HU), and for whom a comprehensive database has been established.

A 25 year follow-up study of 310 patients with inherited bleeding disorders infected with hepatitis C virus between 1961 and 1985 as a result of treatment with non-virally attenuated blood products has shown that 19% of HIV/HCV co-infected patients progressed to liver disease whilst only 3% of HCV mono-infected patients progressed to liver disease.

In the era of highly active antiretroviral therapy (HAART) a reduction in the incidence of AIDS has been seen within this cohort, but the death rate has remained high as a consequence of the large number of deaths related to liver disease secondary to chronic hepatitis C infection. Patients with haemophilia who were infected with HIV between 1979-1985 are facing new challenges and complications in the era of HAART such as the unique side-effect of the increased bleeding tendency associated with the use of protease inhibitors.

Parvovirus B19, a non-lipid enveloped virus still escapes the virological surveillance system and clinical problems related to this virus have been noted in patients who have contracted this virus through the use of virucidally treated plasma derived clotting factor concentrates.

431 haemophilia A patients of all severities have been followed-up for a total of 5,626 patient years and the frequency of inhibitors was found to be 10% in the severe haemophilia A patients and a third of the inhibitors occurred in children less than 10 years of age.

An inhibitor study of 37 previously untreated children with severe haemophilia A who have had treatment with the first heat-treated factor VIII product produced in the United Kingdom showed absence of inhibitors.

A series of patients with mild and moderate haemophilia A have developed inhibitors more recently in the KDHC&HU and a change in the practice of replacement therapy in haemophilia may well play a role in the development of these inhibitors.

Therapy for haemophilia has progressed substantially not only in the replacement therapeutic materials but also in the treatment strategies and mode of delivery of these therapeutic products. Regular prophylaxis in children with severe haemophilia at our centre has required insertion of central venous catheters because of difficult venous access in some children. A catheter infection rate of 1.74 per 1000 catheter-days has been noted.

Thus optimal comprehensive care for this group of patients requires the haemophilia physician to be aware of the complications of therapy: the sequelae of transfusion transmitted infections; the emerging challenges seen in those patients surviving with HIV in the era of HAART; and the development of inhibitory antibodies to replaced clotting factor proteins.

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Chapter 1

INTRODUCTION

- 1.1 GENERAL INTRODUCTION**
- 1.2 AIMS AND OBJECTIVES OF THE THESIS**
- 1.3 SUMMARIES OF THE STUDIES**

1.1 GENERAL INTRODUCTION

Haemophilia is a bleeding disorder resulting from a congenital deficiency in either factor VIII or factor IX. It is 1800 years since Judah the Patriarch's first reference to the disease in the Talmud in the 2nd century AD, and the more recent history of haemophilia has been through the triumphs and tragedies of transfusion therapy towards the anticipatory dawn of gene therapy. As the 21st century approaches, recent major advances in diagnostic techniques and replacement-factor therapy are already in widespread use; yet, at a time when the cure of haemophilia is rapidly becoming more probable than possible, we still grapple with issues of the main complications associated with haemophilia replacement therapy: the sequelae of the blood borne viral infections from the past, and the development of neutralising antibodies against the replaced clotting factor proteins.

The Royal Free Hospital Haemophilia Centre was established in 1964 and is now one of the largest comprehensive haemophilia centres in the United Kingdom. Dr Katharine Dormandy, who pioneered the unit in 1964, introduced the era of modern haemophilia therapy in UK with the introduction of cryoprecipitate produced in-house in 1966 (Bennet et al 1967) and also initiated the concept of home therapy using cryoprecipitate in a home treatment setting since 1969 (Le Quesne et al, 1974).

All retrospective and prospective studies presented in this thesis have been conducted on a large population of 580 haemophilia patients registered through out the years from 1964 to the present time at the KDHC&HU, and for whom a comprehensive database has been established. A computerised database was introduced in 1980.

Patients with severe haemophilia are seen six monthly and those with other inherited clotting deficiencies annually. From 1985 onwards human immunodeficiency virus

(HIV) sero-positive patients have been seen at three-four monthly intervals. Assessment included medical history, review of home and in-patient treatment records, physical examination and standard blood tests. From 1979, a serum/ plasma sample has been taken at each clinic visit and stored at - 40°C.

The information collected on the HIV infected haemophilia cohort at the Royal Free Hospital is as follows:

Demographics – date of birth, sex, haemophilia diagnosis and severity.

Clinical events – dates and type of all AIDS-defining events, date and cause of death.

Laboratory measures – dates and values of all CD4 and CD8 counts, Beta 2 microglobulin (B2M), IgA, IgM, p24 antigen and HIV RNA levels.

Treatment – dates of starting and stopping all antiretroviral drugs.

Other – date of first HIV positive and last HIV negative test results, estimated date of seroconversion.

All information is stored anonymously, without any personal identifiers, on a computer database.

1.2 AIMS AND OBJECTIVES

The purpose of the work described in this thesis is to gain more insight into the natural history, clinical, epidemiological and patho-physiological aspects of the two main complications associated with haemophilia replacement therapy: transfusion transmitted viral infections and their sequelae and the development of neutralising antibodies to the replaced clotting factor proteins.

1.3 SUMMARY OF THE STUDIES

a) A short summary of the studies undertaken on the various aspects and new challenges of transfusion transmitted infections seen within our haemophilia cohort is given below:

1.3.1 Hepatitis C virus infection

The natural history of hepatitis C infection appears to differ according to geography, alcohol use, virus characteristics (e.g. viral genotype and viral load), coinfection with other viruses and other unexplained factors.

Data on the natural history of hepatitis C are limited. Prospective cohort studies are few and typically small, include relatively few subjects whose date of infection can be well documented (e.g. blood transfusion recipients and victims of accidental needle-sticks), and have relatively short follow up duration.

Therefore a natural history study of 310 haemophilia individuals who have been infected with hepatitis C infection between 1961 and 1985 and followed up in a single comprehensive haemophilia centre for 25 years has been undertaken and is described in the thesis.

1.3.2 Surviving with HIV in the era of HAART: emerging challenges.

Combination therapy with protease inhibitors and nucleoside analogues potently suppress plasma HIV-1 RNA levels in HIV infected individuals and have resulted in dramatic decreases in the progression of HIV infection to AIDS and death. There are now new difficulties associated with long term HIV infection. The combined effect of powerful antiretroviral drugs and sophisticated techniques for assessing disease progression has created a range of new and often contradictory challenges for patients and their families. These developments have allowed hope to emerge. The improved

wellbeing in patients has resulted in an opportunity to reappraise reproductive choices, the possibility of career training and employment.

The clinical outcomes and response to highly active antiretroviral therapy in a cohort of closely monitored 111 HIV infected haemophilia patients and the unique side effect of one of the powerful HIV medication (protease inhibitors) seen in patients with bleeding disorders have been studied.

A study of the risk factors associated with heterosexual transmission of HIV infection in monogamous relationships in HIV sero-discordant haemophilia couples has been undertaken in an attempt to provide information for family planning in the era of HAART.

1.3.3 Parvovirus B19 infection

The disastrous consequences of blood-borne virus infections in haemophiliacs have provided the impetus for the development of safer plasma derived clotting factor concentrates, yet the search for absolutely safe plasma products must be tempered with the recognition that such a goal is probably unattainable. The transmission of parvovirus B19 infection through clotting factor concentrate in two of our patients is presented because of the potential pathogenicity of this virus and the possible significance as a “sentinel virus” for other as yet unknown organisms. The HIV epidemic caught the plasma fractionators unawares, and so might another equally dangerous virus in the future.

b) A short summary of the studies undertaken on the development of inhibitors to the replaced clotting factor concentrates is as follows:

1.3.4 Epidemiology of factor VIII inhibitors

In the United Kingdom, a national database was started in 1969 by the UK Haemophilia Centre Directors Organisation (UKHCDO) (Biggs 1974). A recent updated report from this database by the UKHCDO showed a prevalence of factor VIII inhibitors in haemophilia patients of all severities of approximately 6% with an annual inhibitor incidence of 3.5/1000 severe haemophilia A patients (Rizza et al 2001). Thus most haemophilia centres in the UK have only very small numbers of inhibitor patients.

We have undertaken a retrospective study of all factor VIII inhibitor patients followed-up over three decades in one of the largest comprehensive haemophilia centres in the UK, in an attempt to understand the natural history of factor VIII inhibitor patients and also to get some insight into the pathophysiology of factor VIII inhibitor formation.

1.3.5 The inhibitor study of the first heat-treated factor VIII concentrate produced in the United Kingdom.

Concentrates of both FVIII and FIX, derived from the UK volunteer donors, have been available ever since the early 1960s. These are manufactured by Bio Products Laboratory (BPL; Elstree, Herts, UK) and the Protein Fractionation Centre (PFC; Edinburgh, UK). Both agencies are an integral part of the National Health Service, and are thus enterprises supported by the government. The first heat-treated FVIII, 8Y heated at 80° for 72 hours, produced in the UK became available in July 1985.

Several studies published prior to 1985 indicate that 6-15% of patients with severe haemophilia A develop inhibitors (Brinkhous et al 1974; Gill 1984). When heat

treatment for viral inactivation of FVIII concentrates was introduced in the 1980s, there was concern that heat treatment could produce neoantigens on the factor VIII protein that might increase the risk of inhibitor induction.

We have carried out a study of inhibitor development in a cohort of 37 boys with severe haemophilia A (FVIII:C < 2 u/dl) exposed to the first heat-treated FVIII concentrate produced in the UK.

1.3.6 Inhibitors in mild and moderate haemophilia A patients

Inhibitor formation is an increasingly recognised problem in patients with mild and moderate haemophilia A. The UKHCDO inhibitor register reported 57 new cases of inhibitors during January 1990 to January 1997, of which 16 (28%) were in patients with mild and moderate haemophilia A (Rizza et al 2001). This finding may have resulted from improved data collection or it is also possible that recent changes in clinical practice, such as the use of continuous infusions and high purity or recombinant FVIII, are associated with increased inhibitor formation. A small series of inhibitors in patients with haemophilia A of mild and moderate severity observed recently in our haemophilia centre is reported in this thesis.

1.3.7 New Treatment Strategies

Therapy for haemophilia has progressed substantially not only in the introduction and widespread use of safe clotting factor concentrates but the advancement has also been seen in the change of treatment strategies and the mode of delivery. In the majority of developed countries replacement therapy has moved on from on-demand infusion treatments to using prophylactic therapy especially in patients with severe haemophilia. Improvements in the stability of the clotting factor concentrates have also made it

possible to change the mode of delivery of the clotting factor concentrates from bolus injection to the use of continuous infusion techniques. Continuous infusions of clotting factor concentrate is very useful in certain clinical situations such as life-threatening bleeds and during surgical procedures. The complications which could be associated with prophylaxis have been undertaken in our haemophilia cohort and is included in this thesis.

Chapter 2

OVERVIEW OF INFECTIOUS COMPLICATIONS OF BLOOD PRODUCTS

- 2.1 TRANSMISSIBLE VIRUSES BY CLOTTING FACTOR
CONCENTRATES**
- 2.2 VIRAL REDUCTION STRATEGIES APPLIED TO CLOTTING
FACTOR CONCENTRATES**
- 2.3 IMPACT OF HIV AND HCV INFECTION IN THE UK HAEMOPHILIA
COMMUNITY**

INFECTIOUS COMPLICATIONS OF BLOOD PRODUCTS

The risk of acquiring diseases from the transfusion of blood and blood products is well recognised. Haemophilic patients receive clotting factors derived from cell free plasma and are therefore susceptible to infectious agents transmitted in plasma. As a result of plasma pooling, individuals with haemophilia are also at significant risk of an infection even if the agent has a low prevalence rate in the blood donor population. Infectious organisms such as those associated with red cells or with leucocytes (cytomegalovirus, Epstein Barr virus and toxoplasmosis) are generally less of a threat to haemophilia patients.

2.1 VIRUSES TRANSMISSIBLE BY FACTOR CONCENTRATES

The major threats to haemophilic patients are transmissible viruses which have a plasma phase and which cause serious and/or chronic disease. The actual and potential pathogens and their characteristics are shown in Table 2.1.

The major threats in terms of both prevalence and pathogenicity of the transfusion transmitted infections are HIV and the hepatitis B and C viruses (HBV and HCV).

Other transfusion transmitted viruses

Among other potential viral threats are the agents believed to be responsible for the small proportion (probably less than 10%) of post-transfusion hepatitis which is not caused by hepatitis A, B, or C viruses. Two early candidates were the hepatitis G virus/GB virus (HGV/GBV) and the TT virus (TTV). The evidence now suggests that neither is associated with chronic hepatitis (Sheng et al 1997; Lefrere et al 2000), but that both these viruses have been transmitted via haemophilia replacement products. HGV is likely to be susceptible to the viral inactivation strategies which are effective for

other enveloped viruses, although this remains to be established. Even if this is confirmed, the efficacy of these procedures could be compromised if there are high initial viral titres in unscreened plasma pools. TTV is widely prevalent in blood donors around the world (Prescott & Simmonds 1998; Naoumov et al 1998). It is a non-enveloped single-strand DNA virus, and its susceptibility to standard viral inactivation procedures is questionable, as recipients of exclusively virally inactivated concentrates have been frequently affected (Chen et al 1999). Ion exchange chromatography, nanofiltration, and dry heating appear ineffective in inactivating TTV in factor concentrates; however, factor VIII and IX concentrates purified by immunoaffinity chromatography were negative for TTV DNA (Yokozaki et al 1999).

SEN-V is a recently identified blood-borne DNA virus which appears to cause post-transfusion hepatitis. New SEN-V infection was found in 10 of 12 patients (83%) who developed non-A to E post-transfusion hepatitis, 32 of 94 (34%) transfused patients who did not develop hepatitis, and 3 of 97 (3.1%) non-transfused patients (Umemura et al 2000). The consequences of SEN-V infection (chronicity and natural history) and its implication for recipients of clotting factor concentrates remains to be established.

Non-human viruses are a potential concern, but their pathogenicity in humans is often unclear. For example, porcine factor VIII concentrate has been contaminated with porcine parvovirus (PPV), which is highly endemic in pig herds. Fortunately, PPV is not known to be transmissible to humans, and laboratory and clinical data on recipients of porcine factor VIII have thus far failed to demonstrate that it poses a health risk to humans. Attempts to exclude or inactivate PPV are complicated by its small size and by the fragility of the porcine factor VIII molecule, which does not withstand the vigorous procedures needed to inactivate PPV.

Table 2.1 : Characteristics of human pathogens relevant to clotting factor concentrates

Agent	Blood-borne	Pathogenicity	Screening tests	Inactivation
HIV	Yes	Yes	Ab; Ag; NAT	Yes
Hepatitis C virus	Yes	Yes	Ab; NAT	Yes
Hepatitis B virus	Yes	Yes	Ab; Ag; NAT	Yes
Hepatitis A virus	Occasionally	Yes	Ab; (NAT)	+/-
Parvovirus B19	Yes	Yes	Ab; (NAT)	+/-
Hepatitis G virus	Yes	Questionable	NAT	Probable
TT virus	Yes	Questionable	NAT	Questionable
SEN virus	Yes	Possible	NAT	?
vCJD*	?	Yes	None	?

Ab, antibody; Ag, antigen; NAT, nucleic acid amplification testing.

*The agent of a variant of Creutzfeldt-Jakob disease (vCJD) is not a virus, but a prion and is included in the table owing to potential pathogenicity.

2.2 VIRAL REDUCTION STRATEGIES APPLIED TO CLOTTING FACTOR CONCENTRATES

The vexing problem of viral transmission through blood and blood products has to be addressed in a systematic way. Prevention of viral infection relies on the application of sequential strategies including donor selection, testing of donated blood, partitioning of viruses from therapeutic components, viral inactivation, active immunisation and post marketing surveillance. The responsibility for implementation of these strategies is shared among manufacturers, government regulators, treaters and consumers.

Technologies used to prevent viral contamination of the initial plasma pool from which clotting factor concentrates are fractionated are listed in Table 2.2.

Table 2.2 : Prevention of virus entry into plasma pools used to produce clotting factor concentrates

<u>Donor selection</u> <ul style="list-style-type: none"> • Self-deferral (donor indicates that s/he is a potential blood risk) • Deferral by centre
<u>Screening of individual donor units</u> <ul style="list-style-type: none"> • Surrogate assay • Antibody reactivity • Viral antigen
<u>Screening of pool</u> <ul style="list-style-type: none"> • Viral nucleic acid amplification testing (NAT)
<u>Donor re-testing</u>

Donor selection

The viral burden entering the plasma pool can be limited by careful donor selection, a strategy which non-selectivity reduces the risk of transmitting viruses by selecting against high-risk characteristics and behaviours. Many countries rely on altruistic blood donors, but carefully screened repeat-paid donors in fact constitute an extremely safe pool (Taswell 1987). For optimal safety, the donor selection process should be accompanied by strict criteria for the re-entry of deferred donors into the pool and by a registry which effectively ensures that plasma from deferred donors is not inadvertently released.

Screening of donor units

Surrogate tests have usually been applied as temporary measures in the absence of specific screening tests, although they may have additional; independent value. For example, anti-HB core antibody and alanine aminotransferase (ALT) levels were introduced as surrogates for the putative HCV agent before specific serological assays were available.

Monospecific antibody or antigen detection tests are used to exclude individual donor units contaminated with preselected virus targets. The priority of these assays is sensitivity, so that the ratio of true-positive to false-positive results should be high in selected (i.e. low prevalence) donor populations.

False-negative screening tests may be caused by clerical error or failure of quality control, but more predictably they are the result of subthreshold viral contamination. This may occur during an asymptomatic 'window period' early in the course of infection, or else in a late chronic carrier stage when viraemia or the host serological response has declined. One approach to close this early window is the practice of

donor-re-testing, in which frozen plasma is quarantined for sufficient time (e.g. three months) to allow re-testing of donors who initially test negative. Virus transmission during the window period is rare. For example, the Retrovirus Epidemiology Donor Study (REDS) in the USA found that risk of contracting viral infection from a single antibody-negative donor unit is approximately 1:500 000 for HIV, 1:100 000 for HCV, and 1:63 000 for HBV (Schreiber et al 1996). The risk for pooled products, such as clotting factor concentrates, can be magnified, as a single contaminated unit in a large pool (which may include plasma from tens of thousands of donors) can potentially contaminate an entire lot of product. On the other hand, dilution of contaminating virus in these large pools might reduce the infectiousness of the final product and might also enhance the efficiency of viral inactivation steps.

Screening for HBV surface antigen (HBsAg) is extremely sensitive because large amounts of viral protein are synthesised early in the course of HBV infection. Still, HBV can be infectious at titres below the limit of detection by HBSAg assays (Hoofnagle 1990; Thiers et al 1988), and the sensitivity of assay may also be reduced in the presence of antibody (Ferguson et al 1996). HIV antigen testing is of limited value, but screening for the HIV p24 antigen is commonly performed in the hope of excluding antibody-negative donations during the window period. This strategy may be particularly useful in countries where there is a high incidence of newly acquired HIV infection. HCV antigens have not been targets of screening tests, as only small concentrations of antigen circulate during the early phase of infection (Ulrich et al 1990). However, new more sensitive methodologies may make this a feasible approach (Peterson et al 2000).

Nucleic acid testing (NAT) of donated blood has been introduced in many European and North American countries. The rationale for NAT is its potential to identify

infectious donor units that are seronegative. The most common explanation for this discrepancy is the window period phenomenon, but insensitivity of serological tests is an additional limitation. NAT can reduce the window period by as much as 59 days for HCV, 25 days for HBV, and 11 days for HIV (Schreiber et al 1996). NAT on pools of 16-512 plasma samples typically achieves sensitivity limits in the range of approximately 1000 viral genome equivalents (GE)/ml and is practical for implementation in blood banks (Roth et al 1999). The limitation of even this sensitive technology is illustrated by the report of transmission of HCV by a platelet concentrate from a donor who was negative even by single-unit NAT (Schuttler et al 2000). The impact of NAT is greatest for HIV and HCV infections and less so for the detection of HBV, in which viral titres during the window period may be as low as 10^2 - 10^4 /ml. More sensitive techniques and the introduction of single-donor testing could enhance the value of NAT for excluding HBV.

As HIV, HBV, and HCV are all exquisitely sensitive to both solvent detergent (S/D) and thermal viral inactivation, NAT will add little to the safety of serologically screened, virally inactivated clotting factor concentrates. Indeed the major impact of this approach will be on the safety of cellular components and untreated plasma derivatives. However, NAT could also enhance the safety of clotting factor concentrates with respect to viruses such as HAV and parvovirus B19, which are relatively resistant to viral inactivation methodologies.

Removal and inactivation of viruses

All clotting factor concentrates are processed by using methodology designed to remove and/or inactivate viruses. These techniques are not specific for individual agents. They serve to eliminate recognised pathogens in window period donations and unrecognised

pathogens for which screening tests are not performed. The validated techniques are shown in Table 2.3. The International Association of Biological Standardisation recommends that plasma derivatives be treated with at least two independent viral inactivation steps (Willkommen & Lower 1993). However, the efficacy of viral removal or inactivation is finite, and in any event these steps must compromise some degree of viral kill in order to avoid excessive denaturation of the clotting factor protein. For example, heat treatment typically reduces the yield of factor VIII activity by 10-15% despite the addition of chemical stabilisers such as amino acids, citrate, or sugars. Another potential liability is a higher risk of inhibitor antibody development, presumably caused by subtle molecular changes (Rosendaal et al 1993; Peerlinck et al 1997).

Table 2.3: Techniques for removal or inactivation of virus in clotting factor concentrates

Incidental removal during protein purification
Specific virus removal by filtration
Thermal inactivation <ul style="list-style-type: none"> • 80-100°C for 0.5-72 h (dry) • Pasteurisation, 60°C for 10 h (liquid) • Heat under pressurised steam
Chemical inactivation <ul style="list-style-type: none"> • Inorganic solvent + nonionic detergent • Sodium thiocyanate
Photochemical inactivation <ul style="list-style-type: none"> • Beta-propiolactone/ultraviolet light • Ultraviolet C irradiation

Physical partitioning of viruses from clotting factor concentrates occurs incidentally during their purification and formulation. Substantial amounts of virus are removed by steps, such as cryoprecipitation, chromatographic separation (in particular immunoaffinity chromatography), and lyophilization. Filtration techniques are also applicable as dedicated viral removal steps where the protein of interest is small enough (typical less than 100 kDa) to allow efficient separation from target viruses. This is not applicable to the complex of factor VIII with its binding protein von Willebrand factor, but ultrafiltration and 'nanofiltration' membranes are now being used to exclude not only HIV but also viruses as small as HAV and parvovirus B19 from concentrates of factor IX and factor XI (Hoffer et al 1995; Burnouf-Radosevich et al 1994).

Viruses have varying thermal sensitivity, and heat treatment is a widely used viral inactivation procedure. The effectiveness of heat as a virucidal treatment is a function of many factors, including time, temperature, the physical state (dry or in solution), the salt content, the rate of temperature change, and the nature and concentration of the stabilizers (Suomela 1993). Effective protocols include pasteurisation (60°C in the liquid phase) and higher temperature protocols applied to lyophilized concentrates (80-100°C for 0.5-72 h).

Viruses with lipid coats (including HIV, HBV, and HCV) can be efficiently disrupted by exposure to an organic solvent (usually tri-(n-butyl)phosphate, TNBP) in the presence of a nonionic detergent, either Tween 80, sodium cholate, or Triton X-100 (Horowitz et al 1985). These chemical agents are potentially toxic, and they must be removed from the final product. As is the case for heat treatment, the efficiency of solvent detergent (S/D) protocols is time and temperature dependent (Suomela 1993). S/D causes rapid and complete inactivation of lipid-coated viruses, and the safety record

of S/D-treated blood products with respect to these viruses is excellent. Many S/D-treated products are now further treated with a terminal heat treatment step. This broadens the spectrum of viral inactivation to include non-enveloped viruses, while retaining the advantage of the potent activity of the S/D treatments. The experience to date with these dual-treated products has been good, with no evidence that the added manipulation has enhanced their immunogenicity (Smith et al 1997; Hart et al 1994; Robinson 1994).

Other approaches have been used for viral inactivation in concentrates of factor IX, which is more stable to manipulate than factor VIII. These techniques are generally less active individually, but are of some use when combined with other methodologies. They include sodium thiocyanate, photochemical inactivation by ultraviolet irradiation with a chemical sensitising agent, and ultraviolet C irradiation.

The combined application of all the strategies outlined above have for all practical purposes virtually eliminated the risk of transmission of virulent organisms by plasma-derived clotting factor concentrates.

2.4 THE IMPACT OF HCV AND HIV INFECTION IN THE UK HAEMOPHILIA COMMUNITY

The true impact of viral transmission through clotting factor concentrates was not appreciated until the magnitude and consequences of the HIV epidemic in haemophiliacs became evident in the mid-1980s. By the end of that decade it was found that virtually all treated individuals had contracted the hepatitis C virus, including those who had been fortunate enough to escape infection with HIV. It is therefore a tragic irony that the same plasma derivative which brought independence and life-saving

therapy to thousands of people also devastated a generation of haemophiliacs and their families.

In the UK during 1969-85, 4865 haemophilia individuals are known to have received virally contaminated blood products (Darby et al 1997). As serum samples for this large population were not stored systematically, and sensitive tests to detect antibodies to HCV antigens became available only in the 1990s the HCV status cannot be formally classified in this large group. However studies in small groups of haemophilic individuals have shown that virtually 100% of those treated with non virus inactivated large pool concentrate are infected with HCV, and that a single exposure usually causes infection (Fletcher et al 1983; Kernoff et al 1985; Watson et al 1992). Of those individuals 80% (3662/4865) had explicit evidence that they received non-virus inactivated large pool concentrate, and many of the remaining 1203 would also have received this treatment. Therefore approximately 4000 haemophilia patients in the UK have been infected with HCV and 30% (1227/4000) of those HCV infected individuals are also co-infected with HIV.

Most individuals treated before the introduction of donor screening for hepatitis B virus in 1972 were exposed to HBV (MRC report BJH 1974) and about 2% of HBV exposed haemophilic individuals developed persistent HBs Ag positive infection (Telfer et al 1994b). 10-20% of haemophilic individuals are also chronic carriers of hepatitis G virus (HGV) (Jarvis et al 1996) although a causal role for it in liver disease has not been shown.

Chapter 3

HEPATITIS C VIRUS (HCV) INFECTION

- 3.1 OVERVIEW OF HEPATITIS C VIRUS**
- 3.2 CLINICAL SPECTRUM OF THE DISEASE**
- 3.3 DIAGNOSIS OF HEPATITIS C INFECTION**

3.1 OVERVIEW OF HEPATITIS C VIRUS - NATURE OF THE VIRION AND THE VIRAL GENOME

Non-A, non-B hepatitis (hepatitis C virus) was first recognised in 1975 but it was not until 1989 that the genome of the virus was first cloned and sequenced, and expressed viral antigens were used to develop serological assays for screening and diagnosis (Purcell 1997). Hepatitis C virus (HCV) is in a separate genus of the virus family *Flaviviridae*. It is a spherical enveloped virus of approximately 50 nm in diameter. Its genome is a single-stranded positive sense linear RNA molecule of 9,500 bases coding for 3000 amino acids. It has no DNA intermediate and hence cannot integrate into the host genome, but does use a negative strand RNA in its replicative cycle within the liver. The RNA consists of a 5' noncoding region, a single large open reading frame, and a 3' noncoding region. The open reading frame encodes at least three structural and six non-structural proteins. The most important characteristic of the HCV genome is its sequence heterogeneity (Bukh et al 1995). In this respect, it resembles the human immunodeficiency virus, a member of the virus family *Retroviridae*. The genetic heterogeneity of HCV is not uniform across the genome and is greatest in the amino-terminal end of the second envelope protein (hypervariable region 1). This region may represent a neutralisation epitope that is under selective pressure from the host's humoral immune response.

Genetic heterogeneity: Types, Subtypes and Quasispecies

Based on their genetic heterogeneity, HCV strains can be divided into major groups, called types or genotypes. Six major genotypes have been identified and at least partially characterised. These have been designated types 1 through 6. Subtypes have been given letter designations. The major genotypes of HCV differ in their distributions

worldwide. Genotypes 1, 2 and 3 and their subtypes are distributed worldwide. In contrast, genotype 4 appears to be a Pan-African type, and genotype 5 has been found to be the principal genotype in South Africa. Genotype 6 and its many variants have been found principally in Asia. The consequence of the genetic diversity of HCV is, that a virus has the ability to escape the immune surveillance of its host, leading to a high rate (>80%) of chronic infections and lack of immunity to re-infection in repeatedly exposed individuals. Both chronicity and lack of solid immunity probably result from the emergence of minor populations of the virus quasispecies that vary in sequence, especially in the hypervariable region-1 (HVR1). A quasispecies is defined as a heterogeneous population of individual virions, each of which may be different in at least one genomic site. Therefore, HCV still remains a challenge both to the physician and the virologist.

3.2 CLINICAL SPECTRUM OF DISEASE

Hepatitis C virus is not easily cleared by the host's immunological defences. Thus, a persistent infection develops in perhaps as many as 85% of patients with acute hepatitis C. This inability to clear the virus by the infected host sets the stage for the development of chronic liver disease. The spectrum of disease after hepatitis C infection is broad. In contrast to hepatitis A and B, there is no effective vaccine to prevent acquisition of hepatitis C infection.

Mode of Transmission

Hepatitis C is transmitted primarily by the parenteral route, and sources of infection include injection drug use, needle-stick accidents, and transfusions of blood products.

Since 1990 and the introduction of tests for antibodies to HCV (anti-HCV), new cases of post-transfusion hepatitis C have virtually disappeared.

Acute infection

After initial exposure, HCV RNA can be detected in blood in one to three weeks. Within an average of 50 days (range 15 to 150 days), virtually all patients develop liver cell injury, as shown by elevation of serum alanine aminotransferase (ALT). The majority of patients are asymptomatic and anicteric. Anti-HCV almost invariably becomes detectable during the course of illness, and can be detected in 50% to 70% of patients at the onset of symptoms and in approximately 90% of patients three months after onset of infection. HCV infection is self-limited in only 15% of cases. Recovery is characterised by disappearance of HCV RNA from blood and return of liver enzymes to normal.

Chronic infection

A propensity to chronicity is the most distinguishing characteristic of hepatitis C, occurring in approximately 85% of HCV-infected individuals. These patients fail to clear the virus by six months and develop chronic hepatitis with persistent, although sometimes intermittent viraemia and abnormal ALT levels that can fluctuate widely. Approximately one third of patients have persistently normal serum ALT levels. Antibodies to HCV or circulating viral RNA can be demonstrated in virtually all patients. A small proportion of individuals with chronic hepatitis (<20%) develop non-specific symptoms, including mild intermittent fatigue and malaise. Symptoms first appear in many patients with chronic hepatitis C at the time of development of advanced liver disease. Chronic hepatitis C is typically an insidious process, progressing, if at all,

at a slow rate without symptoms or physical signs in the majority of patients during the first two decades after infection. The rate of progression is highly variable. Long-term studies suggest that most patients with progressive liver disease who develop cirrhosis have detectable ALT elevations; however, these can be intermittent. The relationship is inconsistent between ALT levels and disease severity as judged histologically. Although patients with HCV infection and normal ALT levels have been referred to as healthy HCV carriers, liver biopsies can show histological evidence of chronic hepatitis in many of these patients.

Cirrhosis

The major serious complication of chronic hepatitis C is the development of cirrhosis. Cirrhosis can develop rapidly, within one to two years of exposure, or slowly, within two to three decades (Hoofnagle 1997). In studies with 10 to 20 years of follow-up, cirrhosis develops in 20% to 30% of patients (Seeff 1997; Vaquer et al 1994; Yano et al 1996). It is unclear whether the remaining patients will eventually develop cirrhosis or whether the numbers of patients developing cirrhosis will level off with time. Thus, chronic hepatitis C probably does not have one typical course; there are probably multiple different courses ranging from rapidly progressive to slowly progressive to non-progressive.

The determinants of severity in chronic hepatitis C are not known. Features that have been suggestive as predictive of more severe or more rapidly progressive disease are older age, immunodeficiency, concurrent excessive alcohol intake, and in some series, certain HCV strains and high degrees of genetic heterogeneity (Seeff et al 1992; Vaquer et al 1994; Yano et al 1996). Once cirrhosis develops, the symptoms of end-stage liver disease can appear, including marked fatigue, muscle weakness and wasting, fluid

retention, easy bruising, upper intestinal haemorrhage, jaundice, dark urine, and itching. Once end-stage liver disease has developed, the only practical means of restoring health is liver transplantation. Recurrent HCV infection of the new graft occurs in almost all patients who undergo transplantation for hepatitis C, but in many cases the recurrent infection is mild (Gane et al 1996). At present, long-term survival after liver transplantation for hepatitis C is similar to that for other diagnoses, averaging 65% after 5 years (Detre et al 1996).

Hepatocellular Carcinoma

Chronic infection by HCV is associated with an increased risk of liver cancer. Most cases of HCV-related hepatocellular carcinoma occur in the presence of cirrhosis. The risk that a person with chronic hepatitis C will develop HCC appears to be 1% to 5% after 20 years, with striking variations in rates in different geographic areas of the world. Once cirrhosis is established, the rate of development of HCC increases to 1% to 4% per year. Among patients with cirrhosis caused by hepatitis C, HCC develops more commonly in men and in older patients.

Extrahepatic Manifestations of HCV

Patients with chronic hepatitis C occasionally present with extrahepatic manifestations or syndromes considered to be of immunologic origin, including arthritis, keratoconjunctivitis sicca, lichen planus, glomerulonephritis, and essential mixed cryoglobulinemia. Cryoglobulins may be detected in the serum of approximately one third of patients with HCV, but the clinical features of essential mixed cryoglobulinemia develop in only approximately 1% to 2% of patients.

3.3 DIAGNOSIS OF HEPATITIS C INFECTION

Diagnostic tests for hepatitis C infection can be divided into the following two general categories:

- 1) serological assays that detect antibody to hepatitis C virus (anti-HCV); and
- 2) molecular assays that detect, quantify, and/or characterise HCV RNA genomes within an infected patient.

Serological assays have been subdivided into screening tests for anti-HCV, such as the enzyme immunoassay (EIA), and supplemental tests such as the recombinant immunoblot assay (RIBA).

Screening tests

The main screening assay for detecting anti-HCV is the enzyme immunoassay (EIA) (Younossi & Hutchinson 1996). The EIA has many advantages in the diagnostic setting, including the ease of use, low variability, ease of automation, and relatively low expense. The first generation anti-HCV test (EIA-1) contained a single HCV recombinant antigen derived from the nonstructural (NS) 4 gene, designated c100-3. EIA-1 lacked optimal sensitivity and specificity and was subsequently replaced in 1992 by the second-generation test (EIA-2) (Alter 1992). The EIA-2 test contains HCV antigens from the core and NS3 genes in addition to the NS4-derived antigen, and thus represents a multiantigen EIA. In the high-prevalence setting, EIA-2 testing allows detection of approximately 95% of individuals with molecular evidence of HCV. Immunocompromised patients, such as organ transplantation recipients or human immunodeficiency virus-infected patients, may lack detectable antibodies by EIA-2 even in the presence of active viral infection (Lok et al 1993). A third-generation anti-HCV test (EIA-3) has been approved and contains reconfigured core and NS3 antigens

plus an additional HCV antigen NS5 not present in the EIA-2 test. The slight improvement in EIA-3 sensitivity compared with the EIA-2 test has been attributed to reconfigured antigens already present in the EIA-2 test and not to the NS5 antigen. However, testing in high-prevalence populations has indicated that not all patients with active HCV infection (e.g. HCV RNA positive) are identified with the EIA screening tests. Three generations of anti-HCV tests have been developed, and each generation has resulted in an improvement in the sensitivity of detecting anti-HCV.

Supplemental tests for anti-HCV (RIBA)

Recombinant immunoblot assay (RIBA) a supplemental anti-HCV test, is designed to resolve false-positive testing by EIA, and is appropriately used in low-prevalence settings in which false-positive anti-HCV tests remain a problem. The tests use the same HCV antigens in an immunoblot format. Third-generation anti-HCV tests (EIA-3 and RIBA-3, respectively) contain antigens from the HCV core, nonstructural 3, nonstructural 4, and nonstructural 5 genes.

In many infections, isolation of the pathogen by tissue culture is useful for establishing the diagnosis. However, tissue culture propagation of HCV has been extremely difficult, with only a few reports of low efficiency propagation in the research setting. Therefore molecular assays have been developed to detect, quantify, and/or characterise HCV RNA genomes within infected patients.

Molecular assays for hepatitis C can be divided into three general categories:

- 1) Detection of HCV RNA in patient specimens by polymerase chain reaction (PCR) provides evidence of active HCV infection and is potentially useful for confirming the diagnosis and monitoring the antiviral response to therapy. Optimal HCV

PCR assays at present have a sensitivity of less than 100 copies of HCV RNA per millilitre of plasma or serum.

- 2) Tests that assess the quantity of HCV RNA in the blood (quantitative HCV RNA tests). Two main technologies exist for assessing HCV RNA levels or viral load. Quantitative PCR is the most sensitive test for determining hepatitis C viral load, whereas the branched-chain DNA test appears to be the most precise method. Major limitations of the current tests are inadequate dynamic range and high variability of PCR-based assays, and poor sensitivity of the branched-chain DNA test.
- 3) Tests that determine the genetic nature of HCV (HCV genotype tests). This test is useful especially in clinical trials as HCV genotype is an independent predictor of response to therapy. Screening tests for HCV genotype includes: (1) restriction fragment length polymorphism analysis of the highly conserved 5'-noncoding region, (2) reverse dot blot hybridisation analysis of 5'-noncoding region sequences (LIPA assay), (3) nested PCR analysis of the HCV core gene using genotype specific primers.

Chapter 4

THE NATURAL HISTORY OF HCV IN A COHORT OF HAEMOPHILIC PATIENTS INFECTED BETWEEN 1961 AND 1985

- 4.1 INTRODUCTION**
- 4.2 PATIENTS AND METHODS**
- 4.3 RESULTS**
- 4.4 DISCUSSION**

NATURAL HISTORY OF HCV IN A COHORT OF HAEMOPHILIC PATIENTS INFECTED BETWEEN 1961 AND 1985

4.1 INTRODUCTION

There has been much difficulty in designing and undertaking suitable studies that can convincingly define the natural history of chronic HCV infection. Indeed, the difficulties are such that an absolute answer may never be fully realised. With regard to hepatitis C, it seems apparent that certain critical features must be incorporated into the design of a natural history study if it is to be truly informative. These include the need for the following: (1) to accurately establish onset of the initial acute disease to properly determine disease duration; (2) to identify and evaluate the full spectrum of acute disease to avoid the bias of focussing evaluation on the more obvious and more severe forms of illness; (3) to track the process to its resolution or to its adverse disease end point, regardless of the duration of the process, which may extend for decades; (4) to evaluate outcome without instituting treatment that might modify the course; and (5) to assemble a properly matched control group without hepatitis C that can be followed-up with the same zeal and intensity as the study cohort to perform classical case control studies.

Unfortunately, it is difficult, if not impossible in some instances, to adhere to each of these requirements in a natural history study of HCV infection because: (1) the onset of acute hepatitis C is not recognised in the majority, perhaps 60% to 80%, of instances. The time of onset, therefore, has to be inferred, often inaccurately, on the basis of historical information; (2) onset generally occurs without symptoms, and hence most clinically mild cases will be missed unless prospective serologic or biochemical screening is performed; (3) the disease generally progresses very slowly, requiring the

passage of three or four decades, in most instances, to reach recognisable serious endpoints. It therefore represents a daunting task for the clinical investigator, few of whom are willing to devote their research careers to this extended form of investigation; (4) treatment of chronic hepatitis C has become common place in the last few years, potentially modifying the natural history of the disease; and (5) the construction of a matched control group cannot be achieved without the ability to identify onset of the acute bout of hepatitis C.

Indeed, conduct of an ideal natural history study of chronic hepatitis C requires either that at-risk groups, such as transfusion recipients, are screened prospectively for onset of current disease and then evaluated during the ensuing two to three decades, or that serum samples are recovered from outbreaks that can be traced to a common source exposure in the distant past, permitting conduct of a retrospective-prospective, referred to more properly as a non-concurrent prospective, study. The latter fortuitous circumstance is a rare event. Accordingly, much of the reported data on natural history are accrued from retrospective studies of persons with already established chronic liver disease, excluding from analysis the group – possibly the bulk of cases – that do not reach clinical awareness. As noted, this approach tends to bias outcome data in the direction of the more severe disease sequelae.

Nonetheless, a great deal of information has been gathered over the past two decades regarding the natural history of chronic hepatitis C that provides important if not entirely complete or satisfactory information.

The above considerations necessary for conducting an accurate natural history study of chronic HCV infection have been taken into account in the study of the natural history of HCV in a cohort of haemophilic patients infected with HCV and followed up for over two decades.

4.2 PATIENTS AND METHODS

The cohort comprises 310 patients with hereditary bleeding disorders who had been treated with non-virucidally treated blood products. The first large pool clotting factor concentrates were used to treat factor IX deficiency in 1961 and single donor pool cryoprecipitate was used from 1966 to treat factor VIII deficiency (Bennett et al 1967). There was a gradual introduction of large pool factor VIII concentrate from 1976. From 1985, all large pool clotting factor VIII and IX concentrates used in the centre were treated with virucidal processes. Individuals with von Willebrand's disease were treated with cryoprecipitate or desmopressin (DDAVP) before 1985. Patients with factor XI deficiency were treated with fresh frozen plasma (FFP) until a heated large pool clotting factor concentrate became available in 1990 (Bolton-Maggs et al 1992).

Follow-up

The Royal Free Hospital Haemophilia Centre was established in 1964 and therefore it was possible to review the clinical and treatment records for all patients from this time up until September 1st 1999. A computerised database was introduced in 1980. Over this period, patients with severe haemophilia (factor $<2\text{u/dl}$) were seen six monthly and those with other inherited clotting deficiencies annually. From 1985 onwards, HIV seropositive patients were seen at least three monthly. Assessment has included medical history, review of home- and in-patient treatment records, physical examination, a standard panel of blood tests, ultrasound of the abdomen and endoscopy for evidence of varices. From 1979, a serum sample was taken at each clinic visit and stored at -40°C .

Statistical methods

All analysis were done with procedures in SAS (version 6.2). Comparisons between groups were made using the Chi-squared test or Fisher's Exact test for categorical variables, and using the Mann-Whitney U test for continuous variables because of the expected non-normality of these variables. Standard survival analysis methods were used to model progression to death. Person-years at risk were calculated using the date of first exposure to unsterilised concentrate until death or September 1st 1999. For those patients lost to follow up [49 (16%)] the end point was taken as the date when the patient was last reviewed at the centre; information about last attendance at another centre was used where that was available. Kaplan-Meier progression rates and 95% confidence intervals were calculated from 1985 until the censoring date for analyses relating to HIV infection. In order to visually illustrate the impact of HIV infection on survival, Kaplan-Meier plots were also drawn using a baseline date of 12 January 1985 by which time HIV-positive patients had seroconverted (Lee et al 1989b). Cox Proportional Hazard Models were used to compare survival between groups. HCV genotype and age at first exposure to concentrates grouped: <10 years; 10-19; 20-29; >30 years of age were taken as fixed covariates at baseline. Seroconversion to HIV and time of first anti-HCV therapy were considered as time-updated covariates, taking the value 0 prior to the time of seroconversion/starting therapy and 1 thereafter. The first exposure date was known for 228 (74%) patients. For those patients for whom the exposure date was not known, the median exposure date, July 1977, was used. Analyses were repeated including only those individuals with known date of infection. Results of these analyses were unchanged and therefore not included.

Virology and immunology

Fresh or serum stored from 1979 from patients who had received any blood product therapy was tested for antibody to HCV by second-generation enzyme immunoassay, EIA, (Ortho Diagnostics, Amersham, England). RIBA-2 was used as a confirmatory test in those who appeared to have cleared HCV infection naturally and were PCR negative. Confirmation of ongoing HCV infection was made by detection of HCV viraemia either by qualitative reverse-transcriptase PCR (Amplicor HCV test, Roche, Lewes, England) or branched DNA (bDNA) assays. HCV genotyping was performed by extracting serum RNA from which HCV RNA was detected by reverse transcription (RT) and nested PCR using primers derived from the 5' non-coding region (NCR) of the HCV genome. Genotype was determined from the pattern of DNA fragmentation after digestion of the PCR product with the two enzyme pairs Mva 1 + Hinf and Hae III +Rsa 1. Hepatitis B surface antigen (HBsAg) was tested by EIA (Murex Diagnostics, Abbott, Maidenhead, England). Antibodies to HIV were tested by EIA (Wellcozyme, Murex Diagnostics, Abbott, Maidenhead, England), and confirmed by gelatin particle agglutination (Mast Diagnostics, Liverpool, England and Abbott, Maidenhead, England). All the virological tests and HIV antibody tests were done by the Royal Free Hospital virology laboratory and the immunology department.

4.3 RESULTS

Serological and viral testing for HCV: Of the 310 patients who had been treated with non-virucidally treated blood products, 305 were tested for HCV and five patients (who had also been treated with large pool non virucidally treated clotting factor concentrate) had died before HCV antibody tests became available and there were no stored serum samples available for testing. 298/305 (98%) were anti-HCV positive and the

remaining seven (2%) were anti-HCV negative. Of the 200 patients in whom it was possible to determine HCV viraemia 27/200 (14%) were consistently HCV PCR negative in recent and archived specimens. These 27 individuals were thought to have cleared the virus and they have been studied in more detail.

Exposure to HCV, HBV and HIV: Of the 310 patients who were exposed to a high HCV risk blood product the source of the infection was thought to be large pool non virucidally treated concentrate in 287 (93%) and of these the date of exposure was known in 216 (75%), a median date July 1977 (range 1961–1985). One patient was treated with factor IX concentrate as early as May 1961 in Oxford. Of the 22 patients who became infected from cryoprecipitate the date of infection was known in 11, including one patient who was a carrier of haemophilia A and developed fulminant hepatitis C following treatment with cryoprecipitate in 1981 (Lee et al 1985). A patient who was treated with several hundred-donor units of cryoprecipitate at another hospital in 1990 was the only patient to be infected after 1985. Six patients, of whom two had died, were HBsAg positive. Previous study of this cohort of patients using archival specimens has shown that patients were infected with HIV between 1979 and 1985 with a median seroconversion date of 1983 (Lee et al 1989b).

Demographics (Table 4.1): Of the total 310 patients, 223 (72%) were alive at September 1st 1998, 26 (8%) had died of a liver-related death, and 61 (20%) had died from other causes. The inherited coagulation disorders were haemophilia A, factor VIII deficiency, 227 (73%); haemophilia B, factor IX deficiency, 49 (16%); von Willebrand's disease, 25 (8%); and other deficiencies 9 (3%). This distribution reflects the relative prevalence of these disorders in the haemophilic population. There were a

higher number of deaths within the haemophilia A group (85% of 26 liver-related deaths, and 95% of 61 deaths not caused by liver disease). Of the total 87 deaths, 80 patients had haemophilia A, 5 haemophilia B and 2 vWD. Of the total population of 310, 125 (40%) were infected with HIV and of these 54 were alive, 20 had died from a liver-associated death and 51 had died of other, predominantly HIV-related, causes. HCV genotyping was performed in 213 (69%) of the total 310 patients: 135 (63%) were type 1; 29 (14%) type 2; 39 (18%) type 3; and 10 (5%) other genotypes. Of the 189 (83%) living patients who were tested for HCV viraemia, 133 (70%) were positive. Of the 57 patients (including patients who have been treated) who were PCR negative for HCV, 27 (9%) were thought to have cleared the virus. All seven individuals who died a liver-related death were HCV RNA positive. The HCV viral load in serum was higher in patients who had died with liver-related deaths, median 19.1×10^6 compared to 8.1×10^6 genome eq/ml in those still alive ($p = 0.16$).

Table 4.1 : Demographics of the cohort of 310 patients

		Alive		Liver-related deaths		Other deaths		Total	
	Total	223 (100)		26 (100)		61 (100)		310 (100)	
Coagulation Disorder	Haemophilia A	147 (66)		22 (85)		58 (95)		227 (73)	
	Haemophilia B	44 (20)		3 (12)		2 (3)		49 (16)	
	VWD	23 (10)		1 (4)		1 (2)		25 (8)	
	Other	9 (4)						9 (3)	
Age in years at HCV infection	Median	15.3		27.2		23.3		17.4	
	Range	(0.01-61.3)		(6.8-62.8)		(2.0-76.8)		(0.01-76.8)	
HIV	HIV Positive	54 (24)		20 (77)		51 (84)		125 (40)	
HCV genotype	Total tested	159 (71)	(100)	22 (85)	(100)	32 (52)	(100)	213 (67)	(100)
	1	94	(59)	19	(86)	22	(69)	135	(63)
	2	23	(15)	3	(14)	3	(9)	29	(14)
	3	34	(21)			5	(16)	39	(18)
	4	2	(1)			2	(6)	4	(2)
	5	2	(1)					2	(1)
	Mixed	4	(3)					4	(2)
HCV viraemia	Total tested	189 (83)	(100)	7 (27)	(100)	4 (7)	(100)	200 (65)	(100)
	Positive	133	(70)	7	(100)	3	(75)	143	(72)
Last measured HCV viral load X 10 ⁶ genome eq/ml	Total tested	150 (67)		7 (27)		3 (5)		160 (49)	
	Median	8.1		19.1		8		8.3	
	Range	(<0.2-126)		(1.3 – 41.4)		(1.3-16)		(<0.2 – 126)	

Total cohort () percentage

Subpopulation () percentage

Kaplan-Meier analyses: The progression rates to death from any cause and deaths related to liver disease 25 years from exposure to HCV were 47% (95% CI 34-60) and 19% (95% CI 10-27) respectively (Figure 4.1). Patients were infected with HIV during the years 1979-85, by which time virucidal treatment of large pool clotting factor concentrates was in use. Therefore, in order to show the effect of HIV on the progression of HCV-related liver disease, Kaplan-Meier analyses were calculated from 1985 by which time all patients would have seroconverted to HIV. Thus, over a 13.3 year interval from 1985, the progression to death from all causes was 8% (95% CI 4-13) and 57% (95% CI 48-66) for those HIV negative and positive respectively (p= 0.0001; Figure 4.2); the progression to death related to liver disease was 3% (95% CI 0.4-6) and 21% (95% CI 13-31) for those HIV negative and positive respectively (p = 0.0001; Figure 4.3).

Figure 4.1 : Kaplan-Meier progression to liver related and all-cause deaths yearly after exposure to HCV

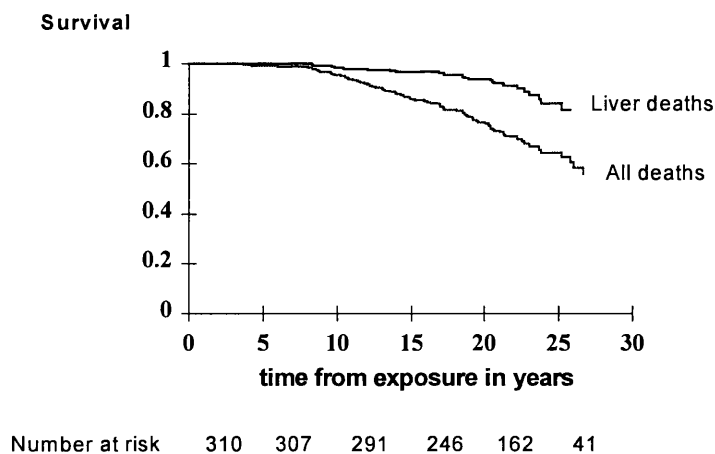


Figure 4.2 : Kaplan-Meier progression to all-cause death from 1985

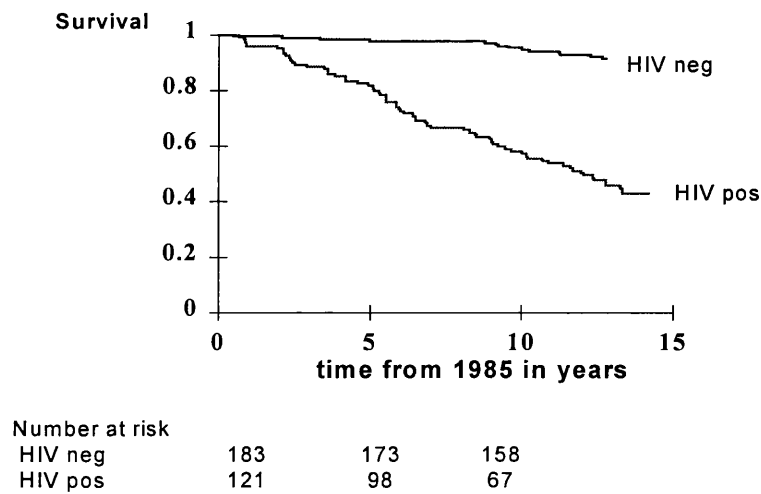
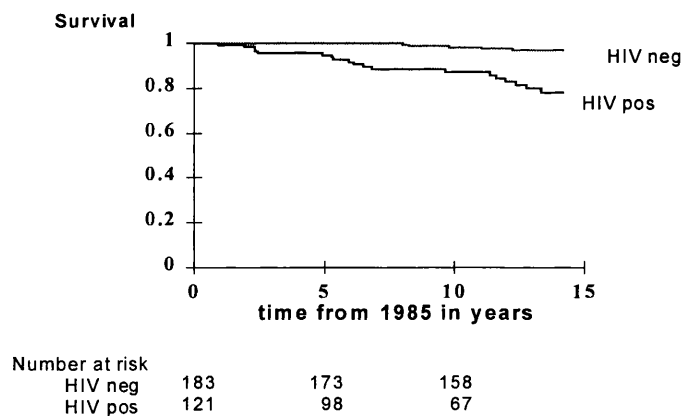


Figure 4.3 : Kaplan-Meier progression to liver-related death from 1985



Cox proportional hazards models: The influence of HCV genotype, HIV co-infection, age at infection and time of first anti HCV therapy were examined using Cox proportional hazard models (Table 4.2). The relative hazards (95% CI) associated with progression to ‘all’ and ‘liver related’ deaths, both unadjusted and adjusted, were calculated. In a multivariate analysis the relative hazard of dying for individuals co-infected with HIV compared to those infected with HCV alone was 19.47 (95% CI 9.22-41.10) after adjusting for age at infection and genotype. The adjusted relative hazard

was 0.99 (95% CI 0.39-2.53), 3.47 (95% CI 1.40-8.63) and 9.74 (CI 3.91-24.26) for the age groups at infection 10-19, 20-29, and >30 years respectively, compared to age group <10 years. The adjusted relative hazard for genotype 1 compared to other genotypes was 2.65 (95% CI 1.36-5.15). The relative hazard of liver related death, adjusted for age and genotype, for those co-infected with HIV compared to those infected with HCV alone was 17.51 (95% CI 5.82-52.72). The adjusted relative hazard for genotype 1 compared to other genotypes was 7.78 (95% CI 1.96-30.83). After adjusting for genotype and HIV co-infection, the relative hazard of liver related death was 1.66 (95% CI 0.17-16.12), 16.04 (95% CI 1.96-131.45) and 45.93 (95% CI 5.47-385.91) for the age groups at infection 10-19, 20-29 and >30 years respectively, compared to the age group <10 years. This clearly shows that the relative hazard associated with progression to death increases with age at exposure to HCV. Thus co-infection with HIV, age at infection, and genotype 1 HCV were the major determinants of death. (Since only six of the total 310 population were HBsAg positive, this was not taken into account in the hazards analysis.)

Table 4.2 : Relative hazards (95% CI) associated with progression to death

		All deaths			Liver related deaths			
Variable		Relative Hazard	95% CI	P	Relative Hazard	95% CI	P	
(a) Unadjusted	Genotype 1	1.91	(1.03 – 3.57)	0.04	3.92	(1.16 – 13.24)	0.03	
	HIV seroconversion*	7.60	(4.63– 12.37)	0.0001	4.64	(2.06 – 10.45)	0.0002	
	Age at HCV infection (in years)	<10	1.0					
		10-19	2.08	(0.96 – 4.51)	0.06	1.59	(0.29 – 8.7)	0.06
		20-29	2.80	(1.25 – 6.29)	0.01	5.95	(1.26 – 28.04)	0.02
	>30	6.43	(3.11 – 13.30)	0.0001	9.18	(2.05 – 41.06)	0.004	
	Time of first anti-HCV therapy*	0.71	(0.32 – 1.58)	0.41	1.22	(0.40 – 3.74)	0.72	
(b) Adjusted	HIV Seroconversion*	19.47	(9.22 – 41.10)	0.0001	17.51	(5.82 – 52.72)	0.0001	
	Age at HCV infection (in years)	<10	1.0		1.0			
		10-19	0.99	(0.39 – 2.53)	0.987	1.66	(0.17 – 16.12)	0.66
		20-29	3.47	(1.40 – 8.63)	0.007	16.04	(1.96 – 131.45)	0.01
		>30	9.74	(3.91 – 24.26)	0.0001	45.93	(5.47 – 385.91)	0.0004
	Genotype 1	2.65	(1.36 – 5.15)	0.004	7.78	(1.96 – 30.83)	0.004	

*Time dependent variables

Patients who died and had a liver-related pathology: Twenty-six patients had died and had liver-related pathology at the time of death. All were male with a median duration of HCV infection of 17 years (range 8 - 25). The median age at death was 47 years (range 23 - 76). Two patients were HBsAg positive. Of the 26 patients 20 (77%) were co-infected with HIV and five (19%) were known to have an increased alcohol consumption, defined as greater than 80 grams of alcohol per day. Thus of the six HIV-negative patients, four had increased alcohol intake, one died from haemorrhage following a liver biopsy performed in 1981 (Lee 1997). HIV infection and/or alcohol were cofactors in majority of the deaths. Seventeen patients died in hepatic failure, seven had AIDS, four had hepatocellular carcinoma (HCC). The details of the patients with HCC are outlined in Table 4.3. All four patients with HCC had underlying cirrhosis. Biopsy or post-mortem studies showed 12 patients had cirrhosis, two chronic hepatitis and one patient had drug related hepatotoxicity.

Table 4.3 : Main features and outcomes of the four patients with HCC

Parameter	Patient 1	Patient 2	Patient 3	Patient 4
Haemophilia (HA) A	Severe HA	Severe HA	Severe HA	Severe HA
Age at HCV infection (years)	46	27	27	21
Duration of HCV Infection (years)	21	17	21	22
Age at HCC Dx (years)	67	44	48	43
HCV-RNA type	Type 1	Type 1	Type 1	Type 1
HCV viral load (genomes eq/ml)	-	-	42 million	7 million
Anti HIV	Negative	Positive	Positive	Positive
HIV VL (copies/ml)	-	11,600	7800	1300
CD 4 (μ L)	1000	500	144	171
HbsAg	Negative but previously infected with HBV	Negative	Negative	Positive
Alcohol	20 units/week	5 units/week	Nil	Nil
AFP at Dx (ku/L)	18	2	506	178
Cirrhosis	Present	Present	Present	Present
Imaging results	CT & MRI numerous space occupying lesions	CT Multifocal HCC	CT, MRI space occupying lesion	CT Unifocal 3.75 cm lesion in right lobe and widespread pulmonary metastases
Treatment	Refused Rx	Short course IFN	Nil	IFN Rx off & on since 1984 (for HBV) for 14 years. Targeted lipiodol ¹³¹ injection
Survival/Yr of death	7 months/1991	6 months/1998	4 months/1998	5 months/1998

Natural immunity: Of the 27 patients who were thought to have cleared the virus, 26 were male and one female. Seven had severe haemophilia A (factor VIII < 2U/dl), seven had moderate/mild haemophilia A (factor VIII > 2U/dl), four severe haemophilia B (factor IX < 2U/dl), four mild/moderate haemophilia B (factor IX > 2U/dl), one was a carrier of haemophilia A and four had von Willebrand's disease. Three, all with severe haemophilia A, of the 27 patients were co-infected with HIV. Three patients had been treated only with cryoprecipitate. The remaining 24 had been treated with both cryoprecipitate and large pool clotting factor concentrate of both US and UK donor pool origin. The age at first exposure was a median 9 years, (range 3 months to 56 years). In 25 patients normal liver serum aminotransferases (ALT) were recorded at least once per year for a median follow-up period of 15 years. One of the two patients who recorded abnormal liver aminotransferases had increased alcohol intake; the other received antiretroviral therapy for HIV and this was thought to be the cause of the abnormalities. Serial measurements of HCV viraemia have been performed on a median of 5 (range 3-10) archived and recent (within the previous eight months) serum samples in these 27 patients. HCV RNA has been undetectable in all 154-serum samples that have been tested.

4.4 DISCUSSION

We reported on this cohort up until 1993, (Telfer et al 1994a) and have more recently extended our observation of the natural history of this infection to 1999 as observation of long-term infection is intrinsic to the successful management of HCV infected individuals.

This study is the follow up a in single haemophilia centre of a large number of patients (310 patients) some of whom have been exposed to the HCV virus for over three decades. We have included detailed individual patient observations, including date of

infection, HCV viral load, genotype as well as cause of death and influence of treatment. Virtually all patients treated with non-virus inactivated clotting factor concentrates during the 1970s were infected with HCV at the time of the first infusion (Kernoff et al 1985) and therefore the onset of infection could be reasonably estimated (first treatment with non-virus inactivated blood products) in the majority of the patients. Since 1986 there has been virtually no transmission of HCV in countries with access to viral inactivated concentrates (Mannucci 1996).

This detailed follow-up of 310 haemophilic patients has shown the lethal combination of HCV and HIV co-infection, with a 47% progression to all cause death and 19% to a liver-related death at 25 years. However, for those HCV-positive individuals without HIV infection, we have shown hepatitis C to be a very slowly progressive disorder with a 3% progression rate to a liver-related death from the time of HIV infection in 1985.

An analysis of 138 patients from Sheffield showed that patients who are HIV positive had a 3.9 fold and 4.2 fold increased incidence of cirrhosis and liver failure respectively (Makris et al 1996). The effect of coinfection was also documented in an American study of 223 haemophiliacs in whom liver failure was found in 8/91 (9%) of coinfecting patients but in none of the 58 HCV+/HIV- patients between 10-20 years after exposure to blood products (Eyster et al 1993). A recent study of the natural history of hepatitis C in a cohort of 102 HIV negative Italian patients with hereditary bleeding disorders revealed that there were only 3.4% liver-related deaths (Franchini et al 2001) confirming our data of the slow progression of HCV infection in HIV-seronegative haemophiliacs.

The mechanism by which HIV infection, which occurred in the haemophilic population up to a decade or more after initial HCV infection (Kasper & Kipnis 1972), may increase the risk of HCV liver disease progression is unclear. The progressive decline in CD4⁺ T lymphocytes, defective CD4⁺ proliferation, and CD4⁺ apoptosis may

weaken the already limited immune response to HCV (Fauci et al 1991). HIV is also a potent activator of immune response through the up-regulation of the production of cytokines (Fauci et al 1991), including IL-6 and transforming growth factor- β , which also are known to promote hepatocyte proliferation and fibrosis (Rockstroh et al 1996). In untreated patients with chronic HCV infection the viral load is relatively stable (Nguyen et al 1996). HCV viral loads have been reported to be higher in co-infected individuals in previous studies in this cohort (Telfer et al 1994b) and by others (Eyster et al 1994). The viral load or level of circulating HCV RNA in serum is a reflection of both the rate of viral replication and the rate of viral clearance in the infected host. The viral loads found in our patients are relatively high, particularly in those co-infected with HIV and presumably this reflects the inability to contain the virus in the face of immunosuppression.

The effect of hepatitis C on progression of HIV-1 infection has been less clear. Most studies have not found a clear effect on immunological indices or progression of opportunistic infections but these studies were done before the era of HAART (Zylberberg et al 1996; Staples et al 1999). Recently a large well characterised cohort of patients with HIV infection has been studied on the clinical progression, survival and immune recovery during antiretroviral therapy and was shown that hepatitis C affects immune reconstitution in HIV-1 infected patients (Greub et al 2000).

There are contradictory results reported with respect to the influence of genotype on progression (Seeff 1997). We have found that HCV genotype 1 was associated with an increased relative hazard of progressing to death from all causes and specifically to death from liver disease, even when this was adjusted for HIV infection and age at infection. Genotype 1 was associated with more severe disease in 4,176 HCV-positive Japanese patients (Yamanda et al 1995), and in a further Japanese study in 140 patients, genotype 1 was associated with deterioration of the liver biopsy (Kobayashi et al 1996).

Dusheiko and colleagues also found more severe disease in type 1, compared to type 2 HCV infection (Dusheiko et al 1994). However, a recent study from Italy has shown no role of genotype in determining disease outcome (Romeo et al 1996). The large-pool clotting factor used to treat these patients was obtained from donors in the UK and US and thus the distribution of genotypes reflects the prevalent genotypes found in North Europe and North America, genotypes 1, 2, and 3 (Jarvis et al 1995). However, in haemophilic patients in the USA 45%, 7% and 42% are reported to have genotype 1, 2 and 3 (Eyster et al 1999), compared to 63%, 14%, and 18% respectively in our cohort. The majority of these patients were exposed over a long period to many batches of clotting factor concentrate, each bottle being made from a donor pool of several thousand donors. It is not clear why a particular genotype becomes the dominant genotype in any one haemophilic patient. The prevalence of genotype 1 may reflect a less robust immune response at the time of infection with HCV, and this in turn could explain the influence of genotype 1 in hastening the progression of HCV; type 1 may have a greater replication competence or may have been epidemiologically more prevalent prior to HIV infection.

We have shown that 9% of these haemophilic patients spontaneously cleared HCV, as have others (Mauser-Bunschoten et al 1995) and in almost half of individuals infected with anti-D immunoglobulin (Dittmann et al 1991; Kenny-Walsh 1999). The majority of the patients who cleared HCV had non-severe haemophilia and therefore infrequent treatment with clotting factor concentrate. Thus the viral variants, including more virulent variants, to which they will have been exposed is likely to have been less. The patients who cleared the virus were young at first exposure and therefore it is possible that their immune response at the time of infection was more effective. Thus, a report from Germany showed that many individuals who had acquired hepatitis C infection in childhood during cardiac surgery had spontaneously cleared the virus after 20 years

(Vogt et al 1999). Of patients who became infected with HIV from clotting factor concentrates most would also have been infected with HCV, either at the same time or earlier. Thus it is remarkable that three individuals co-infected with HIV in our cohort have apparently cleared HCV with consistently negative HCV viraemia.

Two-thirds of the HIV-negative patients who died in this study had increased alcohol consumption. A critical question is whether alcohol and HCV are synergistic in causing a liver injury. The pathogenesis of chronic hepatitis C in those with an increased alcohol intake may be caused by combined alcohol and HCV-induced liver injury or by enhanced injury from hepatitis C caused by the effects of alcohol on the immune system, on viral replication or on hepatocellular responses to cell injury. Alcohol intake in excess of 10g/day has been associated with increased serum HCV RNA and aminotransferase levels (Mendenhall et al 1991; Nishiguchi et al 1991) and chronic alcoholism in patients with HCV appears to cause more severe and rapidly progressive liver disease, leading more frequently to cirrhosis of the liver and hepatocellular carcinoma (Schiff 1997).

HCV infection is now recognised as a major risk factor for hepatocellular carcinoma (HCC) and has been estimated that between 1.9 % and 6.7% of all patients with chronic hepatitis C can be expected to develop HCC over the first two decades of infection (Di Bisceglie 1997). HCC is a terminal complication of chronic inflammatory and fibrotic liver disease. Other risk factors for development of HCC are chronic infection with hepatitis B virus and alcoholic liver disease. In our haemophilia cohort 4/278 (1.4%) with chronic HCV infection developed HCC after two decades of HCV infection and this is likely to become more common in this group of patients who were infected at a median time of 1977. Cirrhosis of any cause is the seedbed of HCC and all four patients in our series had underlying biopsy proven cirrhosis. There is little evidence as yet that co-infection with HIV increases the risk of liver cancer but three of four patients in our



centre were HIV positive for a mean duration of 15 years. The HIV status of these patients on antiretroviral therapy was stable with a mean CD 4 count of 123 cells/ μ L and a mean HIV viral load of 6900 copies /ml. A recent study (Woitas et al 1999) has shown that asymptomatic HIV co-infection alters the HCV specific cytokines towards a greater production of proinflammatory type 1 cytokines and suggested that altered cytokine pattern may contribute to the adverse course of hepatitis C in HIV co-infection. A study up to 1st January 1993 of mortality of liver cancer in haemophilic men and boys in UK has shown that liver cancer has been present in about 10% of the liver related deaths (Darby et al 1997). As HIV antiretroviral therapy makes a dramatic impact on the mortality of HIV infected patients the longer follow up of HIV/HCV co-infected haemophiliacs may show an increase in hepatitis C related liver diseases and liver cancer.

One patient died only eight years after infection with HCV as a result of surgical bleeding after liver biopsy (Lee 1997). It is largely as a result of this unfortunate experience that, after an initial enthusiasm for liver biopsy (Bamber et al 1981), we have chosen to manage this patient group conservatively relying on the detailed clinical history, liver function tests, HCV genotype, viral load and PCR, together with endoscopy and imaging in order to chart the natural history and monitor treatment for HCV. Thus the data on cirrhosis are limited and include those for whom there was a rare biopsy performed in life, or post-mortem liver biopsy. A survey of the management policies of the 100 haemophilia centres in the UK in the early 1990s showed that of the 4000 HCV infected haemophiliacs in the UK, 90 have been biopsied, 194 endoscoped, 293 have had an ultrasound, and only 123 have been offered interferon treatment (Tibbs & Williams 1995).

Unfortunately a large number of the haemophilia patients have bad prognostic factors for successful eradication of HCV infection such as: male sex, high viral load, long

period of infection, type 1 HCV and HIV co-infection. Nevertheless a minority of these patients have responded to alpha interferon alone or combination therapy (Lee et al 1989a; Telfer et al 1995; Dusheiko et al 1996; Bhagani et al 1998).

Clearly the challenge is to provide treatment to delay progression or 'cure' these patients. Guidelines on the diagnosis, management and prevention of hepatitis in haemophilia has recently been published by the transfusion transmitted infection working party of the UK Haemophilia Centre Directors Organisation (Makris et al 2001) and hopefully this will lead to more patients being offered treatment.

Chapter 5

HUMAN IMMUNODEFICIENCY VIRUS

- 5.1 OVERVIEW OF HIV-1**
- 5.2 HIV-1 INFECTION AND HAEMOPHILIA**

5.1 OVERVIEW HUMAN IMMUNODEFICIENCY VIRUS (HIV VIRUS)

The discovery of and testing for HIV

The major breakthrough for the discovery of HIV came in April 1984 when the isolation of a human a new human T-lymphotropic retrovirus from cultured T lymphocytes from two brothers of haemophilia B was described by Montagnier and his group in the Lancet (Vilmer et al 1984). Preliminary biochemical characterisation of the major viral proteins and development of a prototype enzyme-linked immunosorbent assay (ELISA or EIA) and Western blot assay for antiviral antibodies (Sarngadharan et al 1984; Schupbach et al 1984) was followed by licensing of the first commercial enzyme immunoassay (EIA) for detecting anti-HIV antibodies in donated blood and plasma by the US Food and Drug Administration (FDA) in March 1985. The sensitivity of current EIAs for antibody-positive sera is essentially 100%, and the specificities range from 99.8% to 99.95% (Dodd 1994).

The human immunodeficiency virus (HIV-1) has the basic structure of other retroviruses. It contains three major structural gene regions – *gag*, which yields the viral core proteins, *env*, which yields the envelope proteins and *pol*, which yields the reverse transcriptase and integrase enzymes. These enzymes are required for replication of a DNA copy of the viral RNA genome and the integration of this DNA provirus into the genome of the host cell. The structural proteins are the major antigens of HIV-1, particularly the major core protein p24 and its precursor p55, the glycosylated external and transmembrane envelope proteins gp120 and gp41, respectively, and their precursor gp160 (Gallo 1990). As such, HIV-1 antibodies are predominantly directed against these five proteins.

5.2 HIV-1 INFECTION AND HAEMOPHILIA

Transfusion of blood, blood components, and some plasma products from 1978 until 1985/6 resulted in many infections with human immunodeficiency virus type 1 (HIV-1). Persons with haemophilia were at especially high risk by virtue of their need for infusions of factor VIII or factor IX concentrate, each lot derived from the plasma of thousands of donors. The first case of AIDS in a patient with haemophilia was diagnosed in October 1981 and reported by the CDC in July 1982.

HIV-1 Epidemiology in haemophilia

The percentage of patients infected with HIV differs widely from country to country. The highest prevalence is found in countries that predominantly use clotting products manufactured from the plasma of paid donors in the United States (Bottiger et al 1988; Melbye et al 1984; Rouzioux et al 1985). In the United States itself 90% of severely affected haemophilia patients are seropositive, in West Germany 53%, in the United Kingdom 39% (Goedert et al 1985; Erfle et al 1985; UKHCDO 1986). In countries that predominantly used products manufactured from local donors, the numbers of seropositive patients are much lower: Belgium 7% (Haemophilia Study Group 1988), Norway 8% (Evensen et al 1987), the Netherlands 17% (Rosendaal et al 1988), Finland 1%. In France, 50% of haemophilia patients have become HIV positive in spite of a blood product supply predominantly of local origin (Allain 1986).

In the UK it is likely that patients first became infected in 1979: a well documented patient received a first infusion with concentrate of US donor origin on August 23 1979 from which he was subsequently shown to seroconvert following a seroconversion illness in December 1979 (Lee et al 1990). Between 1979 and 1985, when heat-treated concentrates were introduced to the UK, 1227 of the 6278 (19.5%) haemophilic individuals in the country became infected with HIV (Darby et al 1995). Because these

men were infected so early in the epidemic, many died before the introduction of antiretroviral therapy (ART) and few have received highly active antiretroviral therapy (HAART). In the UK the death rate rose steeply from 8/1000 in 1985-1992 in seronegative individuals to 81/1000 in 1991-1992 in seropositive individuals (Darby et al 1995).

Chapter 6

TWO DECADES OF HIV INFECTION IN A COHORT OF HAEMOPHILIC INDIVIDUALS: CLINICAL OUTCOMES AND RESPONSE TO HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART)

- 6.1 INTRODUCTION**
- 6.2 PATIENTS AND METHODS**
- 6.3 RESULTS**
- 6.4 DISCUSSION**

6.1 INTRODUCTION

HIV infected haemophilia cohort of the Royal Free Hospital Haemophilia Centre

Stored serum samples from 525 haemophilia patients registered at the Royal Free Hospital haemophilia centre were first tested for HIV in 1984 with an in-house antibody assay. They were all re-tested with the first licensed commercial enzyme immunoassay (EIA) introduced by the US Food and Drug Administration for detecting anti-HIV antibodies in 1985 and 111 individuals were found to be HIV positive (Lee et al 1989b). 101 patients had severe haemophilia A (FVIII<2u/dl), seven had moderate to mild haemophilia (FVIII>2u/dl), one severe haemophilia B and two von Willebrand's disease. All except two patients had been treated with unheated factor VIII, one had been treated with unheated factor IX and the other with cryoprecipitate. It has subsequently been established that this patient probably acquired the infection sexually.

HIV infected patients have been closely followed up in our centre three to four times annually. Patients are reviewed by a doctor, a social worker and a nurse. At each visit blood samples are taken and some are stored at -40 degree centigrade for future use. High purity factor VIII and IX clotting factor concentrates were used for HIV seropositive patients since the recommendations by the United Kingdom Haemophilia Centre Directors' Organisation in 1992 (UKHCDO 1992).

Patients are offered antiretroviral therapy in line with guidelines in place at the time. From July 1987, 20 patients were started on treatment with zidovudine for advanced symptomatic HIV related disease. From October 1988 patients were entered into the Medical Research Council double-blind placebo controlled trial of zidovudine for asymptomatic patients (Concorde 1 trial). The centre participated in various HIV clinical trials conducted by the Medical Research Council (MRC) over the years.

Since 1988, patients with CD4 counts less than 200 cells/mm³ have been offered prophylaxis against *Pneumocystis carinii* pneumonia (PCP) and candida. In addition, since 1991, patients have been offered prophylaxis against *mycobacterium avium* intracellulare if their CD4 count falls below 50 cells/mm³.

From February 1990 zidovudine was prescribed from the NHS for patients with a CD4 count of 200cells/ml or patients who were symptomatic. Guidelines by the British HIV association on antiretroviral therapy are closely followed and since late 1995, patients have received HAART regimens including at least one protease inhibitor (PI) or non-nucleoside reverse-transcriptase inhibitor (NNRTI). The decision to use PI therapy in patients from this group is made cautiously, due to the possible implications of liver complications in patients co-infected with HCV, and because of a number of reported bleeding episodes in haemophilic men receiving PI therapy (Yee et al 1997a; Ginsburg et al 1997).

Studies on the cohort

The natural history of HIV infection in a haemophilia cohort which was the first description of this group of individuals was published in 1989 and since then studies on the impact of HIV treatment and prophylaxis against opportunistic infections (as it evolves) on the progression of HIV disease have been published (Lee et al 1991).

The earliest person to seroconvert in this cohort did so 19.5 years ago and is currently well with undetectable HIV viral load (< 50 copies/ml) and a CD4 count of 350 cells/ml. Therefore this cohort represents one of the longest prospectively followed cohorts of HIV seroconverters in the world.

The majority of haemophilic men infected with HIV are chronically infected with hepatitis C virus (Kernoff et al 1985), which has implications both for their clinical

prognosis and choice of and response to antiretroviral therapy (Eyster et al 1993; Telfer et al 1994b; Yee et al 1997a).

In this paper, we focus on the clinical status of the above group of patients at April 1999 and on their uptake of and response to antiretroviral therapy.

6.2 PATIENTS AND METHODS

Patients

The cohort of 111 haemophilic men, all initially registered at the RFHHC, seroconverted to HIV between 1979 and 1985 and have previously been described (Lee et al 1989b). The cut-off date for this analysis was April 1999 and the patients had been followed for a median 16.9 (range 8.0 to 19.5) years. Since the start of the study, eleven patients have transferred their medical care to other centres. Vital status and information on AIDS status has been obtained through direct contact with these patients or their treating physicians.

The availability of stored serum samples enabled us to retrospectively test samples for HIV seropositivity and estimate seroconversion dates for each patient (Lee et al 1989b). The patients ranged in age from 1.9 to 77.8 (median 22.6) years at the time of seroconversion. All patients had been exposed to HCV either before or at the time of infection with HIV.

Laboratory methods

As part of a study on the natural history of viral load throughout HIV infection, HIV RNA levels were retrospectively measured on samples collected yearly after HIV-1 seroconversion (Sabin et al 2000a). HIV-1 RNA was measured in serum using the Roche Amplicor HIV-1 Monitor™ v1.0 Assay plus add-in non-B primers (range 400 to 750,000 copies/ml, Roche Diagnostic Systems, Branchburg, NJ, USA). Since 1996,

HIV-1 RNA has been measured prospectively on fresh plasma samples using the Roche Amplicor Monitor™ v1.5 Assay (range 400 – 750,000 copies/ml) and the ultrasensitive version of this assay (range 50-75,000 copies/ml). All HIV-1 RNA measurements are reported on the log₁₀ scale.

Since 1982, lymphocyte subsets have been routinely performed on all fresh samples at the hospital as described previously (Sabin et al 2000a).

Clinical and laboratory data on the cohort has been routinely collected since the 1980s and has been entered into a Statistical Analysis System (SAS) computer data set and updated on a regular basis. The development of non-AIDS clinical conditions (e.g. bacterial infections, herpes zoster, oral candida etc.) and AIDS defining conditions, treatment and laboratory data are collected and a statistician enters all data onto the computer and various analyses are performed.

Statistical methods

Progression to clinical AIDS (a CD4 count < 200 copies/mm³ was not considered AIDS-defining) and survival were described using the Kaplan-Meier method. Patient follow-up was considered from the time of HIV seroconversion until the time of initial AIDS-defining event or death. Follow-up of patients who remained AIDS-free and alive was right-censored on 30th April 1999. For some patients who had transferred their care elsewhere, we were able to obtain information about the patient only at some point prior to 30th April 1999; therefore, in these patients, follow-up was right-censored on the date on which information about their current vital/AIDS status had been received. Where information was not available on current AIDS status, or where the date of development of AIDS was not given, patient follow-up was right-censored three months after the individual's last visit to the RFHHC.

Patient follow-up was stratified into that which occurred in seven different calendar periods (prior to 1987, 1987-1988, 1989-1990, 1991-1992, 1993-1994, 1995-1996 and 1997 onwards). Clinical events occurring in each of these time periods were summed and divided by the total patient-years of follow-up in each period, to give an event rate for each calendar period. Confidence intervals (CI) for these rates were calculated using the exact Poisson distribution when the number of events in a stratum was less than 20, and the Normal approximation to the Poisson distribution when the number of events was larger (Ahlbom 1993).

Comparisons of continuous measurements in patients currently under follow-up who had and had not received ART, were performed using the Mann-Whitney U test; comparisons of categorical measures were performed using the Chi-squared test, or Fisher's exact test where expected frequencies were small.

In order to summarise the information regarding changes in HIV RNA and CD4 count following treatment with HAART (defined for this study as any treatment combination of three or more drugs, including at least one PI or NNRTI), two sets of analyses were performed. Firstly, the mean change in RNA level and CD4 count in each three-month period following the start of HAART (1-3 months, 4-6 months, 7-9 months and 10-12 months) was calculated for each patient. The median and 95% CI of the values at each time point were plotted. These analyses included all follow-up measurements, irrespective of whether the patient had changed or discontinued therapy. Therefore, as a second analysis, we considered the time to an RNA level below 400 copies/ml in those individuals starting HAART with an RNA level above this level at the time of starting HAART. Kaplan-Meier analyses were used to summarise this information. Patient follow-up was right censored if an individual changed their initial HAART regimen, at death, or on 30th April 1999 if they had not had an RNA level below 400 copies/ml at that point.

All analyses were performed using the SAS software package (SAS Institute 1992).

6.3 RESULTS

Progression to AIDS

By the end of April 1999, 57 patients (51.4%) had developed AIDS, a Kaplan-Meier progression rate of 57.0% by 19.5 years after seroconversion (95% CI 46.9%-67.0%, Figure 4.1), with a median time to AIDS diagnosis of 13.6 years. Eighteen patients (31.6% of those with AIDS) developed PCP as their initial AIDS-defining disease. Other common initial AIDS-defining events were oesophageal candida (eight patients, 14.0%), lymphoma (five patients, 8.8%), HIV encephalopathy (four patients, 7.0%), cryptosporidiosis, cytomegalovirus (CMV) disease, toxoplasmosis, and mycobacterium infections (three patients each, 5.3%). The pattern of subsequent events was broadly similar, although mycobacterium infections (eight subsequent events) and CMV infections (seven subsequent events) were much more frequently observed as a subsequent condition, than as initial AIDS-defining events.

The rate of initial AIDS-defining diagnoses peaked in the period 1987-1988 (Table 6.1), with 1.03 events per 10 years of follow-up. The rate then dropped but remained relatively stable at between 0.47 and 0.77 events per 10 years of follow-up until the end of 1996. Since then, only one patient has developed an initial AIDS-defining illness, giving a rate of 0.13 events per 10 person-years of follow-up in the period 1997-1999.

Survival

By the end of April 1999, 65 patients (58.6%) had died, a Kaplan-Meier progression rate of 65.1% by 19.5 years after seroconversion (95% CI 52.7%-77.4%, Figure 6.1), with a median survival of 14.4 years. Death rates from 1987 onwards remained stable

at between 0.56 and 1.00 per 10 years of follow-up (Table 6.1). Noticeably, there has been no drop in death rates since 1997; indeed the death rate in the period 1997-1999 is the second highest over all follow-up periods.

Until 1995, almost all deaths were in individuals with an AIDS diagnosis (43 of 50 deaths, 86.0%). However, since 1995, this proportion has dropped – only 8 of the 15 deaths occurring since 1995 (53.3%) have occurred in individuals who had a prior AIDS diagnosis. When we considered the actual cause of death in these individuals the difference was even more striking. Of the 50 deaths occurring prior to 1995, 35 (70.0%) were directly from AIDS causes, five (10.0%) were due to liver-related causes and ten (20.0%) due to other apparently unrelated causes. In contrast, of the 15 deaths occurring since 1995, only seven (46.7%) were directly from AIDS causes, five (33.3%) were from liver-related causes, and the remaining three (20.0%) were due to ‘unrelated’ causes (p-value for difference 0.09, Fisher’s exact test).

Treatment patterns

Of the 111 patients in the cohort, 80 (72.1%) are known to have received ART (Table 6.2). Of the 65 patients who have died, only 10 (15.4%) received dual combination therapy with NRTIs and five (7.7%) combination therapy including a PI or NNRTI, reflecting the lack of treatments available at the time when many of these patients died. The 35 patients who remain alive and who obtain their haemophilia/HIV care at the RFHHC have experienced a wide range of treatment regimens as ART availability has changed over time. Twenty-eight of these patients (80.0%) have received ART at some stage during infection. There were no significant differences between these 28 patients and the seven who have not received ART in terms of their current age, length of follow-up or previous AIDS diagnosis (Table 6.3). As expected, those who had received ART had a lower median CD4 count at the end of follow-up ($p=0.006$) and

lower nadir count over follow-up ($p=0.0002$) than those who had not received ART. Whilst individuals who had received ART had higher peak RNA levels over follow-up than those who had not received ART ($p=0.008$), there were no significant differences between the most recent RNA levels in these two groups ($p=0.83$). Twenty-four of the 28 patients who have received ART (85.7%) are currently still receiving therapy; 15 are receiving treatment combinations with three or more drugs including a PI or NNRTI, two patients are receiving triple NRTI therapy, and seven are receiving dual nucleoside therapy.

Response to PI/NNRTI therapy

Twenty-two patients in the cohort are known to have received HAART including at least one PI or NNRTI whilst under follow-up at the RFHHC (Table 6.2). Patients started HAART between November 1995 and March 1999. Eighteen patients received a PI-containing combination (ritonavir-11 patients, saquinavir-10 patients, nelfinavir-5 patients, indinavir-2 patients) and four received an NNRTI-containing regimen (nevirapine-3 patients, loviride-1 patient). Seventeen patients were ART-experienced at the time of starting HAART. All of these patients had previously received zidovudine, other antiretroviral drugs previously received were lamivudine (12 patients), didanosine (ten patients), zalcitabine (ten patients) and stavudine (two patients). Twelve of these 17 patients added one or more drugs to an existing treatment regimen, four switched all of their existing drugs to drugs that they had never previously received and one switched all drugs but his new combination included a drug (zidovudine) to which he had been exposed for a period in the past.

Pre-treatment CD4 counts were generally low (median 100, range 0 to 330 cells/mm³) and RNA levels high (median 5.13, range 2.60 to 5.88 log₁₀ copies/ml). One patient had an RNA level below 400 copies/ml at the time of starting HAART.

Over a median of 464 (range 44 to 1248) days since starting HAART, eleven patients made changes to their initial HAART regimen by stopping, switching or intensifying their treatment. This first occurred after a median of 116 (range 7 to 504) days. In addition, three patients took a short treatment break before restarting the same HAART regimen. Four patients have died over follow-up; the causes of death in these four patients were cerebral haemorrhage, liver failure/hepatocellular carcinoma, PCP, and bronchopneumonia.

Eighteen patients had at least one RNA level (median 5, range 1 to 10) after starting HAART. RNA levels dropped by a median of 0.6 log₁₀ copies/ml in the first three months following initiation of HAART and continued to drop thereafter (Figure 6.2), although there was wide inter-individual variability in the response to HAART. Ten of these 18 patients achieved an RNA level below 400 copies/ml over follow-up, although in four cases this occurred after a switch from the initial HAART regimen. After censoring patients at the time of changing their HAART regimen, the Kaplan-Meier rate of having an RNA level below 400 copies/ml was 51.1% by two years after starting HAART. Nineteen patients had at least one CD4 count (median 5, range 1 to 20) after starting HAART. CD4 counts increased by a median of 95 cells/mm³ in the period 3-6 months after starting HAART (Figure 6.2) but this initial increase dropped to around 60 cells/mm³ by 10-12 months after starting HAART.

Clinical side effects of HAART

Eight of the 18 patients receiving PIs have had unusual bleeds. One patient stopped treatment totally, two switched to another PI and one switched to an NNRTI. In all cases, the bleeding improved following changes in treatment. The remaining four patients remained on the same treatment; in these patients the bleeding either improved spontaneously, or the patients took increased doses of factor VIII prophylaxis. One

patient receiving a PI developed diabetes and abnormal lipid levels; an additional patient receiving a PI developed lipomas in both breasts.

Figure 6.1 : Kaplan-Meier plot showing progression to AIDS and death yearly after HIV seroconversion

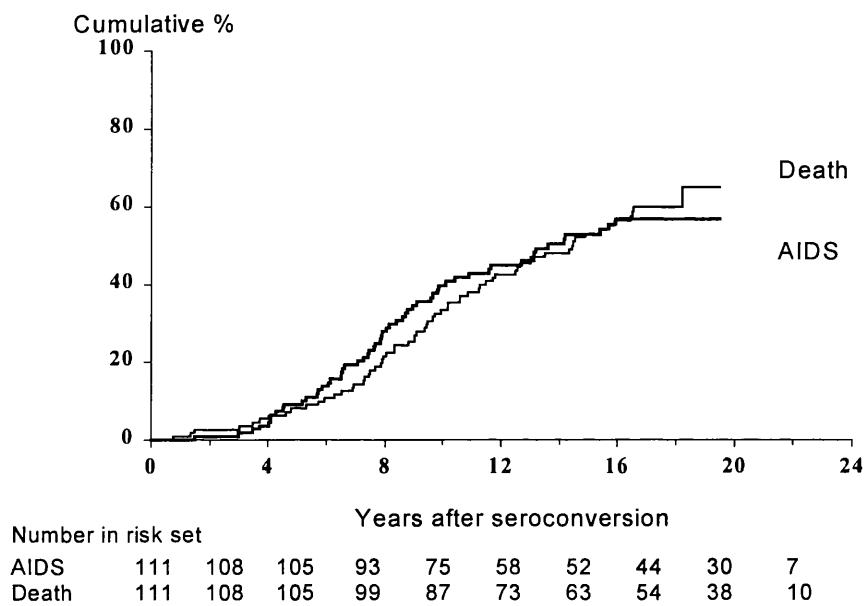
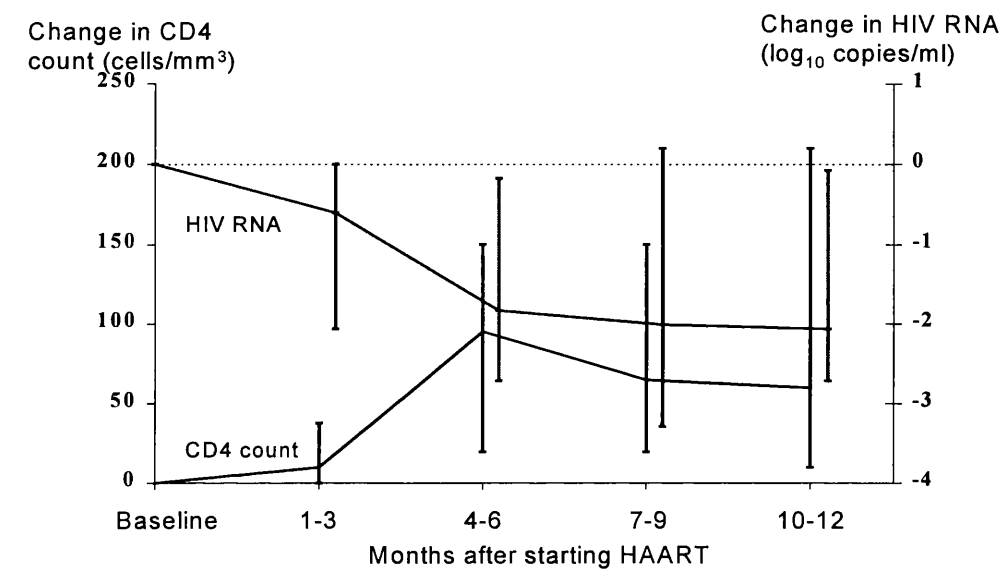


Figure 6.2 : Median (95% Confidence interval) change in HIV RNA level and CD4 count in 22 patients starting HAART including either a PI or NNRTI.



No. of patients with measurement

No. patients under follow-up	22	18	18	16
CD4	15	14	15	11
RNA	12	7	7	11

Table 6.1 : Event rates for initial AIDS-defining illnesses, all AIDS-defining illnesses and death subdivided by calendar period

Calendar period	Initial AIDS-defining events				Any AIDS events*				Deaths			
	Person-years [§]	No. of events	Rate/10 yrs	95% CI	Person-years [§]	No. of events	Rate/10 yrs	95% CI	No. of events	Rate/10 yrs	95% CI	
<1987	520.21	6	0.12	0.04-0.25	522.01	6	0.11	0.04-0.25	6	0.11	0.04-0.03	
1987-1988	184.22	19	1.03	0.62-1.61	197.07	27	1.37	0.85-1.89	11	0.56	0.28-1.00	
1989-1990	149.70	10	0.67	0.32-1.23	174.66	28	1.60	1.01-2.20	11	0.63	0.31-1.13	
1991-1992	120.53	9	0.75	0.34-1.42	139.80	22	1.57	0.92-2.23	14	1.00	0.05-1.68	
1993-1994	106.94	5	0.47	0.15-1.09	123.91	12	0.97	0.50-1.69	8	0.65	0.28-1.27	
1995-1996	91.33	7	0.77	0.31-1.58	105.56	14	1.33	0.73-2.23	6	0.57	0.21-1.24	
1997-1999	79.45	1	0.13	0.00-0.70	95.97	6	0.63	0.23-1.36	9	0.94	0.43-1.78	
Total	1252.39	57	0.46	0.34-0.57	1358.99	115	0.85	0.69-1.00	65	0.48	0.36-0.59	

* Initial and subsequent AIDS events, including any recurrences classified as distinct episodes from first occurrence.

§ Person years of follow-up is censored at time of AIDS diagnosis for initial AIDS events, but is censored at time of death for all AIDS events and deaths. Therefore, person-years for all AIDS events and deaths are the same.

Table 6.2 : Treatment patterns in the cohort

Treatment pattern [§]	Clinical status of patients on 30 th April 1999		
	Dead n (%)	Alive and under follow-up at RFHHC n (%)	Alive, haemophilia care provided elsewhere* n (%)
Number of patients	65	35	11
No known antiretroviral therapy	17 (26.2)	7 (20.0)	7 (63.6)
Zidovudine monotherapy	47 (72.3)	21 (60.0)	4 (36.4)
Other monotherapy	3 (4.6)	2 (5.7)	0 (-)
Dual combination therapy with NRTIs only	10 (15.4)	21 (60.0)	0 (-)
Dual combination therapy with PI/NNRTI	1 (1.5)	1 (2.9)	0 (-)
Triple or more with NRTIs only	0 (-)	2 (5.7)	0 (-)
Triple or more with PI/NNRTI	4 (6.2)	18 (51.4)	0 (-)

* Represents treatment status when last seen at RFHHC

[§] Each patient may be included in the table more than once

Table 6.3 : Clinical, virological and immunological details of patients alive and currently under follow-up at the RFHHC

	Total	Treated with ART	Not treated with ART	P-value
Number of patients under follow-up	35	28	7	
Median (range) years of follow-up	17.0 (14.0 - 19.5)	17.0 (14.0 – 19.5)	17.0 (14.5 - 17.5)	0.34
Median (range) current age	35.2 (19.7 - 47.1)	36.3 (19.7 – 43.4)	28.3 (21.5 - 47.1)	0.29
AIDS diagnosis: n (%)	5 (14.3)	5 (17.9)	0 (-)	0.56
Median (range) current CD4 count (cells/mm ³)	270 (0 – 1084)	195 (0 – 940)	430 (270 – 1084)	0.006
Median (range) nadir CD4 count (cells/mm ³)	150 (0 – 700)	65 (0 – 340)	380 (200 – 700)	0.0002
Median (range) current HIV RNA (log ₁₀ copies/ml)	3.5 (1.7 – 5.7)	3.3 (1.7 – 5.7)	3.7 (2.6 - 4.9)	0.83
Median (range) peak HIV RNA (log ₁₀ copies/ml)	4.7 (2.6 – 5.9)	4.9 (2.6 – 5.9)	4.0 (2.9 – 4.9)	0.008
Number (%) with HIV RNA < 400 copies/ml	13 (37.1)	10 (35.7)	3 (42.9)	1.00
Currently receiving antiretroviral therapy : n (%)	24 (68.6)	24 (85.7)	0 (-)	

6.4 DISCUSSION

This cohort is one of the longest groups of prospectively followed HIV seroconverters in the world. Cohorts such as this are extremely useful for documenting the long-term effects of HIV infection, both before and after the introduction of antiretroviral therapy. Whilst a number of patients have transferred their care elsewhere, we have been able to obtain vital information on all patients, and information on AIDS progression in the majority.

The median time to the development of AIDS in this cohort is now 13.6 years. Similar to other studies of haemophilic individuals (Goedert et al 1989; Astermark et al 1997), the majority of AIDS-defining diseases tended to be either PCP or oesophageal candida, although these events occurred fairly early in the epidemic, prior to the introduction of primary prophylaxis against these conditions (Sabin et al 1995). No cases of Kaposi's sarcoma have been seen. This condition is rare in haemophilic men (Rabkin et al 1990), and may be explained by the fact that haemophilic men are rarely seropositive for human herpes virus type 8 (Simpson et al 1996), the possible causal agent for Kaposi's sarcoma.

After an initially high rate in the first few years after infection, the rate of new AIDS-defining diseases remained relatively stable until the end of 1996. Since then there has been a marked decline in the incidence of AIDS in this cohort. The most likely explanation for this is the introduction of HAART from late 1995 onwards. However, we have not seen a similar drop in the death rate, even after taking account of the ageing cohort (data not shown). Many of the deaths in the most recent calendar period were from liver-related causes, almost certainly due to co-infection with HCV. Individuals infected with both HIV and HCV have an increased risk of liver disease (Eyster et al 1993; Telfer et al 1994a; Darby et al 1997). The reason for this is unclear, although it is thought that infection with HIV may lead to increased HCV loads (Eyster et al 1994) in

these individuals. One alternative explanation for our drop in AIDS rate is that some of the men who would have developed AIDS died from liver-related causes before they could receive an AIDS diagnosis, leading to an underestimate of the AIDS progression rate. Analyses that take account of this possible bias are underway, although as time goes on the increased availability of HAART may mean that AIDS is no longer a suitable endpoint for studies such as these, as it fails to capture some of the more relevant clinical effects of HIV infection.

The treatment experience in this cohort reflects the changing treatment patterns over time. Many of the patients who remain alive have progressively experienced zidovudine or other monotherapy, dual combination therapy, and combinations of three or four drugs including either a PI or NNRTI, as guidelines have changed. Some of the patients still follow regimens that we would now consider to be less than optimal – a number of patients remain on double nucleoside therapy, for example. The fact that the patients were all infected with HIV and HCV following supposedly safe treatment for haemophilia, and the possibility of spontaneous bleeding following treatment with PIs, has meant that both clinicians and patients are understandably cautious when starting ART.

Few studies have reported response to HAART in patients with haemophilia. Merry et al (1998) reported short-term response to PIs in 20 haemophilic patients in Ireland. Our response rates at three and six months are remarkably similar to those quoted in Merry's paper. RNA changes in the first three months after starting HAART were not as large as may be expected, but by six months the response rates (possibly after changing treatments) were good. The patients in this cohort were often heavily pre-treated with NRTIs, and were at an advanced disease stage at the time of starting HAART. Therefore, they may be expected to show a less impressive response to HAART than patients in other studies (Lederman et al 1998; Kaufmann et al 1998). The relative

infrequency of RNA monitoring (approximately every three months) may also contribute to the slower timing of the response.

Recently, it has been documented that individuals treated with HAART, and in particular PIs, may develop lipodystrophy and other metabolic disorders (Carr et al 1999; Walli et al 1998). To date, none of our patients have developed clinical features suggestive of lipodystrophy, although one patient has developed diabetes and was found to have abnormal lipid levels. The lack of consensus in the definition of lipodystrophy used in other published studies, means that it is difficult to assess whether our rate of abnormalities is any higher or lower than might be expected.

We have previously documented the relationship between PI treatment and unusual bleeding in patients with clotting factor disorders in a number of these patients (Yee et al 1997a). This bleeding was unpredictable and did not generally respond to increased clotting factor prophylaxis. In the 21 patients who have received HAART in this cohort, eight have now experienced unusual bleeding, all whilst being treated with a PI. The bleeds resolved in four patients after treatments had been changed. The remaining four patients remained on the same treatment and either the bleeds resolved spontaneously or prophylaxis was increased leading to resolution of the bleeding.

In conclusion, whilst there has been a reduction in the incidence of AIDS in our cohort, the death rate remains high, due to the large number of liver-related deaths now occurring. Many individuals have chosen not to receive HAART or have reduced the number of drugs taken due to worries about toxicities and bleeding. However, where patients are able to maintain their HAART regimen, the long-term virological and immunological response to HAART in this cohort is good.

Chapter 7

COMPLICATIONS ASSOCIATED WITH HAART

- 7.1 PROTEASE INHIBITOR THERAPY AND BLEEDING**
- 7.2 FAMILY ISSUES IN THE ERA OF HAART**

7.1 PROTEASE INHIBITOR THERAPY AND BLEEDING

7.1.1 INTRODUCTION

HIV protease inhibitor drugs (PIs) are a major component of highly active antiretroviral therapy which have dramatically influenced the morbidity and mortality of human immunodeficiency virus (HIV) infection. However HIV protease inhibitors are associated with a wide range of adverse effects amongst which development of an increased bleeding tendency is a unique finding in patients with hereditary bleeding disorders infected with HIV. The first PI drugs ritonavir, indinavir and hard gel saquinavir became available in the mid-1990s. More recently other PIs including *nelfinavir*, *kaletra*, and *amprenavir* have been introduced. Soon after the introduction of PIs the Food and Drug Administration (FDA) in the US issued a circular informing health-care workers that they had received reports of increased bleeding in 15 HIV positive haemophilic patients who were being treated with PIs (Racoosin & Kessler 1999). At about the same time the WHO reported in their Drug Information bulletin a similar observation in association with all three of the first available PIs in nine French haemophilic patients (WHO 1996). Recently a possible linkage of *amprenavir* with intracranial bleeding in an HIV-infected haemophilic patient has been reported (Kodoth et al 2001). As new PIs are introduced they should be under close surveillance for adverse effects especially bleeding in patients with inherited bleeding disorders.

Three haemophilia patients with unusual bleeding patterns following protease inhibitor therapy were investigated as to the underlying mechanism for the increased bleeding tendency and are reported here.

7.1.2 CASE REPORTS

Case 1

A 20 year old patient with severe haemophilia A infected with HIV had *indinavir* 800 mg tds added to *stavudine* and *lamivudine* dual therapy in October 1996 as his CD4 count was low and the drug became widely available in the UK. One week later he developed difficulty in swallowing, bleeding from the throat and a spontaneous bleed in the left knee. *Indinavir* was stopped as there were reports of increased bleeding in haemophiliacs on protease inhibitors in the drug data sheet. He was admitted and treated with twice daily infusions of 2000 units of Monoclate P for a sublingual haematoma which resolved after 48 hours of treatment.

Case 2

A 39 year old patient with severe haemophilia A and asymptomatic HIV infection (CD4 350×10^6 L-1) had *ritonavir* 1200 mg twice a day added to dual therapy with *zidovudine* and *zalcitabine* in June 1996 as part of a clinical trial. The patient was on regular FVIII prophylaxis and had very infrequent bleeds. Two weeks after starting *ritonavir* he developed an unusual bleed into the left calf muscle as well as a spontaneous muscle haematoma on the arm. *Ritonavir* was stopped and a massive total dose of 33470 units of factor VIII was required over a period of 14 days for the bleeds to settle down.

Case 3

A 19 year old severe haemophilia A patient with advanced HIV infection was started on *indinavir* 800 mg tds in addition to his previous combination therapy with *zidovudine* and *lamivudine*. He developed right-sided loin pain within four hours of the first dose of *indinavir* and the same pattern of pain occurred after each dose. After the fourth dose there was also frank haematuria. Previous medical history did not reveal any renal

problems and he had never experienced haematuria in the past. Factor VIII requirement in this young man was approximately only twice a month because of infrequent bleeds. Clinical examination was unremarkable. Urine microscopy revealed numerous red cells but no casts, crystals or malignant cells were detected. Blood calcium, urea and creatinine were all normal. Abdominal x-ray showed no evidence of nephrolithiasis and the ultrasound scan revealed good blood flow in the renal vasculature. *Indinavir* has been associated with nephrolithiasis (Chodakewitz et al 1995; Gulick et al 1996), but the rapidity of the onset of loin pain and haematuria after therapy in this patient and the absence of radiological evidence of calculi makes this an unlikely cause of his symptoms. Nonetheless, in view of the strong temporal association between the patient's symptoms and indinavir therapy, the drug was stopped and the haematuria resolved with a single dose of factor VIII concentrate 2000 units.

Investigations

A full bleeding state work-up was performed on these patients which included a full blood count, prothrombin time, partial thromboplastin time with kaolin, thrombin time, fibrinogen, factor VIII levels, vWF antigen and activity, factor VIII inhibitor screen and platelet function tests. All the tests were within normal limits.

7.1.3 DISCUSSION

PI-associated bleeding usually occurs within the first few weeks of commencing therapy as seen in the above three patients but in some individuals may not become manifest for several months. *Ritonavir* has been associated with the highest incidence, followed by *indinavir* (Racoonsin & Kessler 1999; Wilde et al 1999). The common haemorrhagic manifestations are increased frequency of muscle and usual target joint bleeds, and development of subcutaneous, soft tissue and mucous membrane bleeding in unusual

sites which the patients have never previously experienced as seen in patients 1 and 2. Bleeds into small joints of the hands and the soft tissue of palms and soles have also been observed. *Indinavir*-induced haematuria appears to be a direct bleeding effect and distinct from the *indinavir* loin pain syndrome. Intracranial bleeds and bleeds into the cervical and thoracic spine and retina have also been reported. Bleeds may or may not respond to replacement clotting factor concentrate therapy or larger doses may be required to settle bleeds as seen in case 2. Short courses of prophylaxis have been effective in some individuals but in others with troublesome bleeding PIs had to be discontinued. Interestingly, substitution with another PI usually does not result in a recurrence of the increased bleeding tendency. In many patients who persevere with their first-line PI therapy the bleeding tendency resolves with time. In a large collaborative study undertaken on behalf of the UKHCDO in UK in which our haemophilia centre participated a significant increase in factor concentrate usage during the first six months of PI therapy was observed compared with the six-month period pre-treatment but usage returned to pre-treatment amounts towards the end of the six months (Wilde et al 1999).

It is very important to be aware of the possibility of increased bleeding in any patient on PI therapy undergoing a dental or surgical procedure, regardless of whether or not they have had a previous bleeding problem associated with these drugs. The mechanism of increased bleeding associated with PI therapy remains unexplained. The reduced efficacy or even failure of factor replacement therapy in haemophilic patients and the occurrence of bleeding in non-haemophilic individuals suggests that PIs do not directly exacerbate the inherent increased bleeding risk of low factor VIII and factor IX levels (Racoosin & Kessler 1999). Furthermore, investigation of patients with PI-associated bleeding has not revealed evidence of disturbance of coagulation factor, von Willebrand

factor or fibrinogen function levels as seen in our three patients and other studies (Ginsburg et al 1997; Mandalaki et al 1998; Stanworth et al 1998).

It has been proposed that the effect of this class of drugs on haemostasis may be mediated through interaction with the cytochrome P450 system. In support of this is the observation that *ritonavir* and *indinavir*, powerful inhibitors of some P450 subtypes are associated with a high incidence of bleeding whereas *nelfinavir*, a weaker P450 inhibitor would appear to have less effect on haemostasis. However, the mechanism by which inhibition of P450 may bring about disturbance of coagulation has not been elucidated. Cytochrome P450 has been shown to play an important catalytic role in many of the pathways involved in arachidonic acid metabolism including the generation of thromboxane A₂ via cyclo-oxygenase activation (Capdevila et al 1992). It has, therefore, been proposed that through inhibition of P450, PIs may increase the risk of bleeding by disrupting prostaglandin-mediated platelet activation (Racoosin & Kessler 1999). Whether this mechanism operates within the human platelet has yet to be determined. Results of platelet function investigation in haemophilic patients on PIs are conflicting. Bleeding times and platelet aggregation studies performed on three patients reported by us and others (Stanworth et al 1998) were normal. An enhanced fibrinolytic response has also been postulated as the cause of the bleeding tendency associated with PIs. A 12 amino-acid sequence (aa 19-30) surrounding the active site of HIV-1 protease has been shown to have 63% homology with one of the lipid binding domains of low density lipoprotein-receptor-related protein (LRP) (Carr et al 1998). This hepatocyte membrane receptor is involved in the clearance of postprandial chylomicrons and numerous endogenous proteases including tissue plasminogen activator (tPA) (Herz 1993). It has been postulated that PIs may inhibit the function of LRP and the subsequent impairment of tPA clearance may result in excessive fibrinolysis (Carr et al 1998). However, there was no evidence of enhanced fibrinolysis in the three patients

that were studied (Ginsberg et al 1997) all of whom had normal fibrinogens, clot lysis times and D-dimers before and 3 hours after ritonavir. Further fibrinolytic studies in 16 patients had all been normal (Mandalaki et al 1998).

The lack of consistent evidence that PIs increase the tendency to bleed by the disturbance of clotting factor or platelet function suggests that PIs act in a way that is independent of the circulatory component elements of the haemostatic balance.

In conclusion, PI therapy is associated with a high incidence of increased bleeding in patients with hereditary bleeding disorders. Although factor concentrate replacement therapy is often poorly efficacious, prophylactic administration may control the frequency of bleeds. It would appear that the bleeding tendency settles with time and as the benefits of PI therapy are likely to outweigh the risks of bleeding these drugs should be continued unless a major bleeding problem develops.

7.2 FAMILY PLANNING IN THE ERA OF HAART

7.2.1 INTRODUCTION

As HIV is sexually transmitted, sexual relations will often be impaired in individuals who are HIV infected. HIV constitutes a formidable obstacle with regard to establishing relationships with the opposite sex and for many patients the idea of raising a family with their own biological offspring seems unthinkable. However highly active antiretroviral therapy has improved the quality of life of HIV-infected individuals, and family planning is now one of the issues brought up for discussion during review clinics. Currently there are 36 patients with inherited bleeding disorders and HIV infection being followed up in our haemophilia centre and the median age of this group of men is 36 years (range 20-48 years). Carers of HIV-infected individuals need to be aware of this change in the perceptions of these patients in order to provide support.

The UKHCDO annual returns for 1995 had identified HIV seropositivity in 51 wives/partners of 1321 haemophilic patients in the UK (Winter et al 2000).

A study to analyse some of the risk factors associated with heterosexual transmission of HIV infection in monogamous relationships in a group of individuals with inherited bleeding disorders was conducted in an attempt to provide more information for couples making decisions about childbearing. This was done as an extended study on HIV positive haemophilic men who have fathered children in our haemophilia centre (Goldman et al 1993a).

7.2.2 PATIENTS AND METHODS

In our cohort of 111 haemophiliacs followed up at the Royal Free Hospital Haemophilia Centre the median age at seroconversion was 24 years (range 2-77 years). Many patients were old enough to be married and have families. Patients were encouraged to bring wives or sexual partners and family members to review clinics to address the

issues of haemophilia and HIV on the family. Bearing in mind that all citizens of the United Kingdom, including people with HIV and AIDS, are awarded rights under international law which include the right to marry, the right to found a family, and the right to education. Therefore those couples who wished to have children were counselled using the counselling technique based on that of the Milan Associates (Palazzoli et al 1980). For those who wished to proceed to have a child it was advised that they should practise safe sex and use condoms at all times except at ovulation. The use of ovulation kits was recommended (these have been provided at the Royal Free Hospital since 1988) to reduce the risk of HIV transmission while attempting to achieve pregnancy.

14 out of the 111 haemophilic men had raised 19 biological offsprings before the use of highly active antiretroviral therapy. These men have been studied in detail regarding their immunological, virological and clinical progression of HIV infection at the time of conception and thereafter.

HIV seroconversion dates have been determined as the midpoint between the last negative and the first positive test by using a competitive enzyme immunoassay (Wellcozyme, Wellcome Diagnostics, Dartford, England).

CD4 counts and p24 antigen have been measured at regular follow ups since November 1982 (Lee et al 1989b).

HIV-1 viral load was measured on contemporary and archived serum samples using Amplicor HIV-1 monitor assay (Sabin et al 1998). Viral load at the time of conception has been calculated as the mean of three measurements (two consecutive years prior to conception and the year of conception).

7.2.3 Results

All 19 children and 13 out of the 14 wives were found to be HIV negative. One couple conceived a son who was HIV negative, before being referred to our centre and thereafter had two more pregnancies, both of which were terminated. The wife was found to be seropositive at the time of the second pregnancy.

Details of the fathers at the time of conception is as follows:

- a) duration of HIV seropositivity: median 5.25 years (range 2-16)
- b) CD4 counts: median 550 cells/ μ l (range 250-1300)
- c) viral load: median 4600 copies/ml (range <400-38,800). The viral load at the time of conception has been calculated as the mean of three measurements (results from 2 years prior conception and year of conception)
- d) p24 antigen: all negative

Details of the fathers at the time of conception and the post conception clinical progression are shown in Table 7.1. Six patients progressed to AIDS (Death) 1.75 (2.75), 4 (5), 4 (5), 4.3 (5), 4 (9) and 7 (8) years after conception respectively. One patient died from HCV liver failure 11 years after conception. All except one patient had been antiretroviral naive at the time of conception. Three fathers had more than one child.

Table 7.1 : Fathers' details at time of conception and post conception clinical progression

ID	Age (yrs)	Duration of HIV (yrs)	CD4 count Cells/ μ l	Mean VL Copies/ml	p24 Ag	Post Conception Aids/Death (yrs)	Progression	Present Status
1	33.8	3	500	1325	Negative	4(9)		
2.1	26.7	3.5	600	10,000	Negative			
2.2	29	6	545	38,700	Negative	7(8) * wife infected and pregnancy terminated		wife on Rx
3.1	31.8	3.3	700	3000	Negative			
3.2	33.9	5.25	720	38,800	Negative	4.3(5)		
4	31.1	2.5	500	600	Negative	4(5)		
5	25.3	2	500	9100	Negative			well, on Rx
6	30.1	4	250	26,6 00	Negative	1.75(2.75)		
7	37.7	4	550	23,700	Negative	(11) Liver Failure		
8.1	22.8	3	500	8000	Negative			
8.2	27	7	450	13,766	Negative	4(5)		
9	26.1	5	1300	<400	Negative			well, onRx
10	25.6	6	1100	<400	Negative			well, no Rx
11	28	10	430	2833	Negative			well, on Rx
12	25	8	480	8500	Negative			well,on Rx
13	35	12	400	19,900	Negative			well, no Rx
14.1	24	10	650	660	Negative			well, on Rx
14.2	26	12	600	3100	Negative			
14.3	27	13	600	1400	Negative			
14.4	30	16	590	4600	Negative			
Range	-	2-16	250-1300	<400-38,800	-	1.75-7(2.75-11)		-

7.2.4 DISCUSSION

The risk of sexual transmission of HIV seems to vary among individuals.

Numerous factors have been reported to contribute to this variability: the type of sexual exposure (DeVincenzi 1994), episodes of genital infection (Atkins et al 1996; Cohen et al 1997; Moss et al 1995), immunological and genetic cofactors in the uninfected partner conferring a natural resistance to viral infection (Samson et al 1996) and mucosal disruptions or cervical ectopy facilitating viral infection. In reality, little is known about sexual transmission of HIV. Issues as yet unresolved include the amount of virus required for infection following natural exposure, the viral form(s) (free viral particles or cell-associated virus) involved and the phenotypic characteristics of the viral strains transmitted (Tachet et al 1999).

Some studies of risk factors associated with the heterosexual transmission of HIV have suggested that low CD4 cell count, p24 antigenaemia, symptomatic HIV disease (Goedert et al 1987; Smiley et al 1988) and high viral load (Ragni et al 1998) are associated with a high risk.

Our small study of HIV positive haemophilic men and their families suggests low VL (18/20 conceptions had a VL < 30,000 copies/ml), high CD4 counts (all individuals had CD4 > 250/ μ l) and negative p24 antigen are associated with a reduced risk of HIV transmission by vaginal intercourse. It is of interest that the only patient to transmit HIV infection to his partner had a relatively high VL of 38,700 copies/ml. A similar experience to our centre has also been reported by a study in South Wales, UK. There were 26 apparently healthy children fathered by 18 HIV-infected males at different stages of HIV infection. All the 18 female partners remained negative for HIV infection on repeated testing. These couples had been seen and counselled by local fertility experts and advised to have unprotected sexual intercourse only around the time of ovulation. This experience also reflected efficacy of simple methods in reducing the

risk of HIV transmission in couples determined to conceive despite the potential consequences (Ramsahoye et al 1998). A study in the United States in a group of haemophilia men indicated that high HIV viral load >100,000 copies/ml appears to increase the risk of heterosexual HIV transmission by HIV-infected haemophilic men to their female partners (Ragni et al 1998).

Viral load, CD4 count and p 24 antigen in our study were determined on blood samples before the introduction of antiretroviral therapy (except for one patient) and therefore the low viral load results were not due to the antiretroviral medications.

Although the study was in a small group of patients, there were several advantages of this study. First, this study population is a unique, carefully studied cohort of HIV-infected haemophilic men, of whom seroconversion data were available, in whom monogamy is typical, in whom new HIV infection is not expected, and for whom blood products is the only source of HIV infection. Although a complete and accurate study of sexual transmission in these patients is not possible this small study has shown that the good health of the infected individual and low plasma HIV viral load reduce the risk of sexual transmission. The finding of HIV seronegative state in the female partners of the HIV infected haemophilia patients is encouraging, but should not dissuade female partners of HIV infected haemophilic men from following safe sex guidelines (CDC 1985).

A study in the non-haemophilia population of 104 consecutive pregnancies in 92 HIV-negative women with HIV-positive partners between 1986 and 1996 suggested that male-to-female transmission of HIV is infrequent during natural conception (Mandelbrot et al 1997). These findings were compatible with seroconversion rates in the order of 1 per 1000 episodes of unprotected intercourse reported in longitudinal studies of stable heterosexual couples (de Vincenzi 1994). Most couples in Mandelbrot's study had received non-directive pre-conceptual counselling on the risks

of heterosexual HIV transmission and couples were advised to pinpoint ovulation in order to reduce possible exposure. Most of the men were symptom-free, 14 (13%) had HIV-related symptoms, and one died of AIDS during the pregnancy. The mean CD4 count was 548/ μ L (range 7-1273), and 3 were below 200/ μ L. At the time of conception 21 men were receiving antiretroviral therapy. Seroconversion was observed in two women at seven months of pregnancy and in two others postpartum; all such cases arose in couples reporting inconsistent condom use. Although there have been instances where a single sexual contact has been sufficient for transmission (Laga et al 1989), unprotected sex at the time of ovulation in patients with low or undetectable HIV viral load may be a valuable option for conceiving a child as observed in the above studies. The findings that viral load plays an important role in heterosexual HIV transmission suggest the potential importance of antiretroviral therapy in the prevention of heterosexual HIV transmission.

It is clear that some people choose to have children in spite of their risk of HIV infection, and young couples such as those described will continue to present. Life has many risks, some of which can be avoided. It is for each individual to decide what degree of risk he or she finds acceptable. The role of the counsellor with couples where one partner is HIV positive and one or both wish to have children is to facilitate discussion and ensure that both are in possession of all the available information. Viral load determinations may be helpful in counselling haemophilic couples regarding transmission to female partners. Finally, couples should be assisted in reducing the risk as far as possible while being allowed the basic right to exercise their own choice.

In some cultures where women are valued for childbearing, women face immense pressures to procreate and may find that adherence to risk reduction guidelines are extremely difficult.

Other options for safe parenting for the HIV infected haemophiliac include adoption, foster parenting, artificial insemination using sperm from a healthy donor, or artificial insemination of processed semen from the HIV-infected partner.

Longitudinal virological studies are needed to evaluate whether interventions, including semen preparation, or antiretroviral therapies, can effectively clear cell-associated and cell-free virus from semen, thereby offering real hope for risk-free reproduction in “sero-different” couples.

Chapter 8

PARVOVIRUS B19

- 8.1 OVERVIEW OF PARVOVIRUS B19**
- 8.2 CASE REPORTS**
- 8.3 DISCUSSION**

8.1 PARVOVIRUS B19: OVERVIEW

Parvovirus B19 was first discovered in 1975 in sera of healthy blood donors (Cossart et al 1975) and is the smallest DNA-containing virus (single-stranded DNA of about 5.5 kilobases) that is pathogenic to man (Anderson 1990).

Blood group P antigen is the cellular receptor for the virus, and by utilising specific erythroid transcriptase factors there is active replication of the virus inside the erythroblasts, with eventual death of the red cell precursor. Individuals with the rare 'p' phenotype (frequency 1 in 200,000) who lack the P antigen are resistant to the virus infection (Brown et al 1993). Endothelial cells, megakaryocytes, and fetal heart and liver cells also carry P antigen on their surface but do not allow active viral replication but have other pathophysiological implications.

Transmission is by respiratory secretions, transplacental and transfusion as it is a serum virus and has been shown to be widely transmitted to haemophilic patients by clotting factor concentrates as the virus is sufficiently heat stable to survive in clotting factor concentrates (Azzi et al 1992; Schwarz et al 1991; Corsi et al 1988; Morfini et al 1992).

Parvovirus B19 infection is ubiquitous, common and highly contagious. It is present year round with a tendency to produce spring epidemics among school children 4-10 years old. The disease is not notifiable in the UK and surveillance relies on laboratory-confirmed cases. These show a 3-4 year epidemic cycle with the seasonal peak in the first half of each year. 3000-6000 infections are estimated to occur in an epidemic year but at most 10% of the infections are being reported by PHLS laboratories in England and Wales which highlights the extent to which B19 infection is undiagnosed.

Assuming that immunocompetent adults remain viraemic for an average of seven days, the likelihood of a blood donor being viraemic during an epidemic year is estimated to be around 1 in 10,000. Since the rash develops after the viraemic phase, the clinical

diagnosis cannot be made at the time of blood donation. Around 50% of donors will have IgG antibody from past infection (Miller 1995).

Clinical Findings

The disease manifestations of parvovirus B19 infection vary widely (Table 8.1) with the haematological and/or immunological status of the host.

Table 8.1 : Clinical manifestations of parvovirus B19 infections

Frequently observed manifestations	Rarely observed manifestations
Erythema infectiosum (fifth disease)	Transient pancytopenia
Arthritis – chronic arthritis	Chronic pure red cell aplasia
Transient aplastic crisis	Chronic neutropenia
Thrombocytopenia and Neutropenia	Virus-associated haemophagocytic syndrome (VAHS)
Hydrops fetalis	Hepatitis
Pure red cell aplasia (PRCA)	Myocarditis, Pericarditis
	Encephalitis, Meningitis
	Uveitis
	Pneumonia, Vasculitis

Laboratory Diagnosis

Currently, the corner stone of diagnosis of acute parvovirus infections is the demonstration of antibodies of the IgM class (or of a significant rise in IgG antibodies) by enzyme immunoassay (EIA) or radio immunoassay (RIA). Structural proteins of the parvovirus B19, VP1 and VP2 are produced in *E. coli* or baculovirus systems using recombinant gene technology and are used as antigens for the detection of the

antibodies (Soderlund et al 1992). In general IgM antibodies against the structural proteins VP1 and VP2 are the first serological markers of an acute B19 infection.

They may be detected about six to 10 days after the first contact with the virus.

During the prior interval, high amounts of infectious B19 virus are present in the patients' sera and in respiratory secretions and may be detected by antigen-capture assays or by amplification of viral DNA by polymerase chain reaction (PCR) (Hemauer et al 1996). About 12 days after infection, IgG antibodies follow the IgM response. During the following weeks IgM values drop to undetectable levels, IgG antibodies, however, persist lifelong and are a marker for past B19 infection. Both IgM and IgG contain neutralising antibodies which control B19 infection and are important for virus elimination. Neutralising epitopes have been mapped in VP1 as well as in VP2 proteins. Beyond the immune reactions the temporary destruction of the majority of erythroid progenitor cells as targets for virus replication contributes to the elimination of the virus from the organism.

However, in immunocompromised patients the immune reaction against the structural proteins may be very weak and can therefore not be used for laboratory diagnosis. The best way to detect virus is by nucleic acid hybridisation or PCR for viral DNA, or EIA for viral antigen, in acute phase serum. In some cases B19 virus may have been trapped in the infected organs and consequently viral DNA could only be detected in biopsy material (bone marrow, liver, synovial membranes). Histologically, giant pronormoblasts are found in bone marrow cells. The infected erythroid precursor cells display characteristic large eosinophilic intranuclear inclusions with surrounding margination of the nuclear chromatin. Electron microscopy reveals crystalline arrays of virions in the nucleus. *In situ* hybridisation using cloned parvovirus DNA is useful for demonstrating the presence of the genome in acute or chronic infections including hydrops fetalis (Soderlund et al 1997).

Treatment

Due to the self-limiting characteristics of the disease, treatment is not necessary for the uncomplicated forms of B19 infection. Patients with severe anaemia due to transient aplastic crisis have to be hospitalised and promptly treated with blood transfusions (Serjeant et al 1993). In cases of persistent B19 infections several studies show that intravenous use of immunoglobulins may improve the clinical conditions. Particularly in patients with persistent parvovirus infection associated with anaemia, in transplantation and/or immunocompromised patients intravenous administration of immunoglobulins proved to be successful and resulted in virus elimination (Kurtzman et al 1989). Immunoglobulins have also been given to patients with chronic arthritis. In these cases, relief of symptoms may, however, be only short-termed.

The risk of hydrops fetalis in cases of B19 infected pregnant women has generated high concern. Most maternal infections are without adverse effects for the fetus, but about 9% result in hydrops fetalis and fetal death due to the lytic infection of erythroid progenitor cells and the interruption of erythrocyte production. B19 associated hydrops can also resolve spontaneously with the baby normal at delivery. Intrauterine blood transfusions have been used to treat anaemia of the fetus. Prior to treatment the fetal disease in B19 infected pregnant women is best identified by fetal ultrasound or by the determination of maternal serum α -fetoprotein. Alternatively intravenous immunoglobulin has been successfully applied to the mother of a hydropic fetus (Selbing et al 1995).

The tragedy of AIDS has left an understandable urge to seek absolutely safe products, yet the search must be tempered with the recognition that such a goal is probably unattainable.

We report two cases of parvovirus infection which have occurred in our haemophilia centre in November 1994 and March 1995 to highlight and emphasise the fact that absolute safety of blood products is a mirage.

8.2 CASE REPORTS

Case Report 1

A 33-year-old man with mild haemophilia A (factor VIII 12iu/dl), Hb D trait, and HLA B27 associated ankylosing spondylitis presented in November 1994 with fever, cough and arthralgia. Urethral dilatation had been performed four weeks earlier under cover of 18 000 units of factor VIII concentrate (BPL 8Y, Elstree), which is a stringently heat-treated product (80°C for 72 h of the final product). The patient was not on any medication and there was no history of contact with anyone with a viral illness. On admission he was febrile, pale and had lymphadenopathy, with a truncal macular rash and an enlarged liver. Severe dizziness with no objective neurological signs developed during his hospital stay. Investigations showed a pancytopenia, haemoglobin 8.5g/dl, total white cell count $1 \times 10^9/l$ (neutrophils $0.3 \times 10^9/l$) and platelet count $62 \times 10^9/l$. Aminotransferases were recorded three times the upper limit of normal. Hepatitis A, B and C and HIV (1 and 2) serology were negative. Ultrasound of the liver was normal. Anti-B19 IgM and IgG antibodies were positive on admission, but retrospective testing on stored serum sample from immediately before the first treatment with factor VIII concentrate (26 September 1994) was negative for anti-B19. The batch of factor VIII concentrate received by the patient was positive for parvovirus B19 DNA by nested PCR (Zakrzewska et al 1992). Blood cultures were negative, but the patient had a stormy course, with high fevers requiring broad-spectrum antibiotics and red cell infusions. Eventually he returned to normal health.

Case Report 2

A 31-year-old haemophilia B carrier (factor IX 16 iu/dl) had facial moles removed on 2 March 1995, and the procedure was covered by using factor IX concentrate (Alphanine, Alpha Therapeutic Corporation). This product is purified by ion exchange chromatography and solvent detergent treated for viral inactivation. Surgery was uneventful, but three weeks later she presented with fever, rash, lymphadenopathy and arthralgia. There was no history of contact with persons with viral illness. Serological tests performed at that time revealed anti-B19 IgM and IgG antibodies. The batch of factor IX concentrate received by the patient was positive for B19 DNA by primary and nested PCR, suggesting a high level of B19 DNA $\geq 10^7$ genomes/ml (see Tables 8.2 and 8.3).

Table 8.2 : Clinical and serological findings in two patients with B19 virus infection after infusion of plasma-derived clotting factor concentrate.

Patient (age/sex)	Diagnosis	CFC	Dose of concentrate (units)	Onset of illness post infusion (days)	Anti-B19 (RIA)	IgM	Anti-B19 IgG (ELISA)
33 yr/M	HA (mild)	BPL 8Y F.H.B 4335	18 000	28	> 100		Positive
31 yr/F	HB(carrier)	Alphanine CA 4434A	4200	21	> 100		Positive

Table 8.3 Parvovirus B19 DNA in clotting factor concentrates

Clotting factor concentrate	B19 DNA dot blot	B19 DNA PCR primary	B19 DNA PCR nested
BPL 8 Y FHB 4335	Negative	Negative	Positive
Alphanine CA4334A	Negative	Positive	Positive

8.4 DISCUSSION

The above two patients illustrate that, although both of them were infrequent users of clotting factor concentrates, they contracted a transfusional B19 infection from plasma-derived concentrates. Case 1 in our report demonstrates a B19-induced pancytopenia and severe septicaemia in an immunocompetent adult. It is usually suggested that this is only a risk for immunosuppressed patients. A further immunocompetent 11 year old has also been described who developed a severe disorder associated with severe neurological disturbance following parvovirus infection secondary to treatment with factor VIII concentrate (Coumau et al 1996). Patient 1 also developed disturbances of consciousness and hepatic dysfunction which have been described by Yota et al (Yoto et al 1994).

Case 2 emphasises the vulnerability of female haemophilia carriers to acquiring parvovirus B19 infection via clotting factor concentrates. Of greatest concern is the occurrence of parvovirus infection during the second and third trimester of pregnancy when it can result in miscarriage or hydrops fetalis. B19 is estimated to cause 10% to 15% of all causes of non-immune hydrops. The virus is transmitted via placenta to the embryo in about 33% of cases, and the risk of fetal loss has been estimated at 9% (Anderson et al 1988). Thus for the carrier of haemophilia, the argument for the use of recombinant clotting factor concentrate is compelling.

Studies of human parvovirus B19 in clotting factor concentrates by the PCR method have shown that in 5% to 20% of clotting factor concentrates, B19 DNA can be detected (Zakrzewska et al 1992). Many haemophilic patients are infected with HIV and thus often immunocompromised, therefore transfusional B19 contamination may have severe consequences in this group of patients (Zakrzewska et al 1992; Lefrere et al 1994).

Parvovirus has a 3-4 year epidemic cycle with a seasonal peak in the first half of the year. 60% of UK blood donors are IgG antibody positive from past infection and the

likelihood of a donor being viraemic can be as high as 1 in 260 during the epidemic period. Strategies of prevention of this transfusional risk include screening of blood donors for B19 DNA by PCR (McOmish et al 1993) to create pools of B19 free plasma or end product testing for contaminating infectious agents especially on batches of clotting factor concentrates that are to be used in immunocompromised HIV-infected haemophiliacs and pregnant haemophilia carriers. Recently the diagnosis of B19 infection has evolved into using nucleic acid amplification technology (NAT) assays and currently is the most sensitive test for detecting B19 in plasma pools where the virus may be present in low concentrations. However, NAT assays vary widely in sensitivity and specificity and the use of a well-calibrated standard is essential for comparison of data and the routine introduction of such assays. The first World Health Organisation (WHO) International Standard for NAT assays was first established in 1997 for HCV RNA assays (Saldanha et al 1999) and in 1999 two further WHO International Standards for HBV DNA and HIV-1 RNA assays were established (Saldanha et al 2001, Holmes et al 2001). Recently there has been establishment of the first WHO International Standard for human parvovirus B19 DNA nucleic acid amplification techniques (Saldanha et al 2002).

The removal or inactivation of every virus particle in coagulation factor concentrates is a worthy goal in theory, but in practical terms this may be unattainable. From a practical perspective, the realistic goal is to reduce the pathogenic viral contamination to residual levels which are not infectious.

The issue of preparing for the unknown hazard is very difficult and there is no assurance that the additional safeguards developed would be effective. Clearly there is a need for continuing international surveillance.

Chapter 9

OVERVIEW OF INHIBITORS

- 9.1 INHIBITORS AND HAEMOPHILIA TREATMENT**
- 9.2 QUANTIFICATION OF INHIBITOR ANTIBODY**
- 9.3 CURRENT TREATMENT REGIMENS**
- 9.4 PATHOGENESIS OF FVIII INHIBITORS**

9.1 INHIBITORS AND HAEMOPHILIA TREATMENT

Case reports of haemophilia patients developing a refractory state to the administration of blood, plasma or its derivatives during the course of their haemophilia treatment has been described since the early 1940s (Craddock et al 1947). The development of a circulating anticoagulant after repeated transfusions was observed in these patients and further transfusion caused neither a reduction in clotting time nor clinical improvement. Detailed studies on the nature of the anticoagulant revealed that the anticoagulant activity was associated with the gamma globulin fraction of the plasma.

In the 1940s when the process of Cohn fractionation of plasma was just being developed, a Dr C. Davidson wrote “the refractory state to blood and its derivatives follows, the repeated administration of blood, plasma or the anti-haemophilic globulin fraction (also known as Cohn Fraction 1) and arises during or promptly after a haemorrhagic episode. The exact nature of this refractory state is still obscure, but recent work has suggested that there may be a production of antibodies to the anti-haemophilic substance.” What is more interesting and perhaps a bit prophetic is the footnote that was referenced in this particular passage of the article which states: “Presently available evidence suggests that this refractory state may occur more frequently following the administration of the anti-haemophilic globulin fraction than following administration of blood or plasma. The therapeutic use of the anti-haemophilic globulin fraction cannot be advised, therefore, until further studies have eliminated this hazard” (Davidson et al 1949).

The spontaneous occurrence of factor VIII (FVIII) inhibitors in haemophilic patients is very rare and almost always follows replacement therapy either in the form of plasma, cryoprecipitate or specific clotting factor concentrates. Although tremendous achievement in haemophilia management has resulted in the availability of the most

technologically advanced and safer clotting factor concentrates, inhibitor development persists as a major complication of treatment for a subset of haemophilia patients. These inhibitors are highly specific immunoglobulins which rapidly inactivate infused FVIII, making replacement therapy difficult and challenging. The presence of an inhibitor makes treatment of bleeding episodes difficult, often prevents the possibility of starting a child on prophylaxis, often makes surgical procedures high risk, and makes overall management far more time consuming and expensive.

9.2 QUANTIFICATION OF INHIBITOR ANTIBODY

The first quantitative method for evaluating inhibitor potency in a mixing assay, the Oxford method, was developed by Biggs and Bidwell in the late 1960s (Biggs et al 1959). The method was based on the evaluation of the rate of FVIII inactivation upon incubation of FVIII for a limited period of time with an excess of inhibitory antibody. Characterisation of a series of inhibitor antibodies indicated, however, that the method did not provide quantitative results in all cases. In addition, because the assay was performed in the presence of an excess of inhibitory antibody, the method was not sufficiently sensitive enough to assess clinically relevant low titre inhibitor. The assays to quantify anti-FVIII antibodies were therefore abandoned until the characterisation of the mode of action of inhibitor antibodies led to the development of more generally useful quantitative assays (Biggs et al 1972a; Biggs et al 1972b).

In the New Oxford method, described by Rizza and Biggs in 1973 (Rizza et al 1973), FVIII was added in excess over antibody in the assay and the incubation with inhibitor antibodies was prolonged up to four hours. This extended incubation period allowed accurate detection of inhibitor antibodies characterised by a slow reaction rate with FVIII. The concept at the basis of this method was that for many antibodies, there is a

linear relationship between the inhibitor concentration and the log of residual FVIII following incubation with the inhibitor for a given period of time.

The principle of the New Oxford method was largely adopted for measurement of FVIII inhibitor potency. However, differences in methodology, notably with regard to the incubation time and the source and amount of FVIII used for the assay, brought about difficulties in comparing results obtained in different centres. A standardised procedure, called the Bethesda method, was therefore proposed in 1974 by a group of investigators led by K. Kasper (Kasper et al 1975). An incubation mixture constituted by one part citrated patient (diluted or undiluted) plasma and by an equal part of pooled normal plasma as a source of FVIII is left at 37°C for two hours together with a control consisting of one part normal pooled plasma and one part buffer. Residual FVIII activity of both incubation mixtures is assayed; residual FVIII activity of the control mixture is used as 100% reference in this assay. A dilution of the test mixture containing 50% of FVIII activity relative to this reference after 2 hours of incubation is defined to contain one Bethesda unit (BU) of inhibitor per mL. The amount of inhibitor can be read directly from a semilogarithmic graph. This quantification method is best suited to measure inhibitors arising in haemophiliacs; the designation of BUs in an inhibitor plasma does not imply that an amount of FVIII can be calculated from this that would neutralise the patients' circulating inhibitor (Kasper et al 1975).

Quantification of inhibitors in a standardised way is useful for a meaningful comparison between laboratories: a lower inhibitor titre may give some indication as to which patients can still benefit from treatment with FVIII; a low inhibitor titre at start of immune tolerance therapy is an important predictor for treatment outcome (Mariani et al 1994; DiMichele et al 1999); and quantification of inhibitor titre can be used to study the formation and disappearance of inhibitor in the individual patient. The sensitivity and the specificity of the Bethesda assay are strongly dependent on the pH of the

incubation mixtures (residual FVIII activity in the mixtures decreases with increasing pH). Therefore it is important to use buffered pooled normal plasma (Kitchen & McCraw 2000) or to add 0.1 M imidazole buffer at pH 7.4 to the normal plasma (Verbruggen et al 1995).

Detection of anti-FVIII antibodies by ELISA

In enzyme-linked immunoabsorbent assay (ELISA)-based systems both inhibitory and non-inhibitory antibodies to FVIII are detected. In a recent evaluation of a solid-phase ELISA, (Martin et al 1999) a sensitivity of 97.7% and a specificity of 78.4% for the detection of FVIII inhibitory antibodies was noted. The implications of non-inhibitory antibodies are not well known, although they might have a role in FVIII clearance and half-life (Dazzi et al 1996). Although for current patient management antibodies that inhibit FVIII function seem most important, ELISA-based techniques could have a role as a method of rapid and less labour-intensive screening assay for the presence of anti-FVIII antibodies, with an impressive negative predictive value.

9.3 CURRENT TREATMENT REGIMENS

State of the art replacement products are now made from either plasma derived or recombinant proteins and the evolution of haemophilia replacement therapy over the years is shown in Table 9.1. There has been a dramatic improvement in the therapeutic products available for the haemophilia patients but the relative risk of inhibitor formation with the different products available today is still an ongoing issue.

The mainstay of successful haemophilia therapy for either treatment or prevention of acute haemorrhage is prompt and adequate intravenous replacement of the deficient clotting factor to haemostatic plasma levels. All plasma derived products have similar haemostatic efficacy and undergo a viral attenuation step during the purification

process; however, there is a wide variability among concentrates with respect to final product purity, as defined by units of FVIII specific activity/mg protein.

The intermediate purity FVIII concentrates contain FVIII, von Willebrand factor (VWF) and plasma proteins such as immunoglobulins, fibrinogen, immune complexes, cytokines such as TGF- β and many others. These concentrates are produced predominantly by conventional precipitation techniques and have a factor VIII content of 2-5iu/mg of protein.

High purity FVIII concentrates do not contain other contaminating proteins and are produced either by protein precipitation and chromatographic separation or immuno-affinity chromatography using monoclonal antibodies directed against FVIII or VWF. The former method produces a high purity FVIII product that contains 50-200 iu FVIII/mg protein and a variable amount of VWF. The immunochromatography produces much purer concentrates with FVIII activity of 3000 iu/mg which requires addition of stabilisers such as albumin and sugar to make it suitable for storing and clinical use, resulting in a final FVIII activity of 5-30 iu/mg protein.

The isolation and cloning of the cDNA for human FVIII in 1984 paved the way for the biosynthesis of genetically engineered FVIII in cultured mammalian cells. The currently used recombinant factor VIII (rFVIII) products have been made by the insertion in mammalian cells of the cDNA which encodes the entire FVIII or the B domainless FVIII protein. There are certain differences in the preparation of these recombinant products with respect to the mammalian cell line used to express FVIII. The host cells are Chinese hamster ovary cells for Recombinate and rFVIII-SQ (B domainless FVIII product) and baby hamster kidney cells for the Kogenate product. FVIII is co-expressed with VWF in Recombinate and not with the other two products. The rFVIII which is isolated from the cell line medium and subsequently purified, has a specific activity of 2000-5000 iu/mg and contains traces of hamster proteins (derived

from the mammalian cell lines), bovine proteins (from the culture medium) and mouse immunoglobulin G (from the purification step). However the specific activity of the final recombinant product is much lower (5-20 iu/mg) due to the addition of human albumin to ensure stability. The second generation of rFVIII (rFVIII-SQ) is stable without addition of albumin and the specific activity is extremely high at 15,000iu/mg compared to the first generation products. More second generation rFVIII products without albumin are being manufactured and undergoing clinical safety and pharmacokinetic studies (Abshire et al 2000).

Table 9.1 : History of haemophilia A treatment

Decade	Milestone
1840s	First blood transfusion
1940s	Transfusion therapy established
1950s	Fresh frozen plasma; anti-haemophiliac globulin (Cohn Fraction-1)
1960s	Cryoprecipitate
1970s	Intermediate-purity factor concentrates; DDAVP
1980s	Monoclonal antibody-purified and high-purity FVIII concentrates; effective viral inactivation
1990s	Recombinant FVIII
2000s	Second generation (albumin free) recombinant FVIII products; gene therapy

9.4 PATHOGENESIS OF FVIII INHIBITORS

The pathogenesis of FVIII inhibitors is still not fully understood. The capacity to mount an immune response toward FVIII obviously varies from one individual to another and depends on the interaction of several factors. These can be broadly divided into host related factors and therapy related factors. Several patient-related characteristics that

render an individual susceptible to develop an anti-FVIII response has been examined to some extent (Aledort 1992; Lusher 1993). Obviously the factor VIII gene mutation is one of the most important risk factors as 90-97% of inhibitors occur in patients with severe haemophilia A. Large databases of FVIII mutations have identified certain genotypes, particularly major gene deletions, intron 22 inversions and nonsense mutations to confer an increased risk of inhibitor formation (Schwabb et al 1995). Yet the risk of developing an inhibitor cannot be related to the FVIII genotype alone, as haemophilic patients from the same kindred are often discordant for inhibitor formation indicating that patients with the same gene defect may respond differently to factor VIII treatment i.e. the immunological response of the individual characteristics are also important (Hay et al 1997; Oldenburg 1997). Therapy related factors (e.g. treatment schedule, FVIII product used, factors that modulate the immune response at the time of FVIII infusions) may also play an important role in the development of inhibitors and evidence is accumulating of the role for the non-genetic factors affecting the immune response (Peerlinck et al 1993). Table 9.2 shows the patient and therapy related variables that can influence the inhibitor incidence.

Table 9.2 : Factors influencing the development of FVIII inhibitors

The patient	<ul style="list-style-type: none">• Molecular defect• Immunological response characteristics• Other immunologic challenges at time of FVIII infusion (vaccinations, infections, medications such as interferon)• Ethnicity• Family history of inhibitors
Therapy	<ul style="list-style-type: none">• Type of FVIII product (purity as well as viral inactivation method)• Number of exposures• Pattern of exposures• Cumulative exposure• Effects of exposure to several different products

Chapter 10

FACTOR VIII INHIBITORS IN HAEMOPHILIACS: A SINGLE-CENTRE EXPERIENCE OVER 34 YEARS, 1964-1997

10.1 INTRODUCTION

10.2 PATIENTS AND METHODS

10.3 RESULTS

10.4 DISCUSSION

10.1 INTRODUCTION

Although there have been no formal epidemiological studies of inhibitors in haemophilia, several investigators have reported data on their prevalence and on some characteristics of the affected patients since the early 1950s (Lewis et al 1956; Strauss 1969). In the UK, a data-base on the complications of haemophilia therapy was started in 1969 by the UK Haemophilia Centre Directors' Organisation (UKHCDO) (Biggs 1974) and a recent updated report by the UKHCDO on the prevalence of FVIII inhibitors in haemophilia patients of all severities is approximately 6% with an annual inhibitor incidence of 3.5/1000 severe haemophilia A patients registered with the UKHCDO (Rizza et al 2001). Thus most haemophilia centres in the UK have only very small numbers of inhibitor patients.

We have undertaken a retrospective study of all factor VIII inhibitor patients followed-up over three decades in one of the largest comprehensive haemophilia centres in the UK, in an attempt to understand the natural history of FVIII inhibitor patients and also to get some insight into the pathophysiology of FVIII inhibitor formation.

10.2 PATIENTS AND METHODS

All haemophilia A patients who have been registered and seen in the centre from 1964 to December 1997 were included in the study. Computerised treatment records were started at the centre in 1979. Data of patients with inhibitors have been collected and include the following information:

(1) age of the patient at diagnosis of haemophilia A and detection of the inhibitor to FVIII; (2) ethnic origin; (3) severity of the haemophilia; (4) family history of haemophilia/inhibitor; (5) high (>5BU) or low responder (<5B.U.); (6) nature of the inhibitors (persistent or transient); (7) types of products used and exposure days prior to

inhibitor development; (8) molecular analysis of FVIII gene; (9) HIV/HCV status; (10) age and cause of death for the deceased patients.

All severe haemophilia A patients had at least one inhibitor assessment performed per year at the time of haemophilia review and also were tested whenever clinically indicated in the earlier years. Since mid-1985 all severe previously untreated patients have had inhibitors assessed every 3-4 months.

The inhibitor assessment was by the Biggs and Bidwell method (Biggs et al 1972) in the earlier years and by 1979 was changed to the Bethesda assay (Kasper et al 1975).

Severe haemophilia A patients were screened for FVIII intron 22 inversion by the Southern blot method (Lakich et al 1993).

10.3 RESULTS

431 haemophilia A patients with all grades of disease severity have been exclusively under the care of the Royal Free haemophilia centre for a total of 5626 patient-years.

Demography of the haemophilia population in the centre

Of the 431 patients, 239 (55%) had severe (FVIII:C <2u/dl), 45 (11%) had moderate (FVIII:C 2-5u/dl) and 147 (34%) had mild (FVIII:C >5u/dl) haemophilia A.

Frequency of inhibitors in the centre

27/431 (6.3%) patients attending the centre during a 34 year period had inhibitors to factor VIII. However in 12 patients (ID 1,5,8,9,10,13, 14, 16,17,18,21 and 22 in Table 10.1) initial inhibitor diagnosis was made elsewhere and the patients was referred to the centre later for long-term management. Excluding these 12 patients, 15/149 (3.6%) inhibitor patients were diagnosed at the Royal Free haemophilia centre. 24 of these inhibitors occurred in severe, two in moderate and one in mild haemophilia. Some of the characteristics of the individual patients with the inhibitors are listed in Table 10.1.

Chapter 12

INHIBITORS IN MILD AND MODERATE HAEMOPHILIA A PATIENTS

12.1 INTRODUCTION

12.2 CASE REPORTS

12.3 DISCUSSION

12.1 INTRODUCTION

Patients with severe haemophilia A who do not have measurable FVIII due to gene deletions or other mutations have a higher than average chance of inhibitor development. The infused FVIII acts as a foreign protein and an immune response to the treatment may occur within a short period of exposure to the FVIII concentrate. Immunologic tolerance has been defined as a specific unresponsive state induced by prior exposure to an antigen or antigenic determinant. Therefore patients with moderate and mild haemophilia A, who have circulating plasma FVIII are more tolerant to the FVIII replacement therapy and do not readily develop an immune response to the exogenous FVIII. However inhibitors can develop in patients with mild and moderate disease and the prevalence has been estimated to be between 3-13% (Rizza et al 1983, Sultan et al 1992, Schwaab et al 1995). It is unknown why patients who are primarily tolerant to FVIII later mount an immune response.

Recent United Kingdom data based upon the reports of new inhibitors in the UK over a period of 7 years from January 1990 to January 1997 registered 57 new inhibitors during that period. 16 out of that 57 (28%) arose in patients with mild and moderate haemophilia (Rizza et al 2001).

We have previously reported a low frequency of inhibitors in our cohort of 431 haemophilia patients with all grades of disease severity and followed-up for a total of 5,626 patients years (Yee et al 1999). From 1995 up to the year 2001 six patients with mild and moderate haemophilia A have developed factor VIII inhibitors and their clinical presentation and further investigations are reported. The reason for such an increased incidence of inhibitors recently in this group of patients is, however, not known. We all should be aware of the fact that any patient is susceptible to develop an anti-FVIII immune

response at any time during treatment and the state of immune tolerance to FVIII should be considered as an equilibrium that may be easily broken.

12.2 PATIENTS AND METHODS

FVIII inhibitors :

Inhibitors were measured according to the Bethesda method (Kasper et al 1975) and from 1998, Nijmegen modification of the above assay has been used (Verbruggen et al 1995) to further improve the lower limit of detection and reliability.

Enzyme linked immunosorbent assay (ELISA) was used to detect antibodies directed towards determinants that are not involved in the functional activity of the molecule.

Haemophilia A mutation screening:

FVIII gene mutations were located with the solid phase fluorescence chemical cleavage of mismatch method and characterised by DNA sequencing on ABI Prism 377 DNA sequencer as described previously (Waseem et al 1999).

Epitope specificity and functional characterisation of FVIII inhibitors:

Analysis was performed on two inhibitors from patients number 1 & 5 (Table 12.1). Immunoprecipitation assay was used to measure the antibody binding to FVIII fragments (Thompson et al 1997) and inhibitory activity was determined by inhibitor neutralisation assay (Scandella et al 1989).

Case 1: A 60 year old patient with moderate haemophilia A (FVIII:C:5u/dl) patient developed an inhibitor after intensive replacement therapy for two surgical procedures performed over a four week period. The first exposure to FVIII concentrate was at 44 years of age and the patient had received a cumulative dose of 70,000 iu over a 14 year period from 1980 to 1994. The patient received 120,000 iu of two different high purity FVIII products within 22 days for the two surgical procedures. Preoperative inhibitor screens were negative and post infusion FVIII recovery levels were satisfactory until the third postoperative day of the second surgical procedure, when the inhibitor assay revealed an anti FVIII inhibitor of 5.4 bethesda units (BU) against human FVIII. Infusions of FVIII were stopped and the patient had an uneventful recovery from the surgery. The inhibitor titre gradually rose from an initial anti-FVIII inhibitor of 5.4 BU (H) to a peak titre of 580 BU (H) and 12 BU (P) five months later, even though no further FVIII infusions were given after the diagnosis of the inhibitor. No major bleeding complications occurred until five years later, when rVIIa was required for soft tissue bleeds inside the throat and nose. Immunosuppressive therapy (oral prednisolone & cyclophosphamide & IV IgG) was thus initiated. This resulted in a reduction in the inhibitor titre from 296 BU to 12 BU and baseline FVIII levels rose from <1 to 2u/dl. Because of severe side effects the treatment was stopped and the inhibitor titre rebound to 25 BU (H) and 0.65 BU (P) with FVIII levels declining to <1u/dl.

The factor VIII gene mutation has been identified as Arg2163His in the C1 domain. There was a family history of haemophilia but no history of inhibitors.

The epitope analysis of serial inhibitor plasma samples of this patient over a two year period (1996/97) by immunoprecipitation assay (Figure 12.1) showed that the inhibitor had strong binding to the recombinant FVIII light chain (A3C1C2). The reactivity to the A2

domain is very limited compared to that of the light chain. The epitope specificity had not changed over this period of two years. The neutralisation assay (Figure 12.2) showed that the majority (> 90%) of the inhibitory antibodies were directed against the light chain of the FVIII. Further epitope mapping studies performed at different time-points of inhibitor development revealed that anti-factor VIII antibodies bound predominantly to the C2 domain of FVIII.

Case 2: A 73 year old patient with mild haemophilia A (FVIII:C: 7iu/dl) underwent two coronary angioplasties over a four months time span using FVIII 35,000 units in total. The average annual FVIII consumption of this patient was < 10,000 units. Continuous infusion of recombinant FVIII concentrate was used during the first angioplasty. A high purity FVIII concentrate was given at the local hospital for a severe gastrointestinal haemorrhage that occurred secondary to aspirin therapy post stent insertion. Four months later angina recurred requiring a second angioplasty, which was performed using continuous infusion with another high purity FVIII concentrate. Two weeks after the second angioplasty, the patient developed a large muscle bleed in the right thigh. The FVIII inhibitor assay showed an inhibitor of 2.1BU antihuman and 1.3 B.U. antiporcine. Plasma derived FVIII concentrates resulted in an anamnestic response of the inhibitor and the patient's baseline FVIII levels were reduced to <1u/dl. Recombinant VIIa was used for subsequent bleeding episodes and six months later the inhibitor spontaneously resolved and baseline FVIII levels returned to normal. The FVIII gene mutation was in the C1 domain giving rise to Phe 2101Cys. The patient had a family history of a transient low titre FVIII inhibitor in his younger brother.

Case 3: A 67 year old mild haemophilia A patient, the younger brother of case 2, also underwent angioplasty and had intensive therapy with high purity FVIII continuous infusions and developed a transient low titre inhibitor postoperatively. Three years later he had excision of a mole from the chest and was given DDAVP as well as plasma derived FVIII 9000 units over three days. Four weeks later the patient developed spontaneous bleeds on the left lower lip and left thenar eminence whilst on a ship cruise. Investigations revealed a normal baseline FVIII, negative inhibitor screen and normal platelet count. However an enzyme linked immunosorbent assay (ELISA) designed to detect IgG antibodies reactive with recombinant human factor VIII was positive on blood samples prior to surgery and at the time of the spontaneous bleeds. Therefore the patient had antibodies reactive against FVIII that were non inhibitory or neutralising in nature and escaped detection by the functional Bethesda assay. Antibodies directed towards non functional sites of factor VIII may have pathologic relevance in this patient who developed spontaneous bleeds for which no other cause was found.

Case 4: A 37 year old patient with mild haemophilia A (FVIII:C: 7 iu/dl) developed a FVIII inhibitor after four exposure days with a plasma derived FVIII concentrate given for a lacerated finger and a dental procedure. The only previous treatment was cryoprecipitate in 1983 and DDAVP. The patient developed widespread extensive bruises over the extremities as the baseline FVIII levels became < 1 iu/dl. The inhibitor had anamnestic responses to FVIII containing products and became a high responding type which required intervention. Immunosuppression with oral prednisolone, cyclophosphamide and IV IgG was started six months after diagnosis when the inhibitor titre was 7.7 BU (H) and 8.1 BU

(P). Three weeks post immunosuppression the inhibitor titre declined to 0.9 BU (H) and 2.9.BU (P) with a baseline FVIII level of 7 u/dl. Since this time the patient has been treated with DDAVP resulting in a post treatment FVIII level of 36 iu/dl. Anti-FVIII antibodies derived from the patient's plasma were directed against the FVIII light chain by immunoprecipitation assay. Neutralisation assay revealed that 30% of inhibitory antibodies bound to the C2 domain, whereas 70% bound to the A3-C1 domains of FVIII. The factor VIII mutation was in the C1 domain giving rise to Asp2074Gly.

Case 5: An 85 year old man with mild haemophilia (FVIII: 6 iu/dl) developed an inhibitor after intensive replacement therapy with two different high purity FVIII concentrates in continuous infusion for two surgical procedures performed over a one year period. The first exposure to a FVIII concentrate was at the age of 77 years and the inhibitor developed after 143 exposure days to FVIII. The inhibitor was cross reacting and the baseline FVIII dropped to a level of < 1u/dl. The bleeding pattern of the patient changed to that of acquired haemophilia. (Figure 12.3) and a low dose immunotolerance programme was initiated. The FVIII gene mutation was Arg1997Trpt in the A3 domain of the FVIII molecule.

Case 6: A 51 year old patient with mild haemophilia A (FVIII:C: 17u/dl) was diagnosed for the first time with a bleeding disorder on a preoperative clotting screen for a strangulated hernia in November 2000. The patient gave a history of excess bleeding after dental extractions and varicose vein stripping but had never received any haemostatic interventions. The patient has a brother with mild haemophilia A and this has been confirmed. The hernia repair was carried out under continuous infusion of B domainless recombinant factor VIII concentrate with 14 exposure days. Three weeks later a low titre

FVIII inhibitor of 1.3 BU against human FVIII was detected and the patient's baseline FVIII level was reduced to 5iu/dl. A month later the baseline level was further reduced to 1iu/dl and the inhibitor titre became 2.7 BU. Four months later patient developed haematuria and at this time required FVIII bypassing agent and immunosuppressive therapy with oral prednisolone, cyclophosphamide and IV IgG was started. Currently the patient is on low dose FVIII (FVIII 25 u/kg) three times a week as part of low dose immune tolerance regime. The inhibitor is undetectable by the functional Bethesda assay but the baseline FVIII levels are still low at 7 iu/dl. The enzyme linked immunosorbent assay (ELISA) is positive and the recovery of infused exogenous FVIII levels are also low. The factor VIII gene mutation is in the A3 domain (Cys 1854 Threo).

Demographics of the six patients with mild and moderate severity haemophilia A patients who developed FVIII inhibitors are outlined in Table 12.1.

Table 12.1: Demography of the inhibitor patients

Pt No	Maximum inhibitor titre (B.U.) Human/Porcine	Exposure days prior to inhibitor detection	Blood products received prior to inhibitor development	FVIII mutations	Rx of the inhibitor	Status of the inhibitor
1	568 BU (H)/16(P)	> 50	2 different high purity FVIII concentrates	C1 domain Arg2163His	Pred/Cyclo & IVIgG	High titre till death
2	12BU (H)/ 8 (P)	>50	RFVIII & high purity FVIII	C1 domain Phe2101Cys	Nil	Bethesda -ve ELISA +ve
3	0.74BU (H)	< 20	2 different high purity FVIII concentrates	C1 domain Phe2101Cys	Nil	Bethesda -ve ELISA +ve
4	9BU (H)/ 1.6 (P)	4	Intermediate purity FVIII	C1 domain Asp2074Gly	Pred/Cyclo& IVIgG	Bethesda -ve ELISA +ve
5	12.8 BU (H)	>50	2 different high purity products	A3 domain Arg1997 Trpt	Low dose prophylaxis	High titre till death
6	7.04/ 0	14	RFVIII	A3 domain Cys1854 Threo	Pred/Cyclo/ IVIgG	Bethesda -ve ELISA +ve

DISCUSSION:

We report six patients with non severe haemophilia A (1 moderate and 5 mild haemophilia A) who developed FVIII inhibitors in our centre over a six year period. The median age of the patients at the time of the inhibitor development was 63.5years (range 37-85years). Five patients developed inhibitors after intensive replacement therapy using continuous infusions of either recombinant FVIII or high purity FVIII concentrates. In two of the patients the inhibitors developed within a short period of exposure to a FVIII concentrate and these two patients had not been previously treated with FVIII concentrates. The inhibitors were diagnosed either because of poor recovery of infused FVIII or the change in bleeding pattern.

Inhibitors are an uncommon complication of mild haemophilia, occurring in 3-13% of patients and usually arising in adulthood (Hay et al 1998). It has been reported that mutations in the carboxy terminal end of the C1 domain and the immediately adjacent region of C2 are more frequently associated with inhibitors (Hay et al 1998). Four patients in our small series had a factor VIII gene mutation in the C1 domain and the other two had a factor VIII gene mutation in the A3 domain which has not been described previously in association with inhibitor development. It is possible that the location at which the mutation occurs influences the likelihood that inhibitors will develop.

Some of the other risk factors for inhibitor development in our series are intensive therapy for surgical procedures, switching FVIII products within a short period and using continuous infusion (CI) of clotting factor concentrate. Unusual exposure to large doses of factor VIII within a short time span probably accounted for the development of inhibitor in our group of patients. Hay et al has also shown 14 out of 23 patients with mild and

moderate haemophilia A developed inhibitors following intensive replacement therapy for surgery trauma or muscle bleeding (Hay et al 1996a).

A study on 74 episodes of CI treatment involving 62 patients in our haemophilia centre has been performed to assess if CI influenced the development of inhibitors and logistic regression analysis revealed that mild and moderate haemophilia A was the only variable that emerged as a significant predictor of inhibitor development in association with the use of continuous infusion (Hermans et al 2002).

Interferon-alpha (IFN- α) therapy has been associated with the development of various auto-antibodies and there has been case reports on the possible association of FVIII inhibitors developing in mild haemophilia patients on IFN therapy (Castenskiold et al 1994, Suzuki et al 1995). Two of our patients (Case1&4) had received IFN for chronic HCV infection and the inhibitor developed two years and one year respectively after the interferon treatment. Case 1 (Arg2163His) who had factor VIII gene mutation in the C1 domain developed the highest peak inhibitor titre of 580 BU five months after the last factor VIII infusion. The patient had not received any FVIII containing products since the initial diagnosis of the inhibitor. The low level of factor VIII produced by the patient posed as a chronic antigenic challenge and the endogenous antigenic burden appears to be critical for this patient's immune responsiveness and development of a high titre anti FVIII antibody. Interestingly two unrelated patients with mild haemophilia associated with Arg2150His mutation in the C1 domain of factor VIII were reported with anti-factor VIII antibody inhibiting allogeneic but not autologous factor VIII (Peerlinck et al 1999). These two patients maintained significant factor VIII activity despite the development of high titre anti-factor VIII antibodies. In a detailed study it was shown that these findings were not due to the kinetics

of factor VIII inactivation but rather to epitope specificity of the inhibitor, so that wild-type but not the patient's own variant factor VIII was inhibited.

FVIII inhibitor antibodies with C2 domain specificity are less inhibitory to FVIII complexed with von Willebrand factor (Suzuki et al 1996). Patient number 1 in our series had FVIII inhibitors that bound predominantly to the C2 domain and in patients with this pattern of inhibitor reactivity, intermediate purity FVIII concentrates maybe a better therapeutic option than high purity or rFVIII concentrates which contains little or no VWF. In the two patients (Case1&4) with factor VIII gene mutation in the C1 domain, epitope mapping of the FVIII inhibitors revealed that the inhibitors mainly reacted with the light chain domain of the FVIII (A3C1C2) indicating that the inhibitory antibodies were against the epitopes which were present in or near the mutation site.

Non-genetic factors may have an important role in the development of inhibitors in this group of patients with secondary intolerance to factor VIII. Tolerance to self-factor VIII can be broken in mild/moderate haemophilia A patients by mechanisms similar to those described in auto-immune patients, namely infections or traumas leading to altered processing pathways by unusual antigen-presenting cells attracted to the injured site or abnormal proteolytic cleavage due to enzymes present at such sites. The concurrent exposure to surgery may have posed as an immunologic challenge for this group of patients who developed FVIII inhibitors after intensive treatment for the surgery.

Management of patients with inhibitors is a major therapeutic challenge for haemophilia treaters and the cost of replacement therapy in these patients is increased by a threefold on average (Lipton 1994). Approach to the management of factor VIII inhibitor patients can be broadly divided into treatment of an active bleed and suppression of inhibitors and guidelines on this have recently been published (Hay et al 2000).

Three patients have been given immunosuppressive therapy including oral prednisolone, cyclophosphamide and intravenous immunoglobulins. One patient had partial response and developed side effects from the immunosuppressive therapy. Two patients responded by achieving negative Bethesda assays, one of them has normal baseline FVIII levels, whilst the other still has a baseline FVIII level of 7 iu/dl which is lower than his normal level of 17 iu/dl.

FVIII antibodies are currently evaluated in vitro by their capacity to inhibit the functional activity of FVIII in coagulation (Kasper et al 1975) or chromogenic assays (Svendsen et al 1984). However, antibodies directed towards nonfunctional sites of factor VIII that are not detected by current assays could influence in-vivo the clearance rate of FVIII and therefore be of pathologic relevance (Nilsson et al 1990).

Studies have shown that a majority of the FVIII determinants recognised by human FVIII antibodies are nonfunctional (Gilles et al 1993). The clinical relevance of these antibodies is poorly understood. ELISA methodology allows the detection of antibodies directed towards FVIII determinants that are not involved in the functional activity of the molecule.

We have tested the plasma of patients who have negative bethesda assays for their inhibitor quantitation with an enzyme linked immuno assay (ELISA) and found them to be reactive.

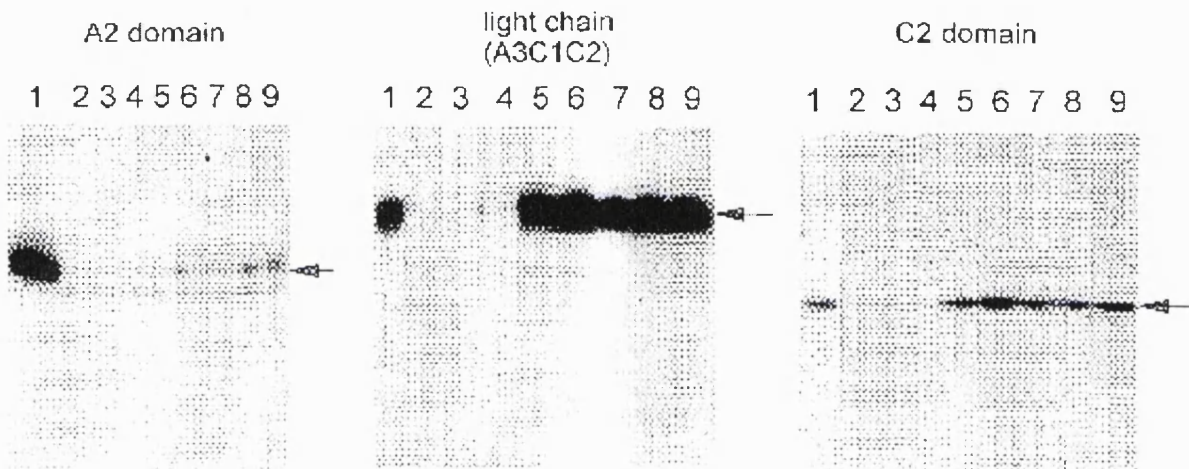
Studies have shown that antibodies to FVIII are frequently directed towards epitopes of FVIII that are not directly involved in the function of the molecule and therefore escape detection in the Bethesda method or chromogenic assay and each patient shows a unique pattern of FVIII epitope recognition. Thereby evaluation of anti-FVIII antibodies by a functional method only does not provide an accurate evaluation of the specific antibody response.

The development of more specific therapies to eradicate FVIII inhibitors is clearly desirable, but requires a more thorough understanding of the mechanisms by which anti-FVIII antibodies are produced as well as an improved assay methodology. The role of the ELISA may go hand in hand together with the conventional methods in assessment and management of FVIII antibodies.

The combination of clinical observations in mild/moderate haemophilia A patients with active testing of hypotheses should provide us with better understanding of the mechanisms by which tolerance to factor VIII is induced and maintained. It is also expected that such knowledge will bring about new insights on tolerance to self in general.

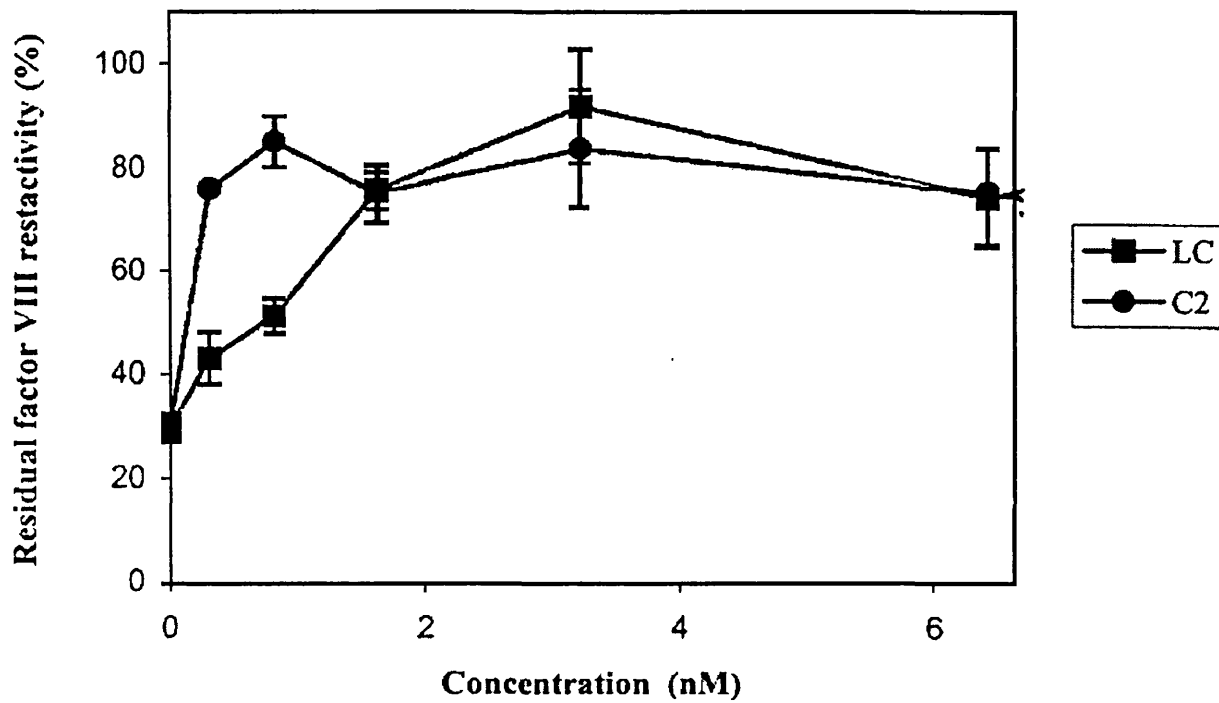
In conclusion inhibitor formation in patients with mild and moderate haemophilia A is becoming more recognised as a major problem in haemophilia care today. DDAVP should therefore be the treatment of choice especially for patients carrying a high risk FVIII genotype or those with a family history of inhibitors.

Figure 12.1 : Immunoprecipitation assay of patient no. 1.



1	positive control (monoclonal antibody)				
2	negative control (1 healthy donor)			6	patient no. 1
3	patient no. 1	special		7	"
4	"	02-01-96		8	"
5	"	15-05-96		9	"
					05-11-96
					07-01-97
					30-04-97
					02-09-97

Figure 12.2 : Neutralisation assay of patient no. 1.



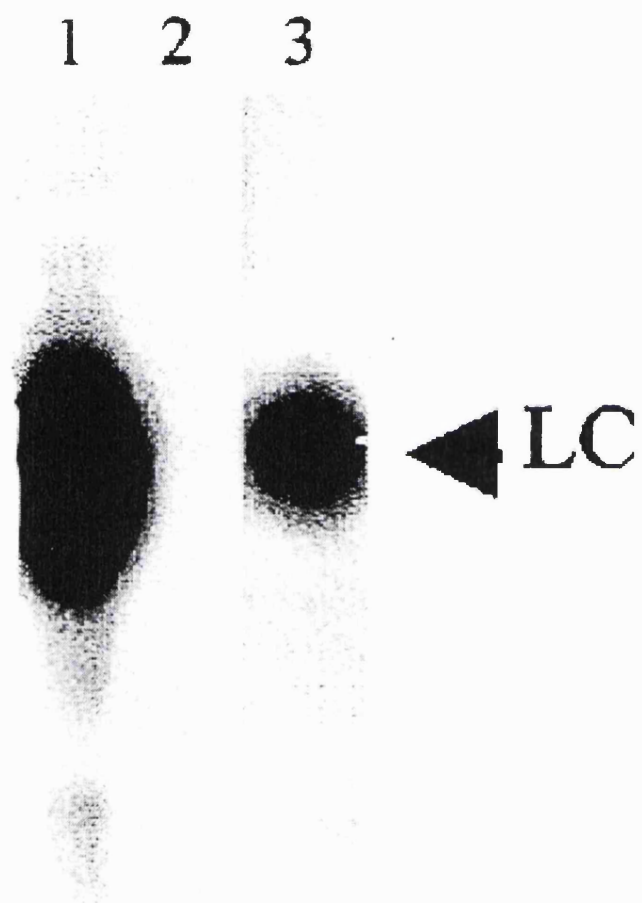
PERCENTAGE OF NEUTRALISATION:

SAMPLE DATE: 15/06/96

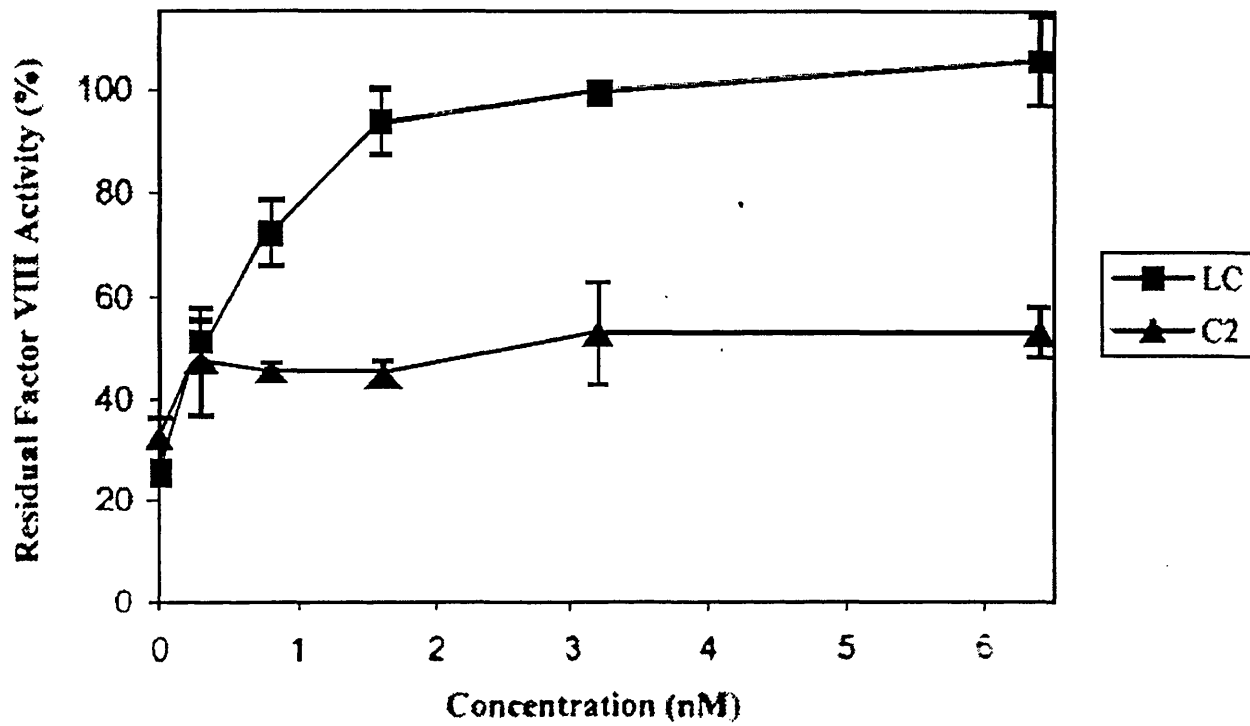
LIGHT CHAIN $90 \pm 20\%$

C2 DOMAIN $80 \pm 20\%$

Figure 12.3 : Immunoprecipitation assay of patient no. 4.



- 1) positive control (monoclonal antibody)
- 2) negative control (normal plasma)
- 3) patient no. 4 17/03/99

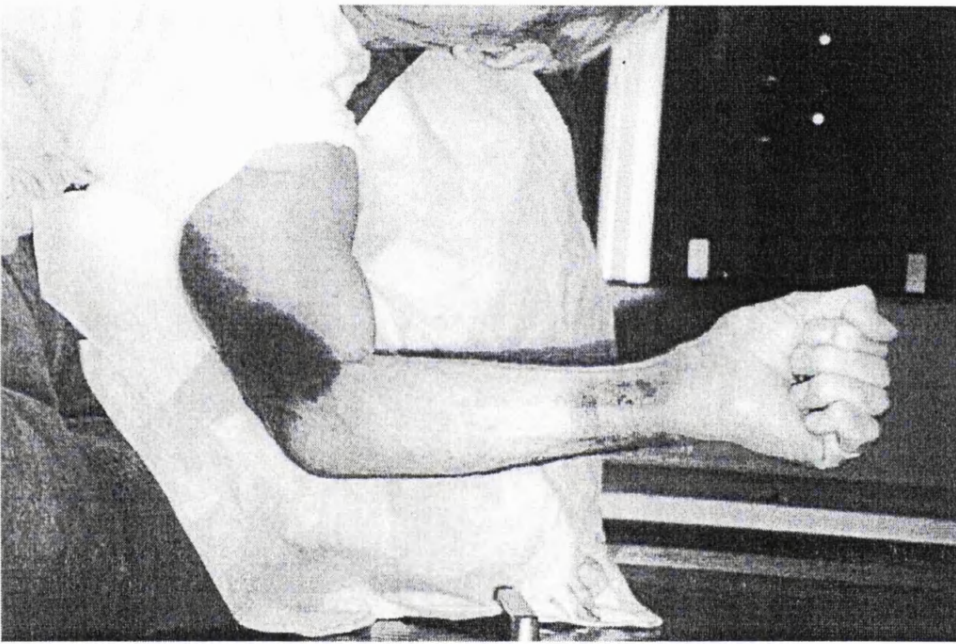
4) **Figure 12.4 : Neutralisation assay of patient no. 4.**

PERCENTAGE OF NEUTRALISATION:

SAMPLE DATE: 17/03/99

RECOMBINANT LIGHT CHAIN (LC) 100 ± 5%

RECOMBINANT C2 DOMAIN (C2) 30 ± 15%



PHOTOGRAPH 12.1 BLEEDING PATTERN OF PATIENT NO: 5

Chapter 13

COMPLICATIONS ASSOCIATED WITH NEW TREATMENT STRATEGIES

- 13.1 OVERVIEW OF NEW TREATMENT STRATEGIES**
- 13.2 EXPERIENCE OF PROPHYLAXIS TREATMENT IN CHILDREN WITH
SEVERE HAEMOPHILIA**

13.1 OVERVIEW OF NEW TREATMENT STRATEGIES

Prophylaxis

Intravenous replacement of the missing clotting factor is the most logical and appropriate treatment of haemophilia. Before replacement therapy was available, bleeding episodes were treated with conservative measures and transfusions with whole blood or plasma in case of severe haemorrhages. However, replacement therapy for haemophilia A on a large scale only became feasible with the development of cryoprecipitate in 1965 (Pool & Shannon 1965). Initially, replacement therapy was only given in case of bleeding episodes (on demand). The advent of self-infusion and home-based therapy permitted prompt treatment of haemorrhages, minimising morbidity and decreasing time lost from school or work and it has been estimated a decrease in the incidence of haemophilic arthropathy by 86% as a result of self-infusion (Levine 1974). Nevertheless, despite aggressive on-demand infusion treatment, progressive arthritis and musculoskeletal disability were not completely eliminated (Arnold & Hilgartner 1977). Therefore the concept of prophylactic therapy for patients with severe haemophilia was investigated first in Malmö, Sweden (Nilsson et al 1992). They are based on the observation that patients with moderate factor VIII or IX deficits rarely present with consequences of the disease such as haemophilic arthropathy. The aim of these prophylaxis programmes is to obtain constant minimum factor levels of over 1-2% in patients with severe haemophilia, through the continuous administration (every 2-3 days) of factor concentrates. Thus, severe haemophilia is converted into moderate haemophilia.

Continuous replacement therapy or prophylaxis programmes applied to patients with severe factor VIII or IX deficiency have become widespread, especially in those countries considered 'developed'. These programmes are achieving excellent results and their

efficacy in preventing the irreversible joint damage arising from haemophilia is now universally accepted.

Continuous Infusion

A second advance in the concept and mode of delivery is the use of continuous infusion techniques. Continuous infusion of plasma fractions rich in blood clotting factors was successfully introduced over 20 years ago (McMillan et al 1970). Clotting factor concentrates that are virally inactivated, lyophilised, stable and of higher purity have recently been made available from sources including recombinant DNA technology. These improved products permit broader applications for continuous infusion techniques by addressing issues raised in earlier trials such as the need for reliable potency, high purity, stability over many hours and sterility when properly reconstituted. Continuous infusion of factor VIII has proved useful in surgical settings and for major haemorrhages that threaten life or limb that are treated in the hospital. As concern about healthcare costs continue, especially in the industrialised nations, continuous infusion techniques can yield real savings by reducing the total amount of therapeutic agent required and decreasing the need for laboratory monitoring of factor VIII levels.

The complications of continuous infusion (CI) therapy are primarily related to central venous catheter infections and thrombosis. Pump failure can also interrupt therapy, and because of their inherent simplicity, the use of less sophisticated mechanical pumps may decrease the frequency of these problems. Few data are however available on the development of inhibitors in patients with haemophilia treated with CI.

Inhibitor formation is an increasingly recognised problem in patients with mild and moderate haemophilia A. While this finding may have resulted from improved data

collection it is also possible that recent changes in clinical practice, such as the use of continuous infusions and high purity or recombinant FVIII, are associated with increased inhibitor formation. Recently reports of factor VIII antibodies developing in mild and moderate haemophilia patients undergoing surgery with clotting factor concentrate given in continuous infusions have been reported (Yee et al 1999, Baglin et al 1998, White et al 2000). The implications of such an association would have considerable impact on the management of patients with mild or moderate FVIII deficiency.

13.2 EXPERIENCE OF PROPHYLAXIS TREATMENT IN CHILDREN WITH SEVERE HAEMOPHILIA

13.2.1 INTRODUCTION

The term prophylaxis means prevention. In haemophilia, preventative treatment is aimed at preventing the consequences of bleeding episodes, in particular, the joint injuries resulting from repeated bleeds into the joint. The aim of primary prophylaxis is to avoid joint bleeds and secondary prophylaxis is to minimise joint injuries once repeated bleeds into the joints have occurred. Today, primary prophylactic therapy may be considered as the gold standard of therapy.

All clotting factor concentrates have to be administered by intravenous injection and have a short half-life. The half-life of factor VIII is about eight hours in children, and about 12 hours in adults and the mean half-life of factor IX is about 24 hours (Messori et al 1988). Due to the pharmacokinetic properties of clotting factor products, prophylactic administration is most effective when given frequently, that is at least three times weekly. However, administering such an intense treatment programme to patients at such an early age may lead to serious difficulties because of the limited availability of venous access.

The insertion of devices that aid the administration of intravenous medication, such as permanently implanted catheters (e.g. the Port-A-Cath) is a significant advance, although they are not free of complications, of which the most serious are infections and thromboses.

It has also been argued that prophylactic treatment can be associated with more side-effects than on-demand treatment. A child on prophylaxis may develop an inhibitor at an earlier age than the child treated on-demand because inhibitors develop during the first 50 infusions of factor VIII (Lusher 2000). The presence of a chronic disorder with a life-long need for frequent intravenous injections, especially in prophylactic treatments, also places a heavy burden on the patient and his family.

The KDHC&HU cares for adults and children and short-term prophylaxis has been in use since the late 1970s. In 1994, following recommendations by the United Kingdom Haemophilia Centres Doctors Organisation (UKHCDO), long-term prophylaxis has been practised for all newly diagnosed children with severe haemophilia.

We have evaluated our experience of prophylaxis in children with severe haemophilia attending our centre and this report forms part of an ongoing study into the long-term efficacy and cost-effectiveness of prophylaxis. The data provided here will form the baseline for continuing prospective study of this cohort of children with severe haemophilia. This study has been included in this thesis as some complications associated with the use of prophylactic regimens have also been addressed.

13.2.2 STUDY POPULATION AND METHODS

Records of all patients with severe haemophilia A and B under 17 years of age on December 1999 who were registered at our haemophilia centre were reviewed. Home

treatment training is managed by specialist haemophilia nurses and the children with severe haemophilia are reviewed by a doctor three to six monthly.

The following variables were collected for analysis: age at start of prophylaxis; home treatment training of carers and patients; use and complications of central venous catheters; frequency of joint bleeds; adverse events whilst on prophylaxis; hospital visits, annual consumption of FVIII/ FIX (IU/kg body weight) and orthopaedic joint scores.

Clinical joint scores were determined by a physiotherapist. Joints are evaluated on a pain scale of 0-3, where 0 is no pain and 3 is severe pain and by means of a physical examination which scores between 0-12, where 0 is a normal joint and 12 is the most severely affected. The physical examination includes assessment of swelling, muscle atrophy, axial deformity, crepitus, range of movement, flexion contracture and instability. According to this method, each of the six joints is scored separately, with a maximum score per joint of 15 and a maximum total joint score of 90 as shown in Table 13.1 (Pettersson 1994).

Table 13.1 : Clinical Joint Scoring System**Joint Evaluation**

PAIN 0-3	
0	<p>No pain.</p> <p>No functional deficit.</p> <p>No analgesic use (except with acute haemarthrosis).</p>
1	<p>Mild pain</p> <p>Does not interfere with occupation nor with activities of daily living (ADL).</p> <p>May require occasional non-narcotic analgesic.</p>
2	<p>Moderate pain.</p> <p>Partial or occasional interference with occupation or ADL.</p> <p>Use of non-narcotic medications.</p> <p>May require occasional narcotics.</p>
3	<p>Severe pain.</p> <p>Interferes with occupation or ADL.</p> <p>Requires frequent use of non-narcotic and narcotic medications.</p>
<p>BLEEDING 0-3</p> <p>This is measured by the number of minor and major haemarthroses per year.</p> <p>Major – Pain, effusion, limitation of motion, failure to respond with 24 hours.</p> <p>Minor – mild pain, minimal swelling, minimal restriction of motion, resolves within 24 hours of treatment.</p>	
0	None
1	No major, 1-3 minor.

2	1-2 major or 4-6 minor.
3	3 or more major or 7 or more minor.
PHYSICAL EXAMINATION This is based on an additive score of 0-12 with 0 being a normal joint and 12 being most affected. An (S) is added after the number if a chronic synovitis is clinically diagnosed.	
Swelling	0 = None 2 = Present (S) = Added after score if chronic synovitis is present.
Muscle atrophy	0 = None or minimal (< 1 cm) 1 = Present
Axial deformity (measured only at knee or ankle)	Knee: 0 = Normal, 0-7° valgus. 1 = 8-15° valgus or 0-5° varus. 2 = > 15° valgus or > 5° varus. Ankle: 0 = No deformity. 1 = Up to 10° valgus or up to 5° varus. 2 = > 10° valgus or > 5° varus.
Crepitus on motion	0 = None 1 = Present

Range of motion	0 = Loss of 10% of total full range of motion (FROM) 1 = Loss of 10-33 1/3 % of total FROM. 2 = Loss of > 33 1/3% of total FROM.
Flexion contracture Measured only at hip, knee or ankle	0 = < 15° FFC (fixed flexion contracture) 2 = 15° or greater FFC at hip or knee or equinus at ankle.
Instability	0 = None 1 = Noted on examination but neither interferes with function nor requires bracing. 2 = Instability that creates a functional deficit or requires bracing.

13.2.3 RESULTS

DEMOGRAPHICS OF THE PATIENTS

The notes of 41 children (34 HA & 7 HB) including six pairs of brothers with severe haemophilia were reviewed. Thirty-eight (93%: 32 HA & 6 HB) of the children were on prophylaxis. Two patients aged three and eight years with severe haemophilia A were not on prophylaxis due to infrequent bleeds. One child with severe haemophilia B developed anaphylactic reactions to FIX with a high responding FIX inhibitor and is treated on demand with recombinant VIIa.

Prophylaxis in 38 children was commenced at a median age 3.7 years (range 0.4-12.7) and 9/38 (24%) started before the age of two years. The follow up period on prophylaxis was a median 4.1 years (range 0.3-11.5).

The patients were divided into two groups (Table 13.2): group 1 consists of nine boys who started prophylaxis at a median age of 1.2 years (range 0.4-1.8); of these two had traumatic intracranial haemorrhage, two experienced one spontaneous joint bleed and five patients had frequent soft tissue haemorrhages but no joint bleeds before the start of prophylaxis. Group 2 (n=29) received prophylaxis at the median age of 4 years (range 2-12.7). In this group the median number of joint bleeds before prophylaxis was initiated was 3.5 (range 1-10) and one patient commenced prophylaxis because of an intracranial haemorrhage.

PHYSIOTHERAPY ASSESSMENT

Joints were examined clinically by a physiotherapist. The clinical joint scores were found to be 0 in 28/38 (73%) children, with a median score of 1.5 (range 1-10) in the remaining ten children. Physical examination identified a total of 18 affected joints in these ten patients and is shown in detail in Table 13.3.

Answers to a questionnaire about sporting activities revealed that all the children participated in sports (such as swimming, football, cricket, tennis) and physical activities (e.g. cycling, climbing trees, roller blading) on a regular basis. Fourteen of the children, aged \leq five years were involved in physical activities but not specific sports. Eighteen of the 22 children (82%) aged 6-16 years, played football, including six who played competitively in a team. Ten of the patients reported bleeds after sporting activities including football, tennis, roller-blading and physical education.

Venous access and associated complications

Currently peripheral venous access is used in 33/38 (87%) patients. Self-administration of treatment was achieved in 11/33 (33%) children, the youngest starting at age eight years. It was necessary to provide port-a-cath access for 8/38 (21%) children using a total of 11 port-a-caths: 5/8 (63%) are still using the devices. Port-a-cath insertion was performed at a median age of 15.5 months (5-36months).

Ten episodes of infection have occurred in 6/11 (54%) port-a-caths, necessitating removal of five catheters. The catheters were removed at a median time from insertion of 18 months (range 2-29 months). One port-a-cath (BardPort: M.R.I. Low Profile Implanted Port) snapped off from the hub and had to be removed from the right ventricle. The port-a-cath infection rate is 1.74/1000 catheter-days. More than one episode of infection occurred in 3/8 (38%) children and included a variety of organisms (Table 13.4 and 13.5). Half of the septic episodes were treated successfully with antibiotics infused through the port. The median survival of the five existing central lines is 13 months (range 8-33).

HOME TREATMENT TRAINING

The period of training, from initiation to competence and independence, was a median of six months (range 3-18 months). Both parents initially learnt the procedure in 20/29 (69%) two parent families but only 8/20 (40%) fathers continued to treat on regular or occasional basis. Nine children were in single parent families. Semi-structured interviews of parents and older patients showed the aspects of training which were particularly valued: continuity of care provided by the same nurse; flexibility of time and place to fit in with family and work commitments; follow up support when administering independently at home and regular review of progress once home treatment was established. Most parents and patients felt with hindsight that the training process was much easier than anticipated. The parents

comments on incentives for home treatment training and prophylaxis were that the children undertook many activities and sports previously not recommended; there was much less parental anxiety; and it was not necessary to rely on local hospitals and health care personnel unfamiliar with haemophilia. Seven of the eleven (64%) who self-administered treatment reported the need for prompting to initiate most treatments.

TYPE AND DOSE OF CLOTTING FACTOR CONCENTRATE

The older children with severe haemophilia A changed from intermediate or high purity FVIII concentrates to recombinant FVIII in 1996 according to the UKHCDO guidelines (UKHCDO 1997). All severe haemophilia B patients were previously treated with plasma derived FIX concentrates until the introduction of recombinant factor IX in 1999. The dose of prophylaxis used in our haemophilia centre is 25-40U/kg three times/week for haemophilia A patients and twice/week for haemophilia B patients. Morning dosing of all prophylaxis was emphasised to each and every patient.

HOSPITAL VISITS

As shown in Figure 13.1 hospital visits (in-patient, out-patient and day-case) decreased steadily as prophylaxis became more established and the small increase in the out-patient and day-case visits noted at the end of the graph were due to a few patients who had come in for minor procedures.

Figure 13.1 : Hospital Visits of the 38 children whilst on prophylaxis

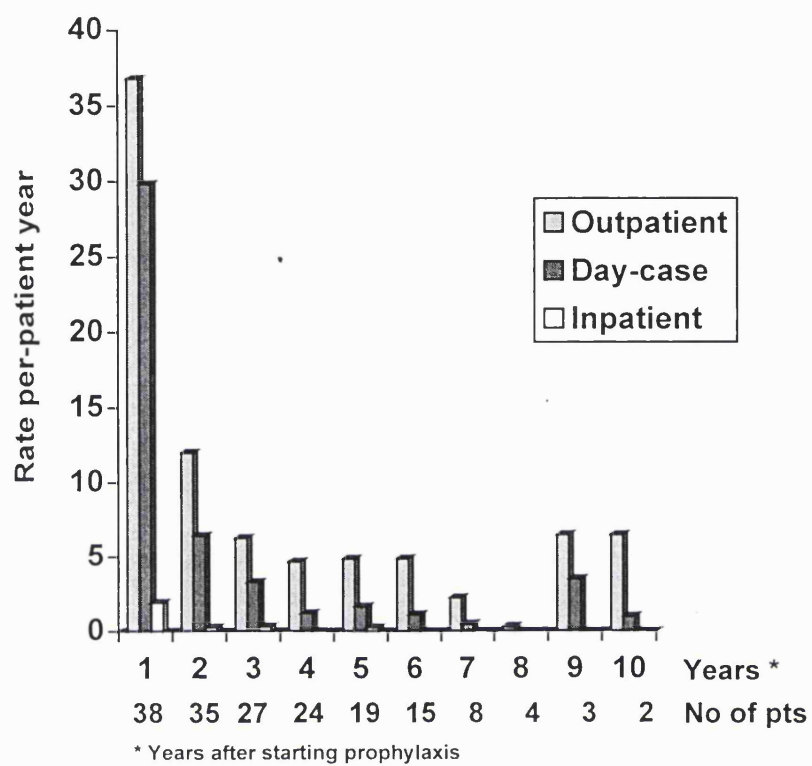


Table 13.2 : Details of 38 children with severe haemophilia who were treated with prophylaxis

Patient details	Group 1	Group 2
Number of patients (HA/HB)	9 (8/1)	29 (24/5)
Duration of prophylaxis (yrs) median (range)	4.3 (1.1-7)	3.8 (0.3-11.5)
Age at start of prophylaxis (yrs) median (range)	1.2 (0.4-1.8)	4 (2.0-12.7)
Age at time of study (yrs) median (range)	5 (2.1-8.2)	9.5 (2.8-16.3)
Dose of FVIII/FIX U/kg/yr at the time of study median (range)	5058 (2522-7726)	4141 (1797-7809)
Inhibitor		
Before prophylaxis	2 (undetectable now)	1 (undetectable now)
On prophylaxis	None	1 (undetectable now)
HIV/HCV infection	Negative	Negative
Orthopaedic joint score:		
Number of patients with score = 0	8 (90%)	20 (70%)
Number of patients with score = range 1-10	1 (median score 1)	9 (median score 1.5)
Joint bleeds: pre-prophylaxis; median (range)	1 (0-1)	3.5 (2-10)
whilst on prophylaxis; median (range) numbers/yr,	0.15 (0-0.30)	0.5 (0-1.76)

Table 13.3 : Joint Scores of ten children who had clinical joint scores of 1-10

Patient	Elbows	Knees	Ankles	Total joint scores
	R/L	R/L	R/L	
1	2/1	0/0	½	6
2*	0/0	0/0	1/1	2
3	3/0	0/0	0/0	3
4	0/0	1/0	0/0	1
5	0/0	0/0	1/0	1
6	0/0	0/0	0/1	1
7	0/2	4/0	0/3	9
8	0/6	0/2	0/2	10
9	0/0	0/0	0/1	1
10	0/1	0/0	0/0	1

Table 13.4 : Patients with Port-a cath.

Dx:	Ethnicity	Age (months) when 1 st port-a cath insertion	Reason	No. of Port-a cath	No. of infections/severe complications
HA	A-C	14	ICH	3	3
HA**	Caucasian	20	IPVA	1	Nil
HA**	Caucasian	30	IPVA	2	1 mechanical failure
HA	African	36	ICH	1	1
HA*	Asian	14	IPVA	1	1
HA*	Asian	15	IPVA	1	2
HA*	A-C	16	IPVA	1	3
HB	A-C	5	ICH	1	Nil

* No longer using port-a cath

ICH- intracranial haemorrhage

** brother pair

A-C – Afro-Caribbean

Dx - Diagnosis

IPVA- inadequate peripheral venous access

Table 13.4 : Patients with Port-a cath.

Dx:	Ethnicity	Age (months) when 1 st port-a cath insertion	Reason	No. of Port-a caths	No. of infections/severe complications
HA	A-C	14	ICH	3	3
HA**	Caucasian	20	IPVA	1	Nil
HA**	Caucasian	30	IPVA	2	1 mechanical failure
HA	African	36	ICH	1	1
HA*	Asian	14	IPVA	1	1
HA*	Asian	15	IPVA	1	2
HA*	A-C	16	IPVA	1	3
HB	A-C	5	ICH	1	Nil

* No longer using port-a cath

ICH- intracranial haemorrhage

** brother pair

A-C – Afro-Caribbean

Dx - Diagnosis

IPVA- inadequate peripheral venous access

TABLE 13.5 Organisms implicated in the port-a-cath infections

Gram Positive organisms		Gram Negative organisms	
Strep Viridans	2	Enterococci	1
Staph Aureus	2	Klebsiella	1
Staph Epidermidis	3	Enterobacter cloacae	1
Total	7	Total	3

13.2.4 DISCUSSION

Studies mainly from Sweden and other European countries have now shown the benefits of long term prophylaxis (Lofqvist et al 1998; Kreuz et al 1998; Van Den Berg et al 2001). In the UK there are 759 children <19 years with severe haemophilia A and B but only one paediatric haemophilia centre in the UK has published the experience of prophylaxis in haemophilic children (Liesner et al 1996).

A retrospective study of the prophylaxis practice in our haemophilia centre has shown that 38/41 (93%) of our children with severe haemophilia are on prophylaxis and 9/38 (24%) of the group fulfil the criteria of primary prophylaxis. In comparison, a recent survey of 20 haemophilia centres in Europe showed 39% of children receive primary prophylaxis (Ljung et al 2000).

Primary prophylaxis has been defined as starting before the age of two years or after the first joint bleed and secondary prophylaxis as starting at age > 2 years or after two or more joint bleeds. The best time to start prophylaxis is still debated. In 1994, the UKHCDO recommended prophylaxis for severe haemophilia in children who have had three

spontaneous haemarthroses or muscle bleeds. However in the Malmo model of prophylaxis in Sweden treatment is begun before the occurrence of the first joint bleed, around the age of one year when the child begins to walk (Nilsson et al 1992).

A majority (29/38, 76%) of the children in our centre started prophylaxis after a median joint bleed of 3.5 and 20/29 (70%) in that group had clinical joint scores of 0. According to Kreuz and co-workers (1998) a few joint bleeds can cause irreversible haemophilic arthropathy but Leisner et al (1996) reported that the joint status of 20 individuals all improved after starting prophylaxis. It is significant that 90% (8/9) of patients in group 1 who started prophylaxis before two years or after one joint bleed had a joint score of 0. The ten patients who had a total of 18 affected joints (Table 13.3) had a median joint score of only 1.5 (range 1-10) out of a total possible score of 90. Nine out of the ten children with joint scores ranging from 1-10 were in the group 2 who started prophylaxis at the median age of four years and after a median of 3.5 joint bleeds. As radiological examination of the joints was not done routinely in our centre, radiological alterations of the joints where bleeding had occurred could have been missed on clinical examination. The joint scoring system (clinical and radiological) currently in use was designed 20 years ago primarily for monitoring adult patients with more severe joint problems and may be inadequate for the follow-up of children of today who are treated more intensively. A more sensitive and refined joint scoring system for children receiving regular prophylaxis (Manco-Johnson et al 2000) with longer longitudinal follow up may determine whether joint problems are progressive in this group of patients.

Patients with severe haemophilia start to bleed at different ages (Pollman et al 1999) and a non-bleeding child may not benefit from prophylaxis. Thus before beginning primary prophylaxis under the age of two years it is important to take into consideration the

bleeding pattern of the patient. The commitment of the parents/carers and accessibility of the peripheral veins are also important issues in deciding when to start prophylaxis.

It has been argued that prophylactic treatment can be associated with more side effects than on demand treatment. In our series of 38 children on continuous prophylaxis only one patient developed a low titre transient inhibitor whilst on prophylaxis. Three patients had low titre (< 5 BU) inhibitors prior to starting prophylaxis which became undetectable whilst on prophylaxis. The low dose FVIII given regularly in prophylaxis may have had a tolerising effect on low titre inhibitors. The Swedish cohort which has the largest number of children on prophylaxis showed that 8% of their patients developed inhibitors whilst on prophylaxis (Nilsson et al 1992). Although there is no indication that prophylaxis is associated with an increased risk of inhibitor, a child on prophylaxis may develop an inhibitor at an earlier age than the child treated on demand since inhibitors develop during the first 50 infusions of FVIII (Lusher 2000). Blood-borne virus infection did not occur as all patients were treated with virally inactivated plasma derived clotting factor concentrates and were later changed to recombinant products.

Two important practical problems in prophylaxis are the route of administration and the high cost of clotting factor concentrates. The use of venous catheters in children were studied in the centres belonging to the European Paediatric Network for Haemophilia Management and it was found that in three out of 19 centres, > 50% of boys under the age of six had a port while none had the device in seven out of 19 centres (Ljung et al 2000). In our haemophilia centre it was necessary to provide intravenous catheter access for 8/38 (21%) children. Difficult peripheral venous access was the reason in five patients, while in the other three patients central venous access was necessary for establishing intensive therapy following intracranial haemorrhage. Some haemophilia centres have advocated

that right atrial catheters are the way forward to facilitate successful implementation of prophylactic regimens (McMahon et al 1998) but only 3/20 (15%) haemophilia centres in Europe preferred central venous lines for prophylaxis in 80-100% of their children (Ljung et al 2000).

Continued reports of complications associated with central venous catheters are being published (Blanchette et al 1997; Bollard et al 2000) and infection necessitating removal is the commonest complication. In our small cohort of 11 port-a cath placed in eight children a port-a-cath infection rate of 1.74/1000 catheter days was noted requiring removal of six (55%). Although the port-a cath infection rate has been calculated, it is difficult to compare with other studies as only eight patients in total have received the port-a cath. Whether this rate of catheter related infection is acceptable in our small series is difficult to determine. In three of the patients central venous catheters were required because of intensive treatment for intracranial haemorrhages. It has been reported by various studies that patients who have inhibitors have a higher rate of catheter related sepsis (Van Den Berg et al 1998). There are no children with high responding inhibitors requiring immune tolerance in our haemophilia centre (Yee et al 1997; Yee et al 1999). Table 13.6 shows the rate of infection in a recently published series of haemophilia patients using central venous lines.

Table 13.6 : The rate of infection in recent series with haemophilia patients using central venous lines.

Study	Number of patients (<i>n</i>)	Rate of infection per 1000 patient days	Comment
Blanchette et al 1996	19	0.7	3 patients with inhibitors, 3 HIV+
Perkins et al 1997	35	1.2 (central) 0.7 (peripheral device)	7/32 inhibitor, 2/32 VWD
Ljung et al 1998	53	0.19	11 patients with inhibitors
Miller et al 1998	41	0.14	Includes external
McMahon et al 2000	58	1.6 (without inhibitor) 4.3 (with inhibitor)	77/86 devices Port-A-Cath 37/58 patients haemophilia

In practising prophylaxis in children the first option is to use a peripheral vein. It is essential to have paediatric nurses with experience and skill in administering intravenous medication to very young children, along with the necessary time, space and calm to carry out the treatment successfully.

The home treatment training programme required a considerable amount of time and cost beyond that of training for on demand therapy. The training programme starts with the teaching of bleed recognition, what prophylaxis is about, basic hygiene, mixing the clotting

factor concentrates and the actual venepuncture. Parents practise venepunctures on adults first and then perform on their respective children. Training is done at the haemophilia centre starting with once a week and after an initial two to four weeks training at the hospital the community nurse visits the home and continues training to fit in with the family and work commitments of the carers of the haemophilic boy. As a result we were able to establish 33/38 (87%) of our children on peripheral venous access and the youngest infant whose family could administer CFC via peripheral vein was 13 months old compared to 4.8 years in another study (Leisner et al 1996).

In our haemophilia centre it has been shown that individuals with mild/moderate haemophilia were 45%, 36% and 70% less likely to have required in-patient, out-patient and day-case visits and also 66% and 23% less likely to have undergone orthopaedic procedures and joint replacements, respectively, compared with individuals with severe haemophilia (Miners et al 2000). Thus providing prophylaxis for our patients with severe haemophilia in an attempt to 'convert' them to moderate haemophilia could significantly reduce the input of expensive medical resources required for individuals with severe haemophilia.

In conclusion, a group of 38 boys with severe haemophilia have benefited from practising regular prophylaxis in terms of marked reduction in the number of joint bleeds, low clinical joint scores, less hospitalisation with improved attendance at school and participation in sports and physical activities. Prophylaxis treatment in our centre has not resulted in any blood borne viral infections or the development of inhibitors. The only complication associated with prophylaxis has been the infections associated with central venous lines.

The children with severe haemophilia who have benefited from prophylaxis may grow up to be adults free of pain and arthropathy enabling them to contribute to the economy of the health care system that had provided them with this therapy.

Chapter 14

CONCLUDING REMARKS

- 14.1 SUMMARY OF MAIN FINDINGS AND IMPLICATIONS REGARDING
BLOOD BORNE VIRAL INFECTIONS**
- 14.2 OVERALL CONCLUSIONS OF FVIII INHIBITORS**

14.1 SUMMARY OF MAIN FINDINGS AND IMPLICATIONS REGARDING BLOOD BORNE VIRAL INFECTIONS

The haemophilia community will be dealing with the tragic consequences of blood-borne HIV and HCV infection for years to come. Haemophilia cohorts followed in various countries have experienced high HIV-related mortality. By the end of 1995 approximately 39% of an estimated 9200 affected patients in the USA had died (Rosenberg & Goedert 1998), and more recent updates from Canada and the UK, report cumulative mortality of 54% (of 658 individuals) and 64% (of 1350), respectively (Walker et al 2000; Winter et al 2000). From 1995 onwards as highly active antiretroviral therapy became widely available the mortality of HIV infection has been markedly reduced.

The study in our haemophilia centre on the clinical outcomes and response to HAART in a cohort of haemophilic individuals infected with HIV for two decades has shown a reduction in the incidence of AIDS since 1997 but the death rate remained high as a consequence of deaths related to liver disease due to chronic hepatitis C infection.

The natural history of hepatitis C infection in our cohort of 310 individuals who have been exposed to virally inactivated blood products from 1961 till 1985 and followed up for 25 years have also shown that HIV and HCV co-infected patients have rapid progression to end stage liver disease.

As HIV therapies improve, the focus of physicians caring for patients with inherited coagulation disorders is to recognise the increased morbidity and mortality associated with HCV infection which has now become the second leading cause of death in this group of patients and this infection is considerably more prevalent than HIV.

The need to treat hepatitis C in HIV patients has recently become a significant issue and adequate treatment of chronic hepatitis C has become a priority in HIV/HCV co-infected patients.

Joint haemophilia/liver/HIV clinics are highly recommended for comprehensive care of the HCV/HIV infected haemophilia patients and from May 2000 to May 2002 we have further treated 30 patients with inherited bleeding disorders (four co-infected) using pegylated interferon and ribavirin (Yee et al 2002).

Clinicians should be made aware of the overlapping toxicities of both treatments and management of them. Timing of treatment for HCV in this co-infected group should be considered in those whose HIV infection is stable and who are not in immediate need of antiretroviral therapy to reduce the risk of toxicity. Alternatively, patients with more advanced HIV disease should be placed on a stable antiretroviral regimen with optimisation of CD4 cell count and HIV viral suppression before starting treatment for hepatitis C. When both treatments have to be administered concomitantly, the choice of nucleotide reverse transcriptase inhibitors must be orientated toward drugs with lower mitochondrial γ polymerase affinity mainly if ribavirin is to be used in combination with interferon. This approach would be useful to increase the safety of coinfection therapy and to avoid cases of fatal lactic acidosis. Studies have shown a ranking of mitochondrial polymerase γ affinity by antiretroviral medications in the order of ddc>ddi> d4t> 3TC> AZT> Abacavir (Kakuda 2000).

The needs and new challenges of those with long term HIV infection is also changing in the era of HAART. Carers of HIV infected individuals need to be aware of this change in the perceptions of these patients in order to provide support.

We and others have shown that high HIV viral loads to be a risk factor for heterosexual transmission of HIV infection in haemophilia sero-discordant couples (Yee et al 1999; Ragni et al 1998). HAART therapies could significantly lower viral load levels to less than 50 copies/ml and viral load determinations may be helpful in counselling haemophilic couples regarding transmission to female partners. Couples should be assisted in reducing the risk as far as possible while being allowed the basic right to exercise their own choice.

The disastrous consequences of blood-borne virus infections in haemophiliacs have provided the impetus for the development of safer clotting factor concentrates, with truly remarkable results. The current haemophilia replacement products have asymptotically approached the theoretical limit of absolute freedom from transmission of blood-borne viruses. Recognition of these facts should not promote an attitude of resignation or complacency. Continued surveillance is needed to monitor unexpected transmission of known agents and to recognise the emergence of new viral threats which will predictably arise, perhaps originating as zoonoses.

We have discussed the two patients in our haemophilia centre who received virally treated clotting factor concentrates in the mid 1990s and contracted acute parvovirus infection through the concentrates. The removal or inactivation of every virus particle in clotting factor concentrates is a worthy goal in theory, but in practical terms this may be unattainable. Clearly there is a need for continuing surveillance.

The availability of safe concentrates also does nothing to diminish the importance of assuring that susceptible individuals are vaccinated against known blood-borne pathogens. Vaccines are currently available for HBV and HAV. At the same time, further

technological innovations will be needed to assure that clotting factor concentrates do not transmit nonviral 'infectious' agents, such as the prions, associated with transmissible spongiform encephalopathies.

The development of powerful methods in molecular biology, such as immunoscreening of nucleic acid libraries, representative differential amplification (RDA) and other methods have made the molecular identification of new viruses feasible in most research laboratories and therefore the stage is set for the discovery of new viruses at a rapid pace. Virus identification no longer requires culture in susceptible cell lines but only the application of standard genomic amplification techniques adaptable to most blood centre laboratories. For each new virus discovered, relevance to transfusion will need to be evaluated.

Future developments should be towards producing recombinant clotting factor concentrates, in which no human and animal plasma derived substance is used in the final formulations or in any stage of the fractionation process and some of these third generation products are undergoing clinical trials (Tarantino et al 2002). This will eliminate the real risk of viral transmission as well as the theoretical risk of transfusion transmitted prion diseases, in particular the recently recognised variant form of Creutzfeldt-Jakob disease (vCJD).

Trials of gene therapy for haemophilia A and B are now in progress. This approach uses the recipient as the bioreactor for producing factor VIII or IX, thus eliminating any risk of exogenous contamination of the replacement protein. The safety of gene therapy approaches, especially those which use viral vectors for gene delivery, will need to be proven.

Prevention of viral infection relies on the application of sequential strategies including donor selection, testing of donated blood, partitioning of viruses from therapeutic components, viral inactivation, active immunisation, and postmarketing surveillance and it is important for all those concerned in the care of haemophilia patients (manufacturers, treaters and government regulators) to take the responsibility for implementation of these strategies.

Lastly but not the least is the provision of virally safe products of low purity such as cryoprecipitate, plasma and prothrombin complex concentrates for developing countries. This is still a critical priority, so that the goal of universal access to effective and safe haemophilia treatment will be achieved.

14.2 OVERALL CONCLUSION OF FVIII INHIBITORS

There is still a significant amount of disagreement and debate regarding the relative risk of inhibitor formation in conjunction with the use of FVIII products of different purity. The availability of recombinant concentrates has added an additional dimension to the issue of inhibitor risk of whether the source as well as the purity of FVIII product poses an additional risk to the development of inhibitors.

There is the possibility that repeated switching from one product to another facilitates an immune response. The studies with the highest cumulative inhibitor incidence were those in which the patients regularly switched products (Ehrenforth et al 1992; Addiego et al 1993). Switching could not only facilitate immune recognition of FVIII, it could make it much more difficult to recognise that specific products are more immunogenic than others.

A retrospective study of FVIII inhibitors experienced in a single comprehensive centre over three decades (1964-1997) have shown a relatively low frequency of inhibitors. There was

a period between 1988-1995 (8 years) where there was no new incidence of FVIII inhibitors in the centre. The treatment policy of exposing patients to a single product only and avoiding switching to several different products maybe one of the reasons for this low incidence of inhibitors.

From 1985 till 1995 all previously untreated severe haemophilia children who have the highest risk of developing inhibitors were treated solely with a plasma derived intermediate purity factor VIII concentrate and a study on these PUPs showed absence of inhibitors. It is possible that the residual and contaminating proteins in the intermediate purity products have immunomodulatory effects on the immune system of the recipient thus reducing the likelihood of inhibitor formation. The well-preserved von Willebrand factor multimers may block epitopes on the light chain of the FVIII molecule to which most of the alloantibodies to FVIII react. There is an increasing amount of in vitro and in vivo evidence suggesting that VWF may play a role in the reactivity of FVIII inhibitors with FVIII (Littlewood et al 1996; Berntorp et al 1996; Suzuki et al 1996; Kallas et al 2001).

Six recombinant FVIII studies in PUPs including a total of 360 severely affected PUPs have been reviewed. Although the trials differed somewhat in trial design there were important similarities in results to date and have shown that 22-31% of severely affected PUPs treated with rFVIII develop inhibitors (Lusher 1999).

The development of inhibitors in six haemophilia A patients with mild and moderate severity disease has been observed in our haemophilia centre recently. Since 1995 there has been a change in our clinical practice of using continuous infusions of either high purity

FVIII products or recombinant clotting factor concentrates in patients undergoing surgery or in certain clinical conditions where continuous treatment is necessary. Five out of the six patients who developed inhibitors were treated intensively for surgery with continuous infusions of different high purity and recombinant FVIII concentrates over a short time span. A study on the development of inhibitors in 62 patients treated with 74 episodes of continuous infusion did not show that the new treatment strategy of administering clotting factor concentrates continuously was related to the development of inhibitors in haemophilia patients. Unusual exposure to large doses of factor VIII and exposure to different FVIII products at a time of another immunologic challenge (surgery) probably accounts for the development of inhibitor in a significant proportion of patients with mild haemophilia A.

The development of inhibitors to factor VIII is a universal risk for individuals with haemophilia receiving clotting factor concentrates. The factors that control the response of patients with haemophilia to factor VIII are likely to be multiple. Genetic factors at the locus bearing the haemophilia mutation and other loci (notably HLA) are important factors in the development of inhibitors (Schwabb et al 1995; Oldenburg et al 1997).

The above mentioned studies in the thesis emphasise, in addition, the potential effect of non-genetic factors such as therapy related factors, the type of factor VIII products used and the factors that modulate the immune response at the time of factor VIII infusion may also play an important part in the development of inhibitors. Evidence is accumulating of the role for non-genetic factors affecting the immune response.

There are still a number of unresolved issues regarding the type of FVIII concentrate as a potential risk for the development of FVIII antibodies. A number of essential questions still remain to be explored on the immunogenicity of FVIII and the mechanisms that modulate and condition the antigenicity of FVIII products. It is difficult to answer the question as to whether some products are safer with regard to the reduction of the risk of inhibitor formation because there are many confounding factors. The issue of purity of products or the source of the product, pose, as a risk factor for inhibitor formation can only be addressed by the pursuit of prospective randomised trials involving controls in which the inhibitor incidence among recipients of concentrate A versus concentrate B is compared.

More pre-clinical assessments on the antigenicity of FVIII concentrates should help anticipate their immunogenicity and provide means to select and design FVIII preparations having the lowest chance of inducing inhibitors.

In conclusion, there clearly is no simple answer to the question how some haemophiliacs develop antibodies. Since high-titre inhibitors remain a major threat to haemophiliacs despite new therapeutic approaches, mechanisms of inhibitor development will remain a major area of haemophilia research in the years to come. Findings may even have implications for the management of other antibody-mediated disorders.

In our series of 38 children < 17 years of age who are on continuous prophylaxis, only one patient developed a low titre transient inhibitor while on prophylaxis. Three patients who had low titre inhibitors (< 5 B.U.) prior to starting prophylaxis, became undetectable whilst on prophylaxis. The low dose FVIII given regularly may have had a tolerizing effect on

low titre inhibitors. It has been argued that prophylaxis can be associated with more side-effects than on-demand treatment. A child on prophylaxis may develop an inhibitor at an earlier age than the child treated on-demand because inhibitors develop during the first 50 infusions of FVIII (Lusher 2000). The Swedish cohort of children on prophylaxis which is one of the largest cohorts showed that 8% of their patients developed inhibitors whilst on prophylaxis (Nilsson et al 1992).

In our small cohort of 11 catheters placed in eight children, an infection rate of 1.74 per 1000 patient days was noted, requiring removal of six (55%). All these children were HIV and FVIII inhibitor negative. Ten episodes of infection have occurred in 6/11 (55%) catheters, necessitating removal of five. There were seven gram positive infections and three gram negative infections. Half of the septic episodes were treated successfully with antibiotics infused through the port. Whether this is an acceptable frequency of infections for this small group of eight patients is difficult to determine. In three of the patients a Port-a cath was required as the babies had intracranial haemorrhages and required intensive continuous treatment. The other complication that we have experienced is a mechanical failure of the catheter, where it snapped off at the hub and had to be removed under general anaesthesia from the right ventricle. Thrombosis which is another main complication of central venous catheters has not been seen in our small series but routine venograms were not performed.

The use of antibiotics in the perioperative period varies between centres and remains controversial. Most of the experience is from cancer patients and in some studies antibiotics have been of value (Lim et al 1993), while in others no such correlation was

found (Ranson et al 1990). Vigorous education in aseptic techniques and follow up and reactivation of the education are keystones in reducing the risk of infection.

It has also been speculated that the common use of EMLATM anaesthetic cream may have a role (Perkins et al 1997). A reduction in infections was found after parents were instructed to scrub the cream off by soap and water in order to remove the residual lipid from the skin. This is probably an experience to include in the education of aseptic techniques.

In conclusion, therefore, this thesis has presented a selection of studies in a well-defined cohort of individuals with inherited bleeding disorders.

The large cohort of HCV and HIV infected haemophilic patients followed up in this haemophilia centre will continue to provide answers to important clinical questions in the future and the observations on inhibitor development need to be added to the experience of other clinicians and scientists in order to provide more understanding.

It is hoped that the findings and issues presented here will contribute to the work of other groups of treaters and patients with inherited bleeding disorders.

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