INHERITED BLEEDING DISORDERS IN

OBSTETRICS AND GYNAECOLOGY

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ABSTRACT

The aim of this thesis is to investigate the obstetric and gynaecological problems and their management in women with inherited bleeding disorders, as well as the role of such disorders in obstetric and gynaecological haemorrhage.

The uptake of prenatal diagnosis and termination of an affected pregnancy is low in carriers of haemophilia. Fetal gender determination has important implications in the management of labour in carriers who do not wish to have specific prenatal diagnosis. The attitude of women towards reproductive choices is influenced by ethnic and cultural issues and family experience with the disease.

Haemostatic response to pregnancy is variable in different types and subtypes of inherited bleeding disorders and in the same patient in different pregnancies. Haemorrhagic complications are confined to post-abortal and post-partum period. The incidence of primary and secondary post-partum haemorrhage was 22% and 11% in carriers of haemophilia, 18.5% and 20% in vWD and 16% and 24% in FXI deficient women, respectively. Women with low factor levels (< 50 iu/dl) and no prophylactic treatment for labour and puerperium are especially at risk.

There are great inter- and intra-individual variations in coagulation markers in women due to different physiological conditions including age, ethnicity, blood group and hormonal changes during different phases of the menstrual cycle.
Women with inherited bleeding disorders suffer from heavy and prolonged menstruation which adversely affects their quality of life. Objectively confirmed menorrhagia is significantly higher in these women (67%) compared with the control group (29%). On the other hand, undiagnosed inherited bleeding disorders can be the underlying cause in a significant proportion (17%) of women presenting with unexplained menorrhagia. The DDAVP nasal spray was shown not to be superior to placebo in the treatment of menorrhagia.

Increased awareness among clinicians responsible for women’s health of these disorders and their morbidity and the availability of management guidelines are essential for optimal care and improvement of the quality of life of these patients.
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<table>
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<td>Activated partial thromboplastin time</td>
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<td>DDAVP</td>
<td>Desmopressin, 1-deamino-8-D-arginine vasopressin</td>
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<tr>
<td>EIA</td>
<td>Electroimmunoassay</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbant assay</td>
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<td>FFP</td>
<td>Fresh frozen plasma</td>
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<td>FVIIa</td>
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<td>IRMA</td>
<td>Immunoradiometric assay</td>
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<td>RiCof</td>
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<td>RFLP</td>
<td>Restriction fragment length polymorphism</td>
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<td>SAS</td>
<td>Statistical Analysis System</td>
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1.1 - GENERAL INTRODUCTION

Hereditary deficiency of each of the 12 coagulation factors have been reported. von Willebrand's disease (vWD), haemophilia A [factor VIII (FVIII) deficiency] and haemophilia B [factor IX (FIX) deficiency] account for 82.6% of all patients registered on the UK Haemophilia Centre Directors' Organisation registry in 1997 and factor XI (FXI) deficiency account for another 4.9%. Other coagulation factor deficiencies account for the remaining 12.5%. At the Royal Free Hospital, the former three disorders account for 62% of all patients registered at the Haemophilia Centre and, as the hospital is located in an area with a very high Jewish population FXI deficient patients comprise almost all (18.8%) of the remaining patients. Therefore the subject of this thesis concentrates on the above mentioned four inherited bleeding disorders.

vWD, which is the commonest inherited bleeding disorder, on clinical grounds appears to be a disease of women. This was reported by Dr Erik von Willebrand in 1926 when he first described the disease. Among 66 members of the bleeder family examined in his study, there were 23 bleeders, 16 of whom were female. In addition, the index case was a female who died of uncontrollable menstrual loss at the age of 13. This disproportionate number of females to males was also seen in the Italian registry of vWD from the first report of 880 patient analysed from a large sample of 25000 cases (Federici & Mannucci 1997). Sramek et al 1995, in a study assessing the usefulness of patient interview in screening for bleeding disorders, reported that among their study population the vast majority (79%) of referred patients with platelet dysfunction and vWD were women. Similarly, 62% of all patients with vWD registered with the Royal Free Hospital Haemophilia Centre are women. As these
disorders are mainly autosomally inherited and an equal sex distribution is expected, it appears that women suffer more from symptoms of bleeding disorders than men.

Because of the mode of inheritance, haemophilia A and B mostly affect males and females are carriers. Most of these carrier females have FVIII and FIX levels within the normal range as they have only one affected X-chromosome. Thus they are unlikely to experience major bleeding problems, although studies have shown them to be at risk of bruising and prolonged bleeding after wounds and following surgery and delivery (Mauser Bunschoten et al 1988). Similarly, women with FXI deficiency (severe or partial) do not generally suffer from spontaneous bleeding, but may do so after haemostatic challenges.

Women are exposed more to haemostatic challenges during their life than men due to monthly menstruation as well as childbirth, which render mild forms of bleeding disorders to be symptomatic. However, the bleeding problems associated with these common events has not been well addressed because women are expected to lose blood during menstruation and after childbirth. There is also difficulty in the objective assessment of blood loss in these situations, especially menstrual blood loss (Hallberg et al 1966b, Harness et al 1977, Framer et al 1984).

There have been several case reports and single institution studies including small numbers of women which have addressed obstetric and gynaecological issues associated with inherited bleeding disorders. The only study with a large number of patients so far published is a multi-centre study (including 16 centres and 44 women) reported by Foster (1995) for the international Society of Thrombosis and
Haemostasis. However, this study only assessed the reproductive health of a small subgroup of women with inherited bleeding disorders (women with vWD unresponsive to DDAVP), the majority of whom had type 2 and 3 disease. Therefore, understandably, the extent of obstetric and gynaecological morbidity among women with inherited bleeding disorders is not known and consequently there is no consensus for their management. In turn, the prevalence of inherited bleeding disorders among patients with menorrhagia or obstetric haemorrhage is also unknown.

1.2 - AIMS AND OBJECTIVES

The Royal Free Hospital has one of the largest haemophilia centres in Europe with 1156 women with various inherited bleeding disorders (including carriers of haemophilia A and B) registered at the centre. The series of studies included in this thesis was designed to address the above unanswered issues and to establish a comprehensive care approach for these women with the intentions of optimising their management and improving their quality of life.

The key topics examined in this thesis include:

1. Assessment of women’s experiences in pregnancy and attitudes towards their reproductive choices including; starting a family, prenatal diagnosis and termination of affected pregnancies and factors that influence these decisions. The aim this study is to provide care givers with a better understanding of women’s
views, thus enable them to provide better care and more effective approaches to counselling.

2. Assessment of obstetric complications and outcomes including; changes in clotting factors in pregnancy, miscarriages and their related bleeding complications, labour and mode of delivery and maternal/neonatal haemorrhagic complications. Management guidelines to minimise these complications and optimise the outcome are also established.

3. Assessment of menstrual blood loss, the prevalence of menorrhagia and other gynaecological complications in women with inherited bleeding disorders and their effect on quality of life in order to increase awareness among the women themselves and their clinicians of these common problems and their management options.

4. Assessment of the frequency of inherited bleeding disorders as an underlying cause of menorrhagia as well as assessment of factors that may predict an increased risk of these disorders.

5. Assessment of DDAVP nasal spray as a treatment option for menorrhagia in women with inherited bleeding disorders.

6. Assessment of changes in coagulation factors in various phases of the menstrual cycle and their clinical implications, as well as the effect of demographic and behavioural factors (such as age, weight, ethnicity, alcohol consumption and
smoking) on these clotting factors. The effect of new low dose oestrogen containing oral contraceptive pills is also assessed.

1.3 - LAYOUT OF THE THESIS

Due to the diversity of the study populations and methods used in each of the studies included in this thesis, there is no specific chapter to describe as a whole, the patients and methods involved in the thesis. However, each chapter contains a detailed description of the patients and methods used in the study presented in that chapter. Similarly, each chapter has a detailed discussion of results and conclusions, although general conclusions relating to the whole thesis are presented in Chapter 10.

This thesis includes 10 chapters; Chapter 1 gives a basic introduction to the thesis and presents the aims, objectives and layout of the thesis. Chapter 2 presents background information and a thorough literature review relating to the topics addressed in the thesis. Chapters 3 and 4 deal with carriers of haemophilia A and B; their attitudes towards reproductive choices, experience in pregnancy and obstetric complications and outcomes. Acquired haemophilia as an unusual cause of post-partum haemorrhage is also included in Chapter 4. Chapter 5 studies the obstetric problems and outcomes in women with von Willebrand's disease or FXI deficiency. In Chapter 6, an assessment of variation in coagulation factors in women is made, concentrating mainly on the effect of different phases of the menstrual cycle. In Chapter 7, the frequency of inherited bleeding disorders in women presenting with menorrhagia is assessed. Chapter 8, presents information on the menstrual blood loss and gynaecological complications in women with inherited bleeding disorders. Their
quality of life during menstruation is also reported. Chapter 9, assesses the efficacy and safety of DDAVP nasal spray in the management of menorrhagia in a randomised placebo controlled trial. Finally in Chapter 10, a brief discussion of general conclusions is presented. Issues in need of further studies in women with inherited bleeding disorders are also raised.

All the studies in this thesis were approved by the Royal Free Hospital Ethics Committee and informed consent was obtained from all women (patients and controls) included in these studies.
Chapter 2

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2.1: VON WILLEBRANDS DISEASE

2.1.1 - HISTORIC PERSPECTIVE

In 1926, Erik von Willebrand (Figure 2.1) described a bleeding disorder in 23 of 66 members of a family from Föglö on the Åland Islands in the Gulf of Bothnia (von Willebrand 1926) which could be distinguished from the classic haemophilia by a prolonged bleeding time and an autosomal inheritance (Von Willebrand 1931). This was called hereditary pseudohaemophilia. Both severe and mild forms of the condition were recognised but, whereas women were affected with either degree of severity, the men who were included only suffered from the milder form of the disease. The predominant symptoms consisted of nose bleeding, bleeding from skin, gums, trivial wounds and the female genital tract. The first patient identified by Erik von Willebrand in 1926 was a girl, Hjördis S., who was five years old when first examined and later died of uncontrollable bleeding from her fourth menstruation at the age of 13 years. Hjördis was one of 12 siblings, all but two of whom had had bleeding symptoms (Figure 2.2). Four of her sisters had died between the ages of two and four of uncontrollable bleeding from the nose, wounds and intestinal tract. Both parents, Oskar and Augusta, had been troubled by severe nose bleeding in their youth; and Augusta, as well as several of her sisters, had had profuse menstrual bleedings.

von Willebrand and Jürgens (1933) using a device called the “capillary thrombometer”, provided early evidence that the basic defect in the disease was related to platelet function but they could not determine whether the abnormality was
intrinsic or extrinsic to the platelet. A vascular defect as the cause of the bleeding was also described by von Willebrand et al 1934 and was later emphasised by other investigators (Macfarlane 1941, Schulman et al 1955, Schulman et al 1956). The name of “vascular haemophilia” was thus given to the syndrome.

Figure 2.1: Dr Eric von Willebrand
Subsequently, several investigators reported the association between a prolonged bleeding time and a deficiency of factor VIII procoagulant activity (FVIII:C) (Alexander & Goldstein 1953, Larrieu & Soulier 1953, Quick & Hussey 1953). In 1956 and 1957a, Nilsson et al also described 10 families in Sweden with a severe bleeding disorder characterised by factor VIII (FVIII) deficiency and a prolonged bleeding time. Nilsson et al 1957b found that administration of plasma fraction 1-0 (containing FVIII) to these patients produced a rise in FVIII level and a temporary shortening of the bleeding time. These findings suggested that the major abnormality responsible for the failure of haemostasis resided in the plasma of these patients. In 1957, von Willebrand's original patients, or their descendants, were reinvestigated independently by Nilsson et al and Jüergens et al. Both groups confirmed an FVIII deficiency in the affected subjects. Jüergens et al claimed to show an abnormality of
platelet thromboplastic function which Nilsson et al failed to demonstrate, having tested the same patients. Nilsson et al also reported normalisation of FVIII concentration and the bleeding time in one of the Åland patients by plasma fraction 1-0. This showed that the inherited bleeding disorder described by several investigators, characterised by prolonged bleeding time and reduced FVIII activity, was identical to that described by von Willebrand.

Nilsson et al 1959 demonstrated a delayed rise in FVIII activity after infusing blood or plasma fraction 1-0 from a patient with classical haemophilia into von Willebrand's patients. Therefore these authors concluded that the impaired haemostasis in von Willebrand's disease (vWD) was due to deficiency of a plasma factor present in both normal and haemophilia A plasma and called it von Willebrand factor (vWF). Administration of this plasma factor not only corrected the prolonged bleeding time but also apparently stimulated the synthesis and release of FVIII. These findings have since been widely confirmed.

From the observation that the platelet counts of blood issuing from a skin wound remains high, Borchgrevink 1960 concluded that platelets of patients with von Willebrand's disease do not stick to damaged tissue in the normal way. Decreased platelet adhesiveness to glass in von Willebrand's disease was also demonstrated (Salzman 1963). Abnormal adhesiveness of platelets was later confirmed by other investigators (Zucker 1963, Odegaard et al 1964). Administration of normal plasma or haemophilia A plasma corrected the decreased platelet adhesiveness parallel with the shortening of the bleeding time (Salzman 1963, Larrieu et al 1968). Howard & Firkin 1971 produced a more quantitative test of platelet adhesiveness with the
antibiotic, ristocetin. Ristocetin causes platelet aggregation in normal blood, but not in the majority of patients with vWD. This abnormality could be corrected both in vitro and in vivo by normal plasma or haemophilia A plasma, indicating that vWF was responsible for ristocetin cofactor activity. In the 1980s, the presence of receptors for vWF on platelets was demonstrated (Hawiger et al 1981, Fujimoto et al 1982, Ruggeri et al 1983, Nokes et al 1984).

2.1.2 - BIOSYNTHESIS, STRUCTURE AND FUNCTION OF VON WILLEBRAND FACTOR

The von Willebrand factor (vWF) gene, is located at the tip of the short arm of chromosome 12, region 12p12-12pter (Ginsburg et al 1985). It consists of 180 kilobases and contains 52 exons (Mancuso et al 1989). A nonprocessed vWF pseudogene has been identified on chromosome 22 (Mancuso et al 1991). vWF is synthesised as a precursor molecule referred to as pro-pre-vWF, by only two cell types: endothelial cells and megakaryocyte (Jaffe et al 1973, Nachman et al 1977, Sporn et al 1985). This tissue-specific expression explains the limited distribution of vWF in the body. Beside plasma, vWF is present in Weibel-Palade bodies of endothelial cells, subendothelium and granules of platelets. The pre-pro-vWF is a 2813-residue precursor polypeptide (Bonthron et al 1986); it consists of a 22-residue signal peptide, a large 741-residue propeptide (also known as vWF antigen II) and the mature subunit of 2050 residues. The pre-pro-vWF is glycosated and dimerised through the creation of disulphide bonds between the carboxyterminal parts of 2 molecules of pro-vWF (Wagner et al 1987, Voorberg et al 1991). The dimers are transported to Golgi and post-Golgi compartments, where they are aggregated to multimers of varying size, first forming non-covalent associations, and then disulphide bonds between aminoterminal parts. The size of the aggregates may vary from dimers to multimers consisting of 50 or more subunits. Multimerisation is associated with cleavage of pro-vWF into the “mature” vWF subunits and the propeptides of 741 amino acids (vWF antigen II) (Fay et al 1986). These propeptides may have a biological function, since they have been shown to inhibit collagen-induced platelet aggregation (Takagi et al 1989).
vWF is secreted constitutively from endothelial cell Weibel-Palade bodies in both luminal and abluminal directions and in a regulated manner in response to stimuli such as thrombin, fibrin, calcium ionophore A23187 (Hattori et al 1989) and hormones such as epinephrine and vasopressin (Mannucci et al 1975); this vWF consists of high molecular weight multimers, which are believed to be haemostatically the most potent. vWF is also released from platelet granules during platelet release reaction. Platelets contain even larger multimers than those seen in the plasma. The presence of such large multimers at a high concentration at the site of vascular injury may be of haemostatic importance.

vWF exists as a series of multimers varying in molecular weight between 0.5 (dimer) and 20 million daltons (multimer) (Ruggeri 1993, Grima et al 1987). The mature vWF subunit contains 2050 amino acid residues and up to 22 carbohydrate side chains, 10 of which are O-linked to serine or threonine residues and 12 N-linked to asparagine residues (Tatani et al 1986, Matsui et al 1992). A characteristic feature of vWF is the high cysteine content, 169 out of the total 2050 residues. These cysteines are essential for the linkage of subunits into higher order structures and conformation of functional domains (Azuma et al 1993). The propeptide (vWF antigen II) and mature subunit of vWF are almost entirely composed of four types of repeating domains, designated A through D (Shelton-Inloes et al 1986), arranged from amino- to carboxyl-terminal in the following order: D1- D2- D3- A1- A2- A3- D4- B1-B2- B3- C1- C2.

vWF plays an important role in haemostasis and has two main properties. First, vWF stabilises FVIII in vitro and in vivo, protecting it from inactivation by activated
protein C or factor Xa; second, it mediates platelet adhesion and platelet aggregation and thrombus formation by bridging platelets to the vessel wall and platelets themselves under high shear stress conditions (Figure 2.3). The sequence of events appears to be the following: binding of vWF to components of the subendothelium, conformational changes of vWF, binding of vWF to platelet glycoprotein (GP) Iß/IX/V complex leading to initial platelet adhesion, transduction of intra-platelet signal leading to the expression of a functional GPIIß/IIIß complex onto the platelet membrane, binding of vWF to GPIIß/IIIß inducing irreversible platelet adhesion, platelet spreading and platelet aggregation (Mazurier & Meyer 1996).

**Figure 2.3 - Function of circulating von Willebrand factor**
The functions of vWF are dependent on the presence of a series of binding sites for specific receptors. A binding site for FVIII is localised at the N-terminal part between amino acids 1 and 272. The presence of two platelet-binding domains has been demonstrated, one interacting with GPIb and the other with GPIIb/IIIa (Ruggeri et al 1983). The GPIb binding site has been localised to a region between amino acids 449 and 728 (Azuma et al 1991). Binding of vWF to GPIb can occur with non-activated platelets and is necessary for the initial contact between platelet and thrombogenic surface, i.e. platelet adhesion. The second platelet binding domain of the vWF is located in the C1 domain at amino acids 1744-1746. This binds to GPII/IIIa platelet receptor on platelets which have already been activated by agonists or shear forces, thereby mediating platelet spreading or aggregation (Ikeda et al 1991). Binding sites for heparin, collagen and sulphatides have also been identified within the A1 domain and a second collagen-binding sites are located within A3 domain.

2.1.3 - PATHOGENESIS AND CLASSIFICATION OF VON WILLEBRAND'S DISEASE

von Willebrand’s disease exhibits significant phenotypic heterogeneity, depending on the particular subtype considered. Two main categories of patients can be identified, distinguished on the basis of whether the main pathogenetic factor is a quantitative (type I and type III) or qualitative (type II) defect of vWF.

**Type 1**

Type I is the most common form of the disease, accounting for approximately 70-80% of all cases. It is characterised by a decrease in synthesis of vWF protein,
however, the structure and function of vWF that is synthesised is normal. Multimer analysis shows that all multimers are present in plasma and are in the same relative proportion as in normal plasma. For most patients with type 1 vWD, the concentration of each multimer is reduced compared with normal plasma, but the reduction is variable among patients and, on different occasions in any given patient, and may be normal. The level of vWF in the plasma is between 10 and 50% of normal and is always accompanied by a parallel decrease in factor VIII procoagulant activity (FVIII:C). The bleeding time and activated partial thromboplastin time (APTT) may either be prolonged or normal.

The mode of inheritance of type 1 vWD is autosomal dominant. It is generally accepted that type I vWD is due to defects in the vWF gene, however, there is very little known about the molecular pathogenesis of this common type of vWD. In view of the phenotypic heterogeneity of these patients, it is likely that more than one pathogenetic mechanism will be identified as the basis for this form of vWD (Ruggeri 1994).

Type 2

Type 2, also named variants of vWD, results from a qualitative abnormality of vWF resulting in most cases in abnormal multimeric structure of the molecule and hence defective platelet-vWF-vessel wall interaction. The plasma concentration of vWF as well as FVIII:C may be only modestly reduced or even normal. This type of vWD accounts for 15-20% of cases. It is phenotypically very heterogeneous and comprises many different subtypes, recently reclassified to four main subtypes (2A, 2B, 2N, 2M) (Sadler 1994), of which the two most common subtypes are type 2A and type
2B. The bleeding disorder is mild to moderate bleeding in the majority of cases (type 2A and 2B) but it can be of variable severity in types 2N and 2M. Type 2 is transmitted as an autosomal dominant, however, recessive forms have also been described (e.g. subtype 2N). Single point missense mutations of the vWF gene have been identified in a number of patients, mainly affected by subtypes 2A and 2B (Ruggeri & Zimmerman 1980, Ruggeri et al 1980).

Type 2A is the commonest vWD variant and accounts for approximately 5-10% of all patients. This variant is characterised by the absence of large vWF multimers in plasma with evidence of increased proteolytic degradation of the molecule (Zimmermann et al 1986); consequently decreased platelet-dependent function of vWF. This means a marked reduction in the ability of vWF to support platelet adhesion and aggregation at sites of vascular injury. Type 2A vWD may arise by two mechanisms (Ginsburg et al 1989, Lyons et al 1992). Group 1 mutations are thought to cause defective intracellular transport of vWF. Large multimeric forms are retained preferentially in the endoplasmic reticulum, thereby inhibiting their secretion. Group 2 mutations appear to cause enhanced susceptibility of vWF to proteolysis in plasma. This results in the degradation of the large multimers. Several missense mutations have been reported by different authors in patients with type 2A vWD, almost all clustered within the A2 domain of vWF (Ginsburg & Sadler 1993).

Type 2B vWD is relatively less common than type 2A, but not rare. It results from a qualitative abnormality of vWF that results in increased binding of vWF to platelet glycoprotein 1b (GP1b), often leading to thrombocytopenia. Different missense mutations of vWF gene leading to single amino acid substitutions have been
identified in several patients (Ginsburg & Sadler 1993), mostly in the A1 domain which is the GP1b-binding domain. Of the reported mutations four account for approximately 90% of type 2B vWD cases investigated (Ginsburg & Sadler 1993).

Type 2M is rare variant which is characterised by decreased platelet-dependent function of vWF. This is not associated with the absence of high molecular weight vWF multimers (unlike type 2A vWD) in the plasma, although multimer binding patterns may be atypical (Sadler 1994). Mutations in the vWF A1 domain (Rabinowitz et al 1992) resulting in decreased affinity of vWF for platelet GP1b have been described as a causative mechanism for this type of vWD.

Type 2N, also known as vWD Normandy, is a recessively inherited disorder and is caused by various mutations in the N-terminal FVIII binding region of the vWF gene. This results in defective binding of FVIII to vWF (Mazurier 1992). Type 2N vWD is difficult to differentiate from mild to moderate haemophilia A as plasma FVIII levels are reduced with normal vWF levels and vWF platelet dependent function. Therefore, type 2N vWD should be considered as a diagnosis in patients with congenital FVIII deficiency that dose not clearly follow an X-linked recessive pattern.

**Type 3**

Type 3 vWD is transmitted as an autosomal recessive bleeding disorder with severe to very severe manifestations. It is a rare disorder with a prevalence ranging from 0.5 to 5 per million population depending on the country. Type 3 vWD is characterised by a marked reduction of plasma and platelet levels of vWF. vWF:Ag and vWF:Ac
are usually undetectable even with very sensitive assays and FVIII levels are also markedly reduced to typically less than 10 iu/dl. Multimeric analysis of plasma shows essentially no multimers because of the marked reduction in vWF.

Various abnormalities of the vWF gene have been detected in several families with type 3 vWD. These abnormalities vary from deletions of various size (from as small as 2.3 kb to deletion of the entire gene, 178 kb) to single nonsense mutations (Ginsburg & Sadler 1993). In a recent extensive analysis of the vWF gene in 53 unrelated families with type 3 vWD from Sweden, Finland and Germany, a vWF gene defect was characterised in 64% of them (Zhang et al 1994, Schneppenheim et al 1994). The majority of these type 3 patients are either homozygous or compound heterozygous for the various reported gene defects. In addition, evidence of mutations affecting vWF mRNA transcription, processing and stability have been demonstrated in some of the cases where a gene defect has not been identified (Nichols et al 1991, Eikenboom et al 1992).

2.1.4 - INHERITANCE OF VWD AND RECONCEPTIONAL COUNSELLING

Inheritance of different types of vWD is discussed above (see pathogenesis and classification). Preconceptional counselling should be provided to women with vWD. The aim is to help these women to understand the genetic implications of their disorder and all aspects of management of future pregnancy. The risk of an affected individual (woman or man) with type 1 vWD of transmitting the disease to her or his child is 50% for any pregnancy, as this type is autosomal dominant. However, because of the variable penetrance and expression of the abnormal gene the risk of
having a clinically affected child is actually less than 50% and has been estimated to about 33% (Miller 1982). The same is true for type 2A and most cases of type 2B. However, the situation is more complicated for the other subtypes of type 2 vWD and extensive family studies are required to assess this risk. Type 3 vWD is an autosomal recessive disorder and affected individuals are either homozygotes or compound heterozygotes. If a child with type 3 has already been born in the family, the risk of a subsequent child being affected is 25%.

Despite the major progress in understanding the molecular basis of vWD and identification of the precise molecular defects in a significant number of cases, genetic diagnosis of vWD remains difficult and does not represent a first-line investigation for diagnosis. In view of this and the complexity of clinical and phenotypic diagnosis in type 1 and 2 vWD, prenatal diagnosis would be quite difficult in these cases. In addition, due to the mild to moderate nature of these type of vWD, there is little demand for prenatal diagnosis. Type 3 vWD is, however, a serious condition. The option of prenatal diagnosis, although seldom necessary, should be considered in affected families and has been successfully performed by fetal blood sampling to evaluate fetal clotting factors (Hoyer et al 1979, Ash et al 1988), and chorionic villus sampling using genetic analysis (Peake et al 1990). Peake et al (1990), reported prenatal diagnosis of type 3 vWD using polymerase chain reaction to detect intron 40 variable-number tandem-repeat (VNTR) polymorphic region of vWF gene. Linkage analysis showed that the fetus had inherited affected vWF gene alleles from both parents. In the few reported cases of prenatal diagnosis of type 3 vWD, the indication for the procedure had been a severely affected child in the family. In these cases accurate carrier assessment for both parents by genotypic
and/or phenotypic analysis is important. Polymorphism based linkage analysis has invariably been used in the diagnosis. In the future, it is likely that analysis of known mutations within the family will be performed without the need for complete family data.

2.1.5 - CLINICAL MANIFESTATIONS

The bleeding tendency in vWD is very variable depending on the type, subtype and severity of disease. Patients with type 1 usually have mild bleeding symptoms. The symptoms often vary markedly from one family member to another, and some affected members detected during kindred studies are symptomless. Patients with type 2 variants may have severe bleedings, in some cases comparable to those in patients with type 3 disease. Patients with type 3 vWD are severely affected and can have spontaneous bleeding from mucous membranes and the gastrointestinal tract that can be life-threatening. Both in severe and mild forms of vWD, the bleeding symptoms are most troublesome during childhood and adolescence, but as plasma levels of vWF increase with increasing age, a remarkable improvement in the bleeding tendency occurs.

The clinical manifestation of vWD is characterised by haemorrhage from delicate mucocutaneous tissues. Mucosal bleeding, particularly epistaxis and gingival bleeding are common as well as cutaneous bleeding and easy bruising. Gastrointestinal bleeding is not infrequent and can be without identifiable cause. In women, the most frequent symptom is menorrhagia, which may be severe and out of proportion to other bleeding symptoms or the only symptom. Menorrhagia is often
A primary coagulation disorder was found in almost 20% of 59 adolescents with such menorrhagia (Claessens & Cowell 1981a) and screening for vWD and platelet disorders has been recommended in these patients (Claessens & Cowell 1981b, Ward 1992). In a small study by Edlund et al 1996, menorrhagia was shown to be a valuable predictor for vWD and can be a guideline in looking for mild forms of this disorders. Six of 30 (20%) women with objectively verified menorrhagia included in the study were found to have mild vWD and in 2 of them menorrhagia was the only bleeding symptom. Another type of bleeding from the female genital tract is bleeding corpus luteum following ovulation. The bleeding is usually mild causing moderate to severe mid cycle abdominal pain which resolves spontaneously. However, the bleeding may occasionally be severe and continuous leading to a drop in haemoglobin or even a hypovolemic shock that necessitates urgent treatment and operative intervention. Assessment of menstrual blood loss and other gynaecological problems in these women has been conducted in this thesis (Chapter 8) as well as assessment of the frequency of inherited bleeding disorders among patients with menorrhagia (Chapter 7).

Post-traumatic, postsurgical and excessive bleeding following dental extraction are also frequent and can be the presenting manifestation of vWD. Pregnancy in women with vWD is usually not associated with excessive bleeding complications as plasma vWF and FVIII levels increase during pregnancy reaching a peak during the third trimester. However, the levels fall rapidly after child birth leading to an increased risk of primary and secondary post-partum haemorrhage. Unlike haemophilia, deep subcutaneous and intramuscular haematomas are uncommon. Haemoarthrosis,
retroperitonal, intracranial and other haemophilia like lesions may occur in the most severely affected type 3 patients without detectable vWF and with very low levels of FVIII.

Acquired inhibitors to vWF in patients with vWD are uncommon and occur only in patients with severe type 3 disease (Lopez Fernandez et al 1988). The prevalence of vWF inhibitor in type 3 vWD patients has been estimated to be 7-8%. The inhibitors are alloantibodies, usually immunoglobulin G (IgG), which inhibit the haemostatic function of vWF. There is almost always a history of prior exposure to exogenous vWF e.g. plasma, cyoprecipitate or vWF concentrate. Patients at higher risk for development of inhibitors are those with vWF gene deletion (Shelton-Inloes et al 1987, Ngo et al 1988) and those with a family history of inhibitor formation (Ruggeri et al 1979). The presence of an inhibitor to vWF in a patient with vWD is expected by the failure to respond clinically to replacement therapy, decreased recovery of infused vWF and the lack of correction of the bleeding time. Confirmation of the presence of an antibody requires demonstration of an inhibitor of ristocetin-induced platelet aggregation in the patient’s plasma. In most case antibodies to vWF also appear to inhibit FVIII activity (Fricle et al 1985). Some antibodies do not inhibit vWF function but accelerate the clearance of transfused vWF. These antibodies can be demonstrated by mixing the patient’s antibody with normal vWF and demonstrating antigen-antibody binding (Lopez Fernandez et al 1988).
2.1.6 - PREVALENCE AND DIAGNOSIS

Von Willebrand's disease seems to be one of the most common hereditary haemorrhagic disorders. The exact frequency of vWD in the general population is difficult to determine because many cases of vWD especially type 1 go undetected until the coagulation system is challenged by an event, such as surgery or major trauma. Several other factors contribute to the difficulty in determining vWD prevalence including the variable penetrance and expressivity of vWD mutations (Bloom 1991), the variability in the level of plasma vWF found in the general population and the temporal variability associated with some of the clinical tests used to diagnose vWD (Bloom 1991, Triplett 1991). Type 1 is the most common form, accounting for approximately 70% of all cases. Rodeghiero et al 1987 reported a prevalence of 0.8% in an ethnically homogenous community in northern Italy and a prevalence of 1.3% was reported by Werner et al 1993 in an ethnically heterogeneous population in the United States. Type 3 vWD, however, is rare with a prevalence of 0.1-5.3 per million (Weiss et al 1982, Berliner et al 1986). Type 2 accounts for about 20% of all vWD (Ruggeri 1991) with type 2A being the most common subtype (15% of all vWD) and type 2B accounting for 5% of vWD cases (Ginsburg & Bowie 1992). Other variants of vWD are rare with few case reports, although the prevalence of the subtype 2N (vWD Normandy) may be substantial.

Diagnosis of vWD and identification of the type and subtype requires an extensive work-up comprising a number of steps:

1. A detailed clinical history concerning the pattern of bleeding tendency and detailed family history. Severe forms of vWD are often easily suspected and diagnosed on the basis of clinical symptoms and pattern of inheritance. In contrast,
the milder forms can go undiagnosed as they can be asymptomatic until subjected to a haemostatic challenge such as major trauma or invasive surgical procedures. It is important to remember that a negative family history does not exclude the diagnosis of vWD (Biggs & Matthews 1963, Miller et al 1979, Rodeghiero et al 1987) as it has been quoted that one-third of the cases are sporadic and have no family history (Strauss 1967). Family investigations should be performed whenever possible.

2. Bleeding time determination: The bleeding time is always markedly prolonged in severe cases, however, it is often normal in patients with mild disease. Therefore, although a prolonged bleeding time is one of the diagnostic criteria, its performance is valuable but not essential for diagnosis of vWD. When performed, this technique should be performed by an experienced operator using a template device and a standardised method. The modified Ivy bleeding time is commonly used. A sphygmomanometer around the arm is inflated to 40 mmHg to standardise the venous pressure. One or two standard incisions are produced on the volar aspect of the arm. The time required for bleeding to cease is determined by carefully blotting the blood emerging from the wounds with a filter paper at 30-second intervals. The test is influenced by a number of factors, including the depth, location and direction of the incision as well as skin thickness. The Duke bleeding time is performed by puncture of the earlobe. This method is less sensitive than Ivy test and in some patients with vWD, the Duke bleeding time may be normal when the Ivy bleeding time is prolonged.

3. The activated partial thromboplastin time (APTT) is usually prolonged in patients with vWD, yet this test may be normal in most of the patients with mild disease and is therefore not sensitive enough to be used for screening and diagnosis of this
disorder. The APTT reflects deficiencies of factors VIII, IX, XI and XII but when
the concentration of these factors are only 30% of normal, the APTT will be
normal in most laboratories (Lusher 1996).

4. Factor VIII coagulation assay (FVIII:C): This can be performed by a standardised
one-stage or two-stage technique or by chromogenic assay. In patients with type 1
disease, FVIII:C levels are variable and may be normal, but if patients are studied
serially, usually at least one abnormal value is observed. In type 2 vWD, FVIII:C
is often normal even if severely affected except in type 2N. In severe type 3
patients, FVIII:C levels are very low, usually ranging from 1-4 iu/dl.

5. vWF antigen (vWF:Ag): This can be measured in plasma by electroimmunoassay
(EIA), immunoradiometric assay (IRMA) or a variety of enzyme-linked
immunosorbent assays (ELISA). In type 1 vWD the plasma vWF:Ag
concentration is decreased, both when measured by ELISA or IRMA (Ruggeri et
al 1976, Lamme et al 1985, Ingerslev 1987). In severe type III, vWF:Ag is absent
or is only present in trace amounts as assessed by IRMA. In type 2 variants,
decreased or normal concentration of vWF:Ag is obtained (Peake et al 1977,

6. vWF activity (vWF:Ac) assay: vWF:Ac levels are reduced in all types of vWD
and the assay is documented to be the single most sensitive assay for screening for
most forms of vWD (Werner et al 1992, Rodeghiero et al 1990). vWF:Ac reflects
the functional ability of the vWF to bind to platelets. The gold-standard method to
measure vWF functional activity is by ristocetin co-factor agglutination assay
(vWF:RiCof assay). The RiCof assay is usually performed by an aggregrometer
using fresh washed or formalin fixed platelets. Recently, a monoclonal antibody
based ELISA assay has been described as a replacement to RiCof assay (Murdock
et al 1997). This is based on the ability of the monoclonal antibody RFF-VIII:R/I to recognise and bind to the GP1b binding region of vWF. A commercial version of this ELISA assay is also available (Shield Diagnostics, now Stago Diagnostica, Dundee, UK).

7. Ristocetin-induced platelet aggregation (RIPA): The antibiotic ristocetin mediates platelet-vWF interaction, resulting in platelet aggregation. The ristocetin-induced platelet aggregation assay uses metabolically active platelets, in contrast to vWF:Ac assay. It is recommended that varying concentrations of ristocetin are used in the assay: 0.5, 0.75, 1.0, 1.25 and 1.5 mg/ml. Platelet-rich plasma should be adjusted to give similar platelet counts for patients and control samples. Ristocetin-aggregation of platelets in platelet-rich plasma is absent in type 3 and reduced in type 1 and 2A. This abnormality can be corrected by addition of a vWF-rich concentrate, such as Hemate P, which distinguishes the defect from that seen in Bernard Soulier platelet syndrome. In type 2B vWD, the platelets aggregate at a lower concentration (0.5 mg/ml and 0.75 mg/ml) of ristocetin than those necessary in normal platelet-rich plasma. This indicates an increased affinity of vWF for platelets.

Interpretation of results:

In the absence of genetic analysis, diagnosis of vWD is made on a clinical basis and from laboratory results of the above mentioned laboratory tests. However, the clinical expression of the disease is variable and the laboratory data may overlap with the normal range and fluctuate with time in a given individual (Abildgaard et al 1980) making the diagnosis, especially of mild forms, difficult and complex (Zhang et al
1995). In patients with mild vWD, FVIII:C, vWF:Ag, vWF:Ac levels are variable and may be normal. Because of this variability of laboratory findings in vWD, the importance of repeated testing to establish the diagnosis of mild vWD has been well documented (Nilsson 1977, Abildgaard et al 1980). In the Haemophilia Centre laboratory at the Royal Free Hospital, all abnormal results are confirmed and a diagnosis of vWD only made after the finding of consistent and significant abnormalities of vWF. Repeated testing is performed in patients with equivocal results or minor abnormalities only of the above tests and where there is a strong personal and family history suggestive of an inherited bleeding disorder in the presence of apparently normal laboratory results. Factor assays are repeated on three occasions and the patient is diagnosed to have vWD if vWF:Ac was < 50iu/dl in two of the three occasions. The blood is analysed within two hours of collection. Normal ranges used are: APTT = 28-38 seconds, vWF:Ac = 50-150 iu/dl, vWF:Ag 50-150 iu/dl and FVIII:C = 50-150 iu/.
Table 2.1: Pattern of results in common types of vWD

<table>
<thead>
<tr>
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<th>Type 1</th>
<th>Type 2A</th>
<th>Type 2B</th>
<th>Type 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII:C</td>
<td>Low*</td>
<td>Low/normal</td>
<td>Low/normal</td>
<td>Low</td>
</tr>
<tr>
<td>vWF:Ag</td>
<td>Low</td>
<td>Low/normal</td>
<td>Low/normal</td>
<td>Very low</td>
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<tr>
<td>vWF:Ac</td>
<td>Low</td>
<td>Very low**</td>
<td>Low/normal</td>
<td>Very low</td>
</tr>
<tr>
<td>RIPA</td>
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<td>Absent</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Bleeding Time</td>
<td>Prolonged</td>
<td>Prolonged</td>
<td>Prolonged</td>
<td>Prolonged/normal</td>
</tr>
</tbody>
</table>

* Usually < 50 iu/dl, ** usually < 5 iu/dl

Further specialised investigations for subtyping and genetic counselling:

1. vWF multimer analysis of platelets and plasma: Determination of vWF multimer composition in plasma and platelets is important in the diagnosis and classification of subtypes of vWD. The multimeric distribution of both plasma or platelet vWF can be determined by gel electrophoresis methods using labelled anti-vWF antibody for detection. Normal platelets contain vWF multimers larger than those seen in plasma. Decreased plasma vWF:Ag concentration with a normal multimeric distribution is consistent with a quantitative deficiency of vWF (type 1). Structural abnormalities, such as loss of high molecular weight and/or
intermediate molecular weight multimers, are consistent with qualitative defects of vWF (type 2). Due to the lack of vWF:Ag no multimers are seen in type 3 vWD. This forms the basis of the primary classification of vWD. Secondary classification is now defined upon absence of high molecular weight vWF multimers, definition of platelet dependent function of vWF, definition of increased GP Ib affinity and definition of FVIII binding. The distinction between type 1 and most forms of type 2 has been shown by a multicentre study to be made by multimer analysis of plasma vWF on low resolution agarose gels, where each vWF multimer may appear as a single band (Mannucci et al 1985). This type of gel would clearly distinguish between the presence and absence of high molecular weight multimers. The subdivision of type 2 variants requires the use of higher resolution gels to resolve the structure of the smaller multimers and minor associated intervening “satellite bands” into at least three bands. At this level of resolution the subtype can be defined. If the precise subtype remains unclear, very high resolution gels should be considered.

2. Analysis of platelet vWF levels: Under the old classification, both quantitative and multimeric analysis of platelets was of diagnostic importance in completing subclassification for type 1 and 2 disease. All platelet vWF:Ag analysis should be performed on washed platelets only to avoid contamination with plasma vWF:Ag. Type 1 vWD was subdivided with regard to platelet vWF content, which may be either normal or low. This distinction has some clinical relevance as patients with low platelet vWF content may respond relatively poorly to DDAVP. Platelet vWF:Ag levels correlate more closely with bleeding time than dose plasma vWF:Ag (Gralnick et al 1986a). Under the new classification type 1 platelet normal or type 1 platelet low are both termed type 1 vWD. Similarly type 2 was
divided into normal and abnormal platelet vWF patterns. Under the new classification all variants associated with the absence of high molecular weight multimers are type 2A vWD. This category covers all non-specific defects of multimer size and presumed different molecular mechanisms are no longer attributed to formal categories. Definition of platelet vWF:Ag multimers remains important for research understanding of vWD variants. Platelet vWF is normal in type 2B disease.

3. Molecular diagnosis of vWD: Precise molecular diagnosis may be performed by screening for common genetic mutation in those vWD variants where clusters of mutations have been identified (type 2A, 2B and 2N) (Inbal et al 1993a). However, the vWD-associated mutation is not known in most cases and gene tracking by analysis of restriction fragment length polymorphisms (RFLPs) or variable number of tandem repeat (VNTR) sequence in the vWF gene is a useful technique for family studies. The cloning of vWF cDNA has led to identification of many RFLPs that have improved genetic studies in vWD (Sadler & Ginsburg 1993). However, the informativity of vWF gene RFLPs is limited by their biallelic nature. The interpretation of RFLP data in autosomally inherited vWD is more complex than in the X-linked hereditary disorders and in order to determine the RFLP halotype associated with vWD it may be necessary to carry out multiple restriction fragment analyses. In addition, the presence of a vWF pseudogene on chromosome 22, to which some vWF cDNA probes may hybridise, complicates the interpretation of Southern blot restriction patterns. Intron 40 of the vWF gene contains several VNTR sequences within a region of repetitive DNA. PCRs for these VNTRs are very informative and useful in gene tracking studies in vWD (Peake et al 1990). Gene tracking techniques have applications in the prenatal
diagnosis of severe vWD, in carrier detection in recessive forms of vWD, and in type 1 vWD families where diagnosis in some individuals may be phenotypically uncertain. The potential for variations in the vWD phenotype, which result from variable penetrance of VWF gene mutations, should always be taken into account when interpreting the results of genetic studies and when carrying out genetic counselling. In some families the variable clinical presentation of vWD may be a consequence of unsuspected compound heterozygosity (Eikenboom et al 1993).

2.1.7 - TREATMENT OF VON WILLEBRAND’ S DISEASE

The aim of treatment in vWD is to correct the dual defects of haemostasis, i.e., the prolonged bleeding time due to abnormal platelet adhesion and abnormal coagulation due to low FVIII levels. There are two main treatment options available: desmopressin (DDAVP) and transfusion therapy with blood products. DDAVP is the treatment of choice in patients with type 1 disease. It raises the endogenous FVIII and vWF correcting the intrinsic coagulation defect and the prolonged bleeding time in most type 1 patients. Type 3 and the majority of type 2 patients do not respond to DDAVP and it is necessary to resort to plasma concentrates containing FVIII and vWF. These concentrates are effective and currently safe, but do not always correct the prolonged bleeding time. Platelet concentrates or DDAVP can be used as adjuvant treatments when there is continued bleeding and poor correction of bleeding time with these concentrates.
Table 2.2: Management of different types and subtypes of vWD *

<table>
<thead>
<tr>
<th>Type</th>
<th>Treatment of choice</th>
<th>Alternative and adjunctive therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1</td>
<td>DDAVP</td>
<td>Antifibrinolytics</td>
</tr>
<tr>
<td>Type 2A</td>
<td>FVIII-vWF concentrates</td>
<td></td>
</tr>
<tr>
<td>Type 2B</td>
<td>FVIII-vWF concentrates</td>
<td></td>
</tr>
<tr>
<td>Type 2M</td>
<td>FVIII-vWF concentrates</td>
<td></td>
</tr>
<tr>
<td>Type 2N</td>
<td>DDAVP</td>
<td></td>
</tr>
<tr>
<td>Type 3</td>
<td>FVIII-vWF concentrates</td>
<td>DDAVP, Platelet concentrates</td>
</tr>
<tr>
<td>Type 3 with alloantibodies</td>
<td>Recombinant FVIII</td>
<td></td>
</tr>
</tbody>
</table>


Desmopressin (DDAVP)

DDAVP, a vasopressin analogue, promotes the release of vWF from the endothelial cells and can increase the plasma levels of vWF and FVIII in patients with mild haemophilia or vWD. DDAVP is most effective in patients with type 1 vWD, especially those with normal vWF in storage sites (Type 1, ‘platelet normal’). In these patients FVIII, vWF and bleeding time are usually corrected within 30 minutes and remain normal for 4-6 hours. In Type 1, ‘platelet low’ patients a poor and short-lasting response is observed (Mannucci 1997b). In type 2 disease, the role of DDAVP
is less clear. In type 2A, FVIII levels are usually increased but the bleeding time is shortened in only a minority of cases. In type 2B, DDAVP is usually avoided since it may cause platelet aggregation and thrombocytopenia. However, some authors (Fowler et al 1989) suggest that a trial of DDAVP is reasonable in these patients and may produce a clinical benefit, even though it results in an increase of abnormal vWF. In type 2N, high levels of FVIII are observed following DDAVP (Mazurier et al 1994). Patients with type 3 vWD are usually unresponsive to DDAVP, although a subgroup of these patients has been identified in whom FVIII become normal with DDAVP treatment, even though the bleeding time remains markedly prolonged (Castaman et al 1995). In responsive patients, DDAVP is the treatment of choice for spontaneous bleeding, trauma and minor surgery. DDAVP is discussed in more detail in Chapter 2.5.

OTHER NON-TRANSFUSIONAL THERAPIES

In addition to DDAVP, there are other non-transfusional therapies used in the management of vWD: antifibrinolytics and oestrogens. Antifibrinolytics interfere with the lysis of newly formed clots by saturating sites on plasminogen, preventing its attachment to fibrin and making plasminogen unavailable within the forming clot. Epsilon aminocaproic acid (50 mg/kg four times a day) and tranexamic acid (25 mg/kg three times a day) are the most commonly used antifibrinolytics. They can be administered orally, intravenously or topically. They are used alone or as adjuncts in the management of epistaxis, oral cavity bleeding, gastrointestinal bleeding and menorrhagia. Tranexamic acid is known to significantly reduce both plasminogen activator activity and plasmin activity in menstrual fluid of women with menorrhagia (Dockery et al 1987) and reduce bleeding in menorrhagia associated with inherited
bleeding disorders (Bonnar et al 1980). Antifibrinolytics are contraindicated in the management of upper urinary tract bleeding because of the risk of uretric colic and obstruction due to thrombus formation. They are also contraindicated in patients with a history of thrombo-embolic disease and patients with an underlying prothrombotic state. Nausea, vomiting and, occasionally, diarrhoea have been reported as adverse effects of treatment with antifibrinolytics. Rapid intravenous injection may cause dizziness and/or hypotension.

Oestrogens increase FVIII/vWF-ristocetin cofactor activity and partially correct the prolonged bleeding time in patients with vWD (Alperin 1982), but as the response is so variable and unpredictable, they not used for therapeutic purposes. However, combined oral contraceptives are effective and are currently the most commonly used treatment of menorrhagia, even in patients with type 3 disease, despite that fact that FVIII-vWF levels are not modified, by decreasing the endometrial thickness.

TRANSFUSIONAL THERAPIES

Transfusional therapy with blood products containing FVIII-vWF is the treatment of choice in patients who are unresponsive to DDAVP. All patients who are potential recipients of blood products should be vaccinated against hepatitis B. Patients who are not immune to hepatitis A should also be vaccinated. FVIII-vWF may be infused as fresh frozen plasma (FFP), but the large volumes required to achieve haemostasis limit its use. Cryoprecipitate contains 5-10 times more vWF than FFP (each bag contains approximately 80-100 iu). Cryoprecipitate has been shown to normalise FVIII, shorten the bleeding time and stop or prevent bleeding (Perkins 1967) and has been the mainstay of treatment for vWD for many years. However, this product has a
small but definite risk of transmitting blood-born infections. Therefore, virus-inactivated concentrates currently play the main role in the management of vWD. These concentrates are classified according their FVIII specific activity as intermediate-purity (FVIII 1-5 iu/mg of total protein) or high purity concentrates (FVIII 50-250 iu/mg). Very high purity concentrates, obtained by immunoaffinity chromatography (FVIII > 2000 iu/mg), contain very small amounts of vWF and are therefore unsuitable for the management of vWD. Recently a chromatography-purified concentrate particularly rich in vWF with a low FVIII content (called very-high purity vWF concentrate) has been produced (Bumouf-Radosevich & Bumouf 1992). In a small cohort study this concentrate was shown to be effective in patients with type 3 disease (Meriane et al 1993). Its efficacy and safety is under evaluation now in a larger number of patients.

The commercially available intermediate and high-purity FVIII-vWF concentrates contain large amounts of FVIII and vWF, therefore, high post-infusion levels of these moieties are consistently obtained. There is also a sustained rise in FVIII, higher than predicted from the dose infused, lasting up to 24 hours. This pattern is due to stabilisation of endogenous FVIII by the exogenous vWF (Cornu et al 1963). Therefore, all products are able to correct FVIII deficiency even though they are not always effective in correcting the prolonged bleeding time (Rodeghiero et al 1992).

There are several reasons for the inconsistent effect of these concentrates on the bleeding time. No concentrate so far contains a completely functional vWF as tested in vitro by evaluating the multimeric pattern and using several functional assays because vWF proteolysis occurs during purification by the platelet or leukocyte
proteases contaminating plasma used for fractionation (Mannuccio et al 1994). Despite their inconsistent effect on the bleeding time, these concentrates are used successfully for the management of vWD patients unresponsive to DDAVP, especially for soft tissue and post-operative bleeding (Rodeghiero et al 1992). The dose of concentrates recommended for patients with vWD undergoing a major or minor surgery is 50 iu/kg and 30 iu/kg, once a day or every other day with the aim of maintaining FVIII > 50 iu/dl and > 30 iu/dl, respectively until healing is complete. When the bleeding persists and the bleeding time remains prolonged despite replacement therapy, other therapeutic options (including DDAVP, platelet concentrates) should be considered. Cattaneo et al (1989) have shown that DDAVP, given after cryoprecipitate, further shortens or normalises the bleeding time in patients with type 3 vWD. Platelet concentrates (given before or after cryoprecipitate, at doses of 4-5 X 10^11 platelets) have also been shown to correct bleeding time and control bleeding in patients unresponsive to cryoprecipitate alone (Castillo et al 1991, Castillo et al 1997). These data emphasise the important role of platelet vWF in establishing and maintaining primary haemostasis.

Patients with type 3 disease may rarely develop alloantibodies due to multiple transfusion. In these cases FVIII-vWF concentrates are ineffective and may also cause anaphylactic reaction due to immune complex formations (Mannucci et al 1981a) which can sometimes be life-threatening (Mannucci et al 1987). Treatment with recombinant FVIII is recommended. As recombinant FVIII is completely devoid of vWF, the treatment does not cause anaphylaxis but has very short half-life. Therefore, infusion in very large doses are required to maintain FVIII > 50 iu/dl to control or prevent bleeding in these patients (Bergamaschini et al 1995).
2.1.8 - TREATMENT OF SPECIFIC PROBLEMS IN VWD

Bleeding from the nose and mouth

Oral cavity bleeding in the form of bleeding gums, bleeding from frenulum tears, bites of the lips and cheeks, which are often seen in young children, may sometimes be controlled by the application of topical thrombin, or by the use of topical tranexamic acid (10 ml of a 5% solution used as a mouthwash six times daily). Prolonged and frequent epistaxis is common in vWD, particularly in children. Local measures such as topical thrombin can be helpful. Nasal cautery can also be used and an anti-fibrinolytic agent may be administered. In severe cases, treatment with DDAVP or vWF concentrates may be required. Oral tranexamic acid may also be helpful.

Dental treatment

A single dose of DDAVP administered together with a fibrinolytic inhibitor, is often sufficient to cover dental extractions. Tranexamic acid can be given orally and/or as a mouthwash for 5-7 days. In patients in whom DDAVP is not suitable, a single dose of FVIII/vWF may be used. Pre- and post-FVIII activity levels should be measured with a Factor VIII level in excess of 50 iu/dl being usually sufficient for extractions.

Menorrhagia

Management of menorrhagia is discussed in detail in Chapter 2.4.
Pregnancy and childbirth

During pregnancy, FVIII and vWF levels rise and in many patients with type I vWD become normal. The majority of patients, therefore, have few problems in pregnancy. A minority of patients have normal levels of FVIII but a significant prolongation of the bleeding time during late pregnancy due to low levels of vWF.

Some patients with type 2 vWD develop thrombocytopenia in pregnancy, which is thought to be due to the increased synthesis of abnormal multimers. In some cases (Giles et al 1987) the development of thrombocytopenia does not appear to exert an additive effect on the underlying haemostatic defect, so that active intervention is not necessary. In others, in whom the platelet count falls to dangerous levels, the use of vWF concentrates can cause a resolution of the thrombocytopenia (Ieko et al 1990).

In patients with type 3 vWD, FVIII and vWF levels do not increase in pregnancy and replacement therapy with FVIII/vWF concentrates will be required to cover delivery or Caesarean section. In type I and type 2 vWD vaginal delivery is usually safe, and it is usually possible to avoid therapy with either DDAVP and/or vWF-containing concentrates. Women delivered by Caesarean section, or those with type 3 disease delivered vaginally, may require sequential treatment with vWF concentrates for the first 7 days post-partum. Cord blood screening of babies of women with type 1 and 3 vWD does not give a reliable diagnosis and the child should therefore be screened later during the first year of life.

Women with type 1 vWD who have corrected their FVIII and vWF levels during pregnancy may sometimes experience bleeding 4-5 days post-partum or prolonged intermittent secondary post-partum haemorrhage as the levels fall. In these situations,
the use of tranexamic acid has been recommended (Bonnar et al 1980). Tranexamic acid is a fibrinolytic inhibitor which competitively inhibits the activation of plasminogen and non-competitively inhibits plasmin thus counteracting increased fibrinolysis in the uterus (Bonnar et al 1980). Combined oral contraceptives are also useful for controlling this type of haemorrhage. Treatment with DDAVP and vWF concentrates may sometimes be required especially in more severe secondary post-partum haemorrhage or patients at risk of thrombosis. Pregnancy in women with vWD is assessed in this thesis in Chapter 5.

Angiodysplasia and Von Willebrand disease

Vascular malformations of the gastrointestinal tract (angiodysplasia) leading to gastrointestinal bleeding have been reported in vWD (Ramsay et al 1976). Both congenital (Ramsay et al 1976) and acquired (Rosborough & Swaim 1978) vWD can be associated with angiodysplasia but it is not clear if the association is casual. An incidence of 6% in congenital and 11.7% in acquired vWD has been reported (Fressinaud & Meyer 1993). The majority of cases have been in adults, with a median age of 55 years, but severe and life-threatening bleeding in children has also been reported. The diagnosis is confirmed by endoscopy, isotope scanning or angiography.

Surgical resection should be avoided unless the bleeding is life-threatening and all other measures have failed. In many patients, severe bleeding episodes can be reduced by prophylactic therapy with a suitable vWF concentrate. Where the angiodysplasia occurs in the stomach or duodenum the use of H2-receptor antagonists may be helpful in reducing bleeding.
Surgery in patients with von Willebrand disease

In all forms of surgery, good liaison between the haematologist and the surgical/anaesthetic team is essential. This helps to assess the operative risk and to assess whether any bleeding is excessive. It is important to remember that abnormal bleeding may be surgical rather than a result of a failure of adequate replacement therapy. Twenty-four-hour laboratory support is essential. All patients require to have FVIII activity levels measured pre-operatively, and before any therapy is given. After the initial treatment, and prior to surgery, FVIII levels should be measured 15 minutes post-vWF concentrates and 60 minutes post-DDAVP. For subsequent treatments, plasma samples pre and post-treatment should be stored so that vWF:Ac and vWF:Ag levels can be measured. Bleeding times may be required after the initial treatment, prior to surgery and if mucosal bleeding persists despite adequate therapy. All type 3 vWD patients should, if possible, be screened for inhibitors to vWF prior to surgery. If the patient is bleeding despite adequate replacement therapy it is useful to check the patient's platelet count and ristocetin-induced platelet aggregation.

DDAVP is an appropriate prophylactic treatment for surgery for patients whose FVIII and vWF levels can be raised above 50% (i.e. responsive to DDAVP). Monitoring of factor levels is essential if treatment is to be continued for more than 2-3 days. When factor concentrates are required for prophylaxis or treatment of surgical bleeding, only concentrates containing vWF should be used. Pre-operatively, FVIII:C levels should be raised to 100%; with the use of most concentrates, this would raise the vWF level to >100%. Treatment may be required 12 hours later, and certainly 24 hours later, to produce a further 50% rise in FVIII:C. FVIII:C levels pre and post-treatment should be assayed. vWF:Ac and vWF:Ag levels should be assayed pre and
post-treatment for the first three treatments, to allow a more informed plan of therapy.
2.2 HAEMOPHILIA A AND B

2.2.1 - MOLECULAR BASIS OF HAEMOPHILIA A AND B

The FVIII gene is 186 kb in length and is situated on the long arm of X chromosome at Xq28. It consists of 26 exons and 25 introns. The processed mRNA is 9kb in length and predicts a precursor protein of 2351 amino acids which in turns leads to a mature FVIII protein of 2332 amino acids. The largest intron, intron 22, is unusual in that it contains a CpG island associated with two further transcripts of part of the FVIII sequence called F8A and F8B. F8A is of opposite polarity to FVIII mRNA and consists of 1.8 kb of intron 22. F8B is of the same polarity as the FVIII mRNA and contains a short first exon plus exons 23-26 of the FVIII gene (Levinson et al 1992).

In terms of the genetic basis of haemophilia A, the presence of two further copies of this gene some 500 kb telomeric to the intron 22 copy (Levinson et al 1990) has considerable significance since intrachromosomal recombination between these homologous sequences is the cause of almost 50% of cases of severe haemophilia.

The mature FVIII protein is preceded by a signal peptide of 19 residues and consists of three types of domain (A, B, C) plus two acidic peptides ($a_1$ and $a_2$) arranged as follows: $A_1a_1A_2Ba_2C_1C_2$. Prior to secretion this undergoes cleavage of the prepeptide and at variable sites within the B domain and at B/a$_2$ boundary as well as post-translational modifications including O-glycosylation, N-glycosylation and sulphation of six tryptines (Vehar et al 1984, Pittman et al 1992). FVIII is a large plasma glycoprotein, stabilised in plasma by its non-covalent association with vWF. Where this association is disturbed, as in type 2N vWD, plasma FVIII levels are...
reduced through a decreased half life. This condition can be confused with mild haemophilia A (Nesbitt et al 1996). Activation of FVIII involves cleavage of the dipeptides Arg372-Ser373 and Arg 1689-Ser1690 and separation from the carrier protein (vWF). Loss of whatever is left from the B domain, that appears to play no part in coagulation, may also occur.

The FIX gene occupies 33.5 kb, completely sequenced, at the boundary between band Xq26 and Xq27 on the long arm of the X chromosome, and has only eight exons (Yoshitake et al 1985, Schwartz et al 1987). The product of the FIX gene is a polypeptide of 415 amino acids preceded by a pre-pro signal peptide. The circulating FIX consists of a gla domain and two epidermal growth factor like domains separated from the serine protease domain by an activation region. This polypeptide undergoes cleavage of the pre- and pro-segment of the signal peptide prior to secretion as well as post-translational modifications that include γ-carboxylation of the first 12 glutamates, O-linked glycosylation at Ser 53, Ser 61, Thr 159 and Thr 169 (Agarwala et al 1994), N-glycosylation at Asn157, and Asn 167, and B-hydroxylation of Asp64. Activation of FIX entails excision of the peptide containing residues 146-180 (Tuddenham & Copper 1994).

2.2.2 - GENE DEFECTS IN HAEMOPHILIA A AND B

Most families with haemophilia A or B carry gene defects of independent origin. Almost half the severe cases of haemophilia A or fifth of all cases are due to frequently occurring inversions caused by homologous intra-chromosomal recombination (Naylor et al 1993 a, b) between repeated sequences. Of the three
repeats one is in intron 22 of the FVIII gene and two are 400-500 kb more telomeric. They are 99.8% similar to each other. Some 75% of haemophilia A is due to small sequence changes. Those observed so far are of types that alter primarily RNA processing, mRNA translation or the fine structure of FVIII (Tuddenham et al 1994). These include splice site, nonsense, frameshift and missense mutations. In 3-5% of cases, haemophilia A is due to gross gene deletions, and 66 different types are reported in the most recent world list (Tuddenham et al 1994). These affect the whole or part of the FVIII gene and usually result in severe haemophilia. An international database of mutations detected in individuals with haemophilia A has been created (Kemball-Cook & Tuddenham 1997). Over 1000 entries are reported and the number is continually increasing.

Haemophilia B is mostly due to small gene defects that cause detrimental effects by impairing mainly or exclusively transcription, mRNA translation or the fine structure of FIX. Gross deletions or rearrangements account for only 2-3% of cases and are usually associated with severe disease. Different types of mutations resulting in haemophilia B include missense 67%, nonsense 13%, splice site 6%, frameshift 5%, gross deletion/rearrangement 3%, cryptic splice site 3%, promoter 3% and in-frame deletion < 1% (Lillicrap 1998). A world-wide mutation database was established in 1990 (Giannelli et al 1990). The most recent, 7th edition, of the database lists 1535 entries representing 597 unique molecular events resulting in haemophilia B (Giannelli et al 1997). Of the mutations that appears more than once in the database, many are at CG dinucleotides and involve a CG to TG or CA change.
2.2.3 - CARRIER DETECTION

Precise diagnosis of female carriers is very important in haemophilia care. Ideally carrier status should be identified and genetic counselling offered before pregnancy. The woman's risk of carriership and of giving birth to a haemophilic child must be established and complete information on prenatal diagnostic techniques including their risks, limitations, the time required to obtain a result and the fact that a conclusive result may not be obtained must be provided.

Family data: risk assessment

As haemophilia A and B are X-linked recessive inherited disorders, in each pregnancy there is a 50% probability that a carrier will transmit the X-linked gene, thus the disorder, to a son and there is also a 50% probability that a daughter of a carrier will herself be a carrier of the condition. Whereas the male offspring of a haemophilic male and an unaffected female will always be normal, the female offspring of a haemophilic male are carriers. If more than one haemophilic individual exists in the pedigree, the case is familial; if the haemophilic individual is the only known case in the family, the case is considered to be sporadic. In families with known haemophilia, genetic obligate carriership can be determined directly from the pedigree and requires no further carriership analysis, although results of DNA analysis may be useful for subsequent prenatal diagnosis of a future pregnancy. An obligate carrier is by definition and has a genetic probability of carriership of 1.0 and should fulfil one or more of the following criteria:

1. She is a daughter of a haemophilic father.
2. She has given birth to more than one haemophilic son (Identical twins excluded) or one haemophilic son and a daughter who has a haemophilic son.
3. She has given birth to a haemophilic son and there are well documented cases of haemophilic men in the maternal line of the pedigree.

Women in families with known haemophilia but who are not obligate carriers, are potential carriers and require further investigation. The first step is to estimate their genetic probability of carriership by tracking back from the consultand, step by step, through the pedigree to the nearest maternal relative who has haemophilia or is an obligate female carrier; each step vertically or horizontally through the pedigree is taken to constitute a factor of 0.5. The product of all these factors is the probability of carriership. The next step is to assess the need for phenotypic or genotypic assessment depending on the probability of carriership and resources available.

A sporadic case of haemophilia may result from transmission of the haemophilia gene through asymptomatic females, from a new mutation in the mother resulting in her being a carrier, or from a new mutation in the affected male himself (i.e. a true de novo mutation). In the overwhelming majority of cases in both haemophilia A and B, the proband’s mother is a carrier due to a new mutation (Ljung et al 1991, Kling et al 1992) and the mutation usually arises from the male gamete i.e. in the proband’s maternal grandfather. The percentage of sporadic cases vary in different countries according to the birth rate. In countries, where the average is two offspring per family, over half of the newly detected haemophilic are sporadic cases, however, in countries with higher birth rates, the proportion of familial cases is greater.
Phenotypic assessment

Phenotypic assessment by measurement of the potential carrier’s plasma concentration of FVIII:C/FIX:C and/or FVIII:Ag/FIX:Ag can be used to modify the genetic probability of carriership. The carriers’ FVIII:C/FIX:C concentration may vary and there is an overlap between non-carriers’ and carriers’ concentrations. Only when a potential carrier’s factor level is very high or very low, can carriership status be concluded with a reasonable certainty. In addition, the use of molecular genetic analysis which enables accurate carrier detection is increasingly available worldwide. Therefore, the combined use of pedigree and laboratory data which provides no more than a probability of carriership is only used for assessment of potential carriers in countries with no or limited resources for genetic diagnosis.

Genotypic assessment

Genotypic assessment is the most accurate method of carrier detection. Ideally, the genetic diagnosis of haemophilia should be based on direct gene analysis (i.e. direct identification of the causative mutation in the FVIII or FIX gene). However, in practice genotypic analysis of haemophilia is by indirect gene analysis in the majority of cases. Indirect gene analysis involves the use of linked polymorphic markers (restriction fragment length polymorphisms, RFLPs) to trace heredity of a haemophilia gene within a pedigree. To offer carrier detection and prenatal diagnosis on the basis of direct gene analysis, the pathogenic mutation needs to be traced and characterised by sampling an affected individual in the family. Once the mutation is known in the family, it is possible to offer carrier detection and prenatal diagnosis at any time in the future without the need to obtain blood samples from family members and even if no living individual with haemophilia remains in the family. Therefore,
this has great advantage over indirect gene analysis, where blood samples for analysis are required from other family members, which may sometimes be both a physical and psychological obstacle for diagnosis. In the United Kingdom, a nation-wide screening programme for haemophilia B mutations has been undertaken and a national patient database is maintained (Saad et al 1994). This information is held centrally and updated every year with details of every patient in whom a mutation is identified. A similar programme for haemophilia A is in progress.

**Timing of carrier testing**

When to identify the carrier status is very controversial. Guidelines from the Genetic Working party of the UK Haemophilia Centre Directors’ Organisation suggests that it is appropriate to determine carrier status as soon as possible in girls with a family history of haemophilia after counselling and a thorough and open discussion with the family, as this would help management of an early unexpected pregnancy. In contrast, clinical geneticists in the UK regard testing healthy young children for conditions which have no immediate implication for their own health unethical (Clarke 1994). In addition, there are also legal implications in the UK because, according to the Children’s Act 1989, testing of young children ignores their rights as it cannot be considered to have been performed with the informed consent of the child concerned. However, there is no dispute that carriership status should clarified and the carrier and her partner counselled regarding their options before pregnancy. The practice in the UK and in most of other countries, is to assess FVIII or FIX levels after birth to identify those with very low levels who might require treatment for trauma and invasive procedures and the actual genetic testing is performed when the child can give consent. However, this policy can be criticised as a significant
proportion of the carriers will be lost and only seen again once pregnant when it is probably too late for genetic counselling and prenatal diagnosis. Because of these controversial issues and ethical dilemmas, it is of paramount importance that the whole process of counselling is well documented in the clinical notes.

2.2.4 - PRENATAL DIAGNOSIS

Prenatal diagnostic techniques

Prenatal diagnosis for haemophilia should be preceded by genetic counselling to provide the prospective parents with adequate information to enable them to reach a decision that is appropriate to their situation, and to provide them with support throughout the process. Chorionic villus sampling (CVS) is the method most widely used today for prenatal diagnosis of haemophilia. The fetal chorion villus sample is first used for fetal sexing; the result is usually available within two days. In the case of a male fetus, further DNA-analysis has to be performed to determine whether or not the fetus is affected. Diagnosis involving the sequencing of a known mutation may take approximately 7-10 days. Diagnosis using RFLPs and the polymerase chain reaction (PCR) technique will yield results within 24-48 hours. CVS is performed at 11-13 weeks under ultrasound guidance and carries a risk of miscarriage of about 1% (Rhoads et al 1989). Limb abnormalities have been reported in association with CVS performed before 10 weeks gestation (Firth et al 1994). Recent years have witnessed successive development of pre-implantation genetic diagnostics. Up to 8-cell stage, every blast cell in an embryo is capable of independent development to a complete individual. One or two of these cells are isolated and the DNA analysed after PCR amplification. Successful attempts have been made in the case of
haemophilia. The method presupposes \textit{in vitro} fertilisation. Another recent
development in the field of prenatal diagnosis is the use of fetal cells in the maternal
circulation (Lo et al 1997). It is possible that in the future these methods will have an
important role in the prenatal diagnosis of haemophilia.

Second trimester amniocentesis trimester can also be used for prenatal diagnosis in
haemophilia. However, CVS has the advantage that termination of pregnancy, if
opted for, can be performed during first trimester which is less traumatic, less
stressful and more acceptable to the patient. In addition, it is often not possible to
obtain adequate DNA for analysis with amniocentesis. In the second trimester it is
also possible to obtain fetal blood for FVIII/FIX analysis by ultrasonic guided
percutaneous method (cordocentesis). This procedure is performed at 18-20 weeks of
gestation and suitable for cases where early genetic diagnosis has for some reason not
been possible or has been inconclusive. The procedure is reported to have a 1.25% risk of procedure related fetal loss when performed for nonchromosomal indications
and by an experienced operator (Wilson et al 1994).

The last method of prenatal diagnosis is fetal sex determination. This was previously
achieved by fetal karyotyping, but now with better resolution of the newer ultrasound
machines it is possible to determine fetal sex accurately during the second trimester
(Plattner et al 1983). Ultrasonic fetal sex determination is helpful in several
situations. If a fetus is identified as female then the mother can be reassured and the
risks of invasive procedures are avoided. When specific prenatal diagnosis for
haemophilia is not possible for a carrier mother because she is not informative on
DNA analysis or because adequate information about the family can not be obtained,
and when a women is unsure of her feelings about termination of pregnancy, knowledge that the fetus is female can be very reassuring (Koerper 1990). Lastly, knowledge of fetal sex is very helpful to the attending obstetrician for labour management of carrier mothers who had not had specific prenatal diagnostic tests. In these situations, the risks of traumatic haemorrhage to a male fetus can be minimised by avoiding invasive monitoring techniques, vacuum extraction or difficult forceps deliveries.

**Laboratory techniques for genetic diagnosis**

Genetic diagnosis in haemophilia can be obtained by either direct identification of the pathogenic mutation (direct gene analysis) or by genetic linkage (indirect gene analysis). Direct gene analysis is the most reliable method for prenatal diagnosis and should be used if available. In families with severe haemophilia A, the natural starting point is to ascertain whether the disease is due to inversion in the X-chromosome. In almost 50% of cases, severe haemophilia A is caused by one or other of two inversions i.e. substantial re-arrangements in the long arm of the X-chromosome. There is reverse orientation of part of the X-chromosome, with one break point in intron 22 of the FVIII gene and the other in either of the two repeats of part of intron 22 sequence that lie telomeric to the gene (Naylor et al 1993a, Lakich et al 1993). Since these inversions can be demonstrated with the Southern blot technique, it is possible to offer carrier and prenatal diagnosis to almost half the families with severe haemophilia A, using methods that are relatively simple and familiar to most laboratories. In the case of families with haemophilia A but without inversions, the disease may be due to any of a large number of different mutations, and the mutation present in a given family must be identified. Due to the size and
complexity of the FVIII gene this involves extensive procedures and is only available at a limited number of laboratories. One technique is to isolate mRNA in leukocytes and reverse transcribe in sections to cDNA. Then cDNA is amplified with PCR and screened for mutations by chemical cleavage mismatch detection. When the mutation is established, direct sequencing in the exon of interest can be used for carrier detection and prenatal diagnosis. In some cases where the mutation is known e.g. in the case of deletions, simpler methods can be used for diagnostic purposes.

In haemophilia B, genotype heterogeneity has been found to be very marked and almost every family have its own unique mutation (Montandon et al 1990). This means that, for the purpose of carrier detection or prenatal diagnosis, the mutation must first be characterised in an affected family member. When using the characterised mutation for diagnostic purposes it is critical to confirm that the mutation found causes haemophilia, as neutral missense mutations have been found that alter an amino acid in the protein molecule without affecting its function or causing haemophilia (Montandon et al 1990). To be acceptable as a cause of haemophilia, a missense mutation must be included in the international databases of haemophilia A and B mutations (Tuddenham et al 1994, Giannelli et al 1997), unless there is no doubt that, owing to its localisation, it has caused such anomalies as conformational changes in the protein molecule. Furthermore, a mother of a sporadic case of haemophilia can not unequivocally be excluded from carriersonship, even if the mutation is not found in her, as there is the possibility of mosaicism (a mixture of normal and mutation carrying cells) in her germinal or somatic cells.
Although the identification of the mutation is the ideal diagnostic tool, it requires access to advanced molecular biological facilities. An alternative option is genetic linkage studies of polymorphisms (Peake et al 1993). Polymorphisms are normally occurring variations in the nucleotide sequence in a gene which can be used to identify the mutant gene and follow its segregation in the pedigree. Owing to this natural polymorphic variation, a cleavage of a restriction enzyme may be altered, and the cleavage fragments obtained when a restriction enzyme cleaves the DNA at a specific site, will vary in length from one individual to another and also between the two X-chromosomes of a given female. This can be used to identify the gene (allele) that carries the mutant gene and trace its segregation in the family.

The logical starting point in haemophilia A is to use the CA-repeat in intron 13, a multiallelic polymorphism, as 80% of Caucasian women are heterozygous with regard to the number of CA repeats (Lalloz et al 1991). In addition, six bi-allelic, intragenic polymorphisms in the FVIII gene may be used - BcII, Hind III, Xba I, Bga I, Msp I and Taq I. The usefulness of a polymorphism is dependent on the frequency of women heterozygous for the allele. For optional diagnostic efficacy, it is necessary to know which polymorphism to use in a given population, since the various alleles are characterised by manifest ethnic variation. In selecting a polymorphism, it must be borne in mind that one allele of a polymorphism usually occurs with a certain allele of another polymorphism (i.e. allele association). Thus, little increase in the detection rate of heterozygosity will be obtained when using certain combinations of polymorphisms. In haemophilia B, suitable combinations of eight intragenic polymorphisms, Taq I, Xmn I, Dde I, Hha I, Mnl I and Mse I are informative in 90% of Caucasian women (Peake et al 1993).
When assessing polymorphisms, blood samples are required from affected family members and a number of other relatives. In sporadic cases where it is unknown whether the disease has existed earlier, carriership can only be excluded in those cases where the allele differs from that in the haemophilic individual in the family. Mosaicism is also a phenomenon to be taken into consideration in indirect genetic analysis.

Assurance of the quality of all the steps of prenatal diagnosis of haemophilia is mandatory. There should be a multidisciplinary approach involving experts in the fields of prenatal medicine, genetic counselling, haemophilia care and molecular genetics who must attain appropriate levels of training. The genetic analysis should ideally be centralised to equality certified laboratories serving a large enough catchment area to allow thorough knowledge of the FVIII or FIX genes and the limitations of the diagnostic procedures.

Prenatal diagnosis: attitudes of carriers of haemophilia and its psychological consequences

Haemophilia is, in general, considered as a severe disorder by families with severe and mild forms of haemophilia (Ranta et al 1994) because of its bleeding episodes, joint problems and its associated limitations in daily life. However, most women in these families do not consider haemophilia to be a sufficiently serious disorder to justify an abortion (Varekamp et al 1990, Kraus & Brettler 1988, Markova et al 1984) and therefore there is a low uptake of prenatal diagnosis and termination of affected pregnancies. Varekamp et al (1990) evaluating the attitudes toward prenatal diagnosis among 549 non-pregnant potential and obligate carriers of haemophilia
found that only 31% of the study group would favour prenatal diagnosis, with the implication of a possible abortion in early pregnancy, and half of them would choose this option even at 16-20 weeks. However, the use of and attitudes towards prenatal diagnosis and termination of affected pregnancies vary widely between different countries, religions and cultures and also according to family experience with haemophilia and carriers’ education and social class. The use of prenatal diagnosis for X-linked conditions is more often considered by carriers who have lived for a long period with the disease, those with higher education and strong career commitments and is least likely to be considered by women with strong religious beliefs (Beeson and Golbus 1985). Catholics, in general, are least likely to choose prenatal diagnosis compared to Protestants and Jews (Krause & Brettler 1988). The attitudes of women in families with haemophilia toward childbearing and prenatal diagnosis and factors affecting their decision making are assessed in this thesis (Chapter 4).

It has been shown that a large proportion of women and their spouses manifest mental and psychosomatic symptoms before and during the process of prenatal diagnosis, particularly while awaiting the test result, and for some time afterwards (Tedgård et al 1989, Tedgård et al 1994). When the fetus is found to be healthy and the pregnancy is continued, carriers experience the period until delivery as very distressing. When the fetus is affected and termination of the pregnancy is opted for a very high frequency of psychological sequelae has been reported for up to 6 months (Tedgård et al 1989). It has also been shown that offering women prospective psychological support and the opportunity to discuss their thoughts and feelings concerning haemophilia, prenatal diagnosis, abortion and its relevance to their self-
esteem, would help coping better emotionally than giving more information and promoting increased knowledge about the disease and prenatal diagnostic procedures (Tedgård et al 1997). Therefore there is a manifest need for support for the woman and her spouse throughout the process of prenatal diagnosis.

The psychological consequences of having to terminate a planned and wished for pregnancy are long-lasting. However, the long-term effects of prenatal diagnosis in haemophilia, especially for couples who undergo termination of an affected pregnancy, is not fully studied. In a study by Tedgård et al 1996, carriers with experience of prenatal diagnosis of haemophilia did not have more signs of psychological distress, as measured by the SCL-90, one year or more after the experience compared with other carriers and non-carriers without prenatal diagnosis. Follow-up of carriers who experience prenatal diagnosis and abortion, at about six years after the abortion, these women also did not have any more signs of psychological distress than the control group. Nevertheless, there must be qualified assistance during the prenatal diagnosis process as well as adequate follow-up after an abortion to help the women to cope with the emotional strain. In addition, the field of prenatal diagnostic techniques is growing very fast; this must be kept under close scrutiny to avoid psychological and ethical problems.

2.2.5 - GENETIC COUNSELLING

In late 1990s, with accurate diagnosis, easier and more effective treatment of haemophilia and gene therapy “on the horizon”, it seems that there is less emphasis placed on counselling aspects in the management of families with haemophilia.
Despite these advances, haemophilia remains a life long incurable disease with potential life threatening bleeds and long-term joint damages. In addition, all current treatments remain invasive, constant, unpredictable and have side effects. A significant proportion of patients have needle phobias, fear side effects and there is a great deal of reluctance to treat. This applies to children and their parents and to adults with haemophilia. Therefore, counselling of potential/obligate carriers remains a central task of all health care providers. The final decision about having children has to be left to women and their partners. However, it is the responsibility of health care professionals to help these carriers make informed choices in the best interests of the individual family without losing sight of the best interests of the child. This is a complex matter for all individuals as there is always a conflict between the best interests of the family as a whole, the mother herself and the child. These issues are universal issues and are difficult even in families without haemophilia. Universal issues, including a wish to have children, concern about continuation of the family and social, cultural and emotional pressures, are all confoundedly affected by normal life stage expectations. Particular issues in haemophilia that require consideration include the family experience of haemophilia, perceptions of ‘normality’ of the carrier, partner and other close family, family concerns about inheritance and, most importantly whether the family is open (i.e. all family members participate in the discussion and management of haemophilia) or has secrets and the carrier sisters are protected and has very little experience.

The counselling of obligate carriers of haemophilia should start from an early age, ideally through an openness about haemophilia in the family with support from those with expert knowledge at the haemophilia centre. The age at which carriers should be
offered counselling depends on many factors such as the degree of their involvement
in the care of the haemophilic individual in the family and the attitude of the family
to haemophilia. However, it should generally be possible to begin to explore
knowledge about haemophilia and respond to questions from the age of 10-11 years.
In early adolescence more explicit information can be given and when the carrier is
of child bearing age, the issue of prenatal diagnosis should then be raised. The main
issues to cover in counselling include: knowledge about haemophilia (what it is and
its consequences), carriers wishes and beliefs about having a child with haemophilia,
options for prenatal diagnosis and their benefits, risks and limitations, views about
termination of pregnancy and how to cope and manage a child with haemophilia. It is
important that during this process partners are not forgotten and their views are
explored as well as the views of maternal parents and affected member(s) in the
family. Treatment issues should also be covered in the counselling; these include the
current available options and their side effects, as well as possible future options
(such as gene therapy) and their certainties/uncertainties. It is also important to
emphasise that although gene therapy is on the horizon, it is not a cure for the disease
and the genetic inheritance does not disappear and has life-long implications for the
carrier and future generations of her family.

Counselling of possible carriers is similar to that of obligate carriers once it is
established that there is a history of haemophilia or bleeding problems in the family.
However, there are some particular issues that should be raised in these carriers
including making contact through the index patient and the need for assessment of
factor VIII and XI levels for trauma or prior to invasive procedures prior to the
carriership status is clarified. Sporadic carriers are often identified through the child.
Some of these children have extensive unexplained bruising and might even have been brought to the notice of social services as sufferers of abuse. If a child has been identified with haemophilia, it is important to remember to counsel the mother about future children. Counselling of these carriers is harder as there is no history or experience in the family.

2.2.6 - BLEEDING SYMPTOMS IN CARRIERS OF HAEMOPHILIA

Although female carriers have only one affected chromosome and the clotting factor level is expected to be around 50% of normal, a wide range of values has been reported (Rapaport et al 1960, Rizza et al 1975) as a result of random inactivation of one of the two X chromosomes i.e. lyonization (Lyon 1962). Rizza et al 1975 reported mean value for FVIII of 54 iu/dl (range 22-116 iu/dl) in carriers of haemophilia compared to a normal mean level of 96 iu/dl (range 44-136 iu/dl). FVIII activity is less than 30 iu/dl in 2% of carriers of haemophilia (Rapaport et al 1960). A small number of haemophilia carriers may have very low factor levels (Lusher et al 1978) due to extreme lyonization, homozygosity for haemophilia gene (Graham et al 1975) or, less commonly, coincidence of carriership and Turner’s syndrome, other chromosomal abnormalities (Mori et al 1979, Neuschatz et al 1973) or coinheritance of a variant von Willebrand factor allele (i.e., Von Willebrand’s disease Normandy).

Because of the possibility of low levels of FVIII or FIX activity, a bleeding tendency is expected in some carriers, especially in those with factor levels below 50 iu/dl. These women have a tendency to increased bruising, prolonged bleeding after dental and surgical interventions, delivery and from small wounds (Mauser Bunschoten et al
Menorrhagia has also been reported in these patients (Lusher et al 1978) although in the study by Mauser Bunschoten et al (1988) there was no significant difference in the percentage of carriers who considered their menstrual loss to be greater than other women compared to a reference group. Assessment of menstrual loss and gynaecological problems in carriers of haemophilia is studied in this thesis in Chapter 8.

2.2.7 - CARRIERS OF HAEMOPHILIA AND PREGNANCY

Carriers of haemophilia A, in general, show a significant rise in FVIII during pregnancy (Kasper et al 1964, Stirling et al 1984, Greer et al 1991). Although the majority of patients develop levels within the normal range, the rise is variable, unpredictable and a small proportion of them still have levels below < 50 iu/dl at term (Greer et al 1991) especially those with very low non-pregnant factor levels. In contrast, in carriers of haemophilia B, factor FIX levels do not rise (Greer et al 1991, Briet et al 1982). Therefore, it is recommended that the mother’s factor VIII or IX level should be checked at booking, and at 28 and 34 weeks’ of gestation. This is especially important in patients with low pre-pregnancy levels. Maternal factor levels do not rise significantly until the second trimester. Thus invasive procedures including CVS, cordocentesis, termination of pregnancy or spontaneous abortion during the first trimester may be complicated by serious haemorrhage. It is important, therefore, that factor levels are checked prior to these procedures and replacement therapy arranged when the levels are less than 50 iu/dl. Monitoring during the third trimester is particularly essential as prophylactic treatment can be arranged during labour and the post-partum period to decrease the risk of post-partum haemorrhage,
especially in patients at risk of rapid labour e.g. ‘grande multipara’ patients and patients with a previous history of rapid labours.

2.2.8 - LABOUR AND DELIVERY

Labour and delivery are critical periods for carriers of haemophilia and their offspring as they are exposed to various haemostatic challenges. Affected male fetuses are potentially at risk of serious scalp haemorrhage, including scalp abrasions, cephalhaematoma, subgaleal haematoma and intracranial haemorrhage from the process of birth, invasive monitoring techniques or instrumental deliveries. Ljung et al (1994) reviewed mode of delivery and perinatal bleeding in 117 children with moderate or severe haemophilia. They concluded that the risk of serious bleeding in normal vaginal delivery is small and delivery of all fetuses known to be at risk of haemophilia by Caesarean section is not expected to eliminate this risk. However, the use of vacuum extraction was shown in the same study to constitute a significant risk factor as 10 of 12 infants with subgaleal and cephalhaematoma were delivered by this instrument. The association of intracranial haemorrhage with traumatic deliveries was also reported by Kletzel et al (1989) in three of four haemophilic infants with post-delivery head bleeding with the use of forceps in two and vacuum extraction in one. Therefore, fetuses at risk of haemophilia should be delivered by the least traumatic method. Prolonged labour, and especially prolonged second stage of labour, should be avoided and early recourse to Caesarean section should be considered. Although vacuum extraction should not be used, low forceps delivery may be considered less traumatic than Caesarean section when the head is deeply engaged in the pelvis and delivery can be achieved as an easy outlet procedure and
performed by an experienced obstetrician. Mid cavity forceps and forceps involving the rotation of the head should be avoided.

Although affected male fetuses are potentially at risk of scalp haemorrhage from the use of fetal scalp electrodes and fetal blood sampling for intra-partum monitoring, there is a lack of published data to support this. No bleeding complications in affected fetuses and neonates have been reported so far from these procedures, probably they have not been used frequently in these situations. However, it is advisable to avoid their use in fetuses at risk.

The use of regional block in patients with bleeding disorders is controversial. Acute spinal cord compression from a haematoma developing in the subarachnoid, subdural or extradural space can rapidly produce irreversible paraplegia (Sage 1990). This is a rare but serious complication of insertion and withdrawal of epidural catheter or spinal anaesthesia in the presence of a coagulation defect. Because of the fear of this catastrophic complication, some anaesthetists and haematologists regard any bleeding disorder as a contraindication to regional block and unnecessarily deny some patients the benefits of these techniques. However, provided the coagulation status is normal and FVIII or FIX levels are maintained more than 50 iu/dl, there is no contraindication for a regional block. It may some times be difficult to assess factor levels when patients present in advanced labour. In this situation provided that factor levels were more than 50 iu/dl during the third trimester, it is sufficient to assess platelet count, activated partial thromboplastin time (APTT), and prothrombin time (PT). However, it is particularly important to check factor levels prior to removal of the epidural catheter because the pregnancy induced rise in factor levels may quickly
reverse after birth and bleeding in the spinal canal may then arise. Regional block in carriers of haemophilia should be performed by an expert anaesthetist with the help of a specialised haematologist for assessment of coagulation status and arrangement of treatment when needed.

2.2.9 - OBSTETRIC HAEMORRHAGE IN CARRIERS OF HAEMOPHILIA

There has been no report of increased risk of antepartum haemorrhage. Thus it seems that the maternal bleeding complications are confined to the post-partum period as the pregnancy induced rise in the clotting factors falls rapidly after delivery. Mauser Bunschoten et al (1988) reported a significantly higher incidence of prolonged bleeding after delivery among haemophilia carriers (22%) in comparison to the control group (6%). Greer et al (1991) also reported 5 post-partum haemorrhages (PPH) and a large perineal haematoma among 43 pregnancies in carriers of haemophilia. To minimise the risk of primary and secondary PPH, therefore, it is essential that factor levels are checked daily and maintained above 50 iu/dl for at least 3-4 days or 4-5 days if Caesarean section has been performed (Walker et al 1994). The risk of primary PPH can be further reduced by active management of third stage of labour and minimising maternal genital and perineal trauma.

In case of any bleeding, correction of hypovolemia, factor replacement therapy or treatment with DDAVP should be instituted with the collaboration and the help of the local haemophilia centre. DDAVP has no effect on factor IX levels and factor IX concentrates would be required in carriers of haemophilia B. High-purity factor IX concentrate should be used as factor IX concentrate containing factor II, factor VII
and factor X are potentially thrombogenic (Magner et al 1979, Lusher 1991). Plasma derived concentrates of coagulation FVIII, treated with the currently available viricidal methods carry a negligible risk of transmitting hepatitis B and C virus. However, they may not be effective against hepatitis A and parvo virus B19 (Santagostino et al 1994). The latter is of particular importance in pregnant women as it can cause severe fetal infection and hydrops fetalis. Therefore, recombinant FVIII or FIX should be used in pregnant mothers when administration of these factors is indicated.

2.2.10 - ACQUIRED POST-PARTUM HAEMOPHILIA

Acquired haemophilia is a rare haemorrhagic disorder, which is induced by FVIII inhibitors in patients who previously had normal levels of FVIII, with a prevalence of 0.2 - 1/million of the population/year (Duran-Suarez 1982, Lottenberg et al 1987). These inhibitors are autoantibodies that partially or completely suppress FVIII procoagulant activity, sometimes leading to serious haemorrhagic complications and a high mortality. The autoantibodies are immunoglobulins of IgG class, predominantly IgG4 subclass, in the majority of patients. IgM and IgA have been observed in very few instances (Hoyer et al 1984, Glueck et al 1987). The kinetics of reaction between these autoantibodies and FVIII in these patients is different and more complex than those observed in haemophilic patients with acquired FVIII inhibitors. In haemophilic patients, the inhibitors inactivate FVIII progressively in direct proportion to their concentration. However, in non-haemophilic patients there is rapid inactivation of FVIII followed by a slower second phase that does not reach a stable end point and some FVIII coagulant activity may still be measurable.
Therefore, in vitro inhibitor assays, such as the Bethesda assay, that depend on the measurement of residual FVIII may underestimate the inhibitors’ potency in these patients (Green 1984).

Acquired FVIII inhibitors may arise in otherwise normal individuals, especially in old age, in the majority (52.3%) of cases (Kessler & Ludlam 1993) or in patients with diseases associated with altered immune function. Rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, lympho-proliferative disorders and multiple sclerosis are most frequently associated immunological disorders. FVIII inhibitors may develop secondary to some other conditions such as asthma, severe dermatologic conditions, malignancies and drug reactions, penicillin, ampicillin and diphenylhydantoin in particular (Hultin 1991, Green & Lechner 1981). Pregnancy/post-partum states are described to be the associated factor in 7% among 65 and 11% among 215 patients with acquired haemophilia by Green & Lechner 1981 and Kessler & Ludlam 1993 respectively.

The risk of developing FVIII inhibitors is highest after the first delivery, although appearance of inhibitors during pregnancy or after several deliveries has been reported. The bleeding symptoms usually become apparent within 3 months after delivery but could be as late as 12 months post-delivery. The bleeding symptoms are usually very severe and occur at several anatomical sites concurrently (Morrison et al 1993). Dramatic ecchymoses, multiple large soft tissue haematomas, post-operative bleeding and vaginal bleeding are the commonest presenting symptoms. Patients with acquired FVIII inhibitors are at a higher risk of death due to uncontrolled haemorrhage than haemophilic individuals with inhibitors. Green & Lechner (1981)
and Lottenberg et al (1987) reported death due to haemorrhage in 22% of 215 and 12% of 16 patients respectively. Complete remission (absence of inhibitors and normalisation of FVIII) is achieved spontaneously in the vast majority of patients with post-partum acquired haemophilia, especially those with no underlying disorders and those who had low levels of inhibitors (Spero et al 1979). While the antibodies usually disappear within a few months, this can be extremely variable and, in some cases, they may persist for years.

There is a need for a high degree of awareness of any circumstances suggesting this condition e.g. unexplained excessive and/or prolonged vaginal bleeding or large soft tissue haematomas during post-partum period. A patient with an autoantibody to FVIII typically has a low FVIII level and a prolonged APTT, but a normal PT. The Ivy bleeding time is usually normal but may be somewhat prolonged for reasons not clearly understood. Platelet function tests are usually normal. Mixtures of patient’s plasma and normal plasma must be incubated and residual FVIII activity measured after a period of incubation in order to confirm the evidence of specific inhibitors to FVIII. Quantitative assay of the inhibitor should then be performed as it helps to decide on the method of treatment and to follow the progress of the patient. The Bethesda assay (Kasper et al 1975) is the most commonly used method world-wide. A Bethesda unit (BU) is defined as the amount of inhibitor that would inactivate half the FVIII in a mixture of pooled normal plasma containing 100 U FVIII/dl and an equal amount of patient’s plasma incubated for two hours at 37 °C. The level of inhibitor against porcine FVIII should also be ascertained, this is required to guide future therapy.
As acquired post-partum haemophilia is very rare, there is no universally accepted method for management and all the available data are from case reports and personal experiences. The first aim of management is to control acute bleeding. Until the disappearance of the inhibitors, these patients are at high risk for recurrent bleeding which could be life threatening. Therefore, the other main goals in managing these patients are to attempt to accelerate eradication of the inhibitors and avoidance of situations that are likely to result in bleeding. These situations include minor traumatic injuries, intramuscular injections, dental procedures and the use of aspirin or other drugs that interfere with blood coagulation and platelet function. Despite the low incidence of underlying disease in patients with post-partum acquired haemophilia, it is important that all these patients are evaluated for any associated systemic disease since the control of the underlying disorder may play an important role in the management of the inhibitor.

Acquired haemophilia is usually diagnosed outside a haemophilia centre, usually in a general hospital. However, because of the rarity of the disorder, the array of treatment modalities and the need for strict monitoring with FVIII assays and inhibitor levels, it is recommended that such patients are best treated in a haemophilia centre by individuals specialised in the management of these disorders.

Control of haemorrhage in patients with acquired FVIII inhibitors is usually achieved by raising the plasma FVIII level, if possible. There are a number therapeutic options, including infusion of human or porcine FVIII with or without measures to lower the inhibitor level if it is very high, recombinant factor VIIa (rFVIIa), activated prothrombin complex concentrates (APCC) and DDAVP infusion. The choice
depends on severity of the bleeding, the level of human and porcine FVIII inhibitor level as well as the safety of each therapeutic method. If the level of inhibitor against human FVIII is very low (under 3 BU) and the bleeding is not immediately threatening DDAVP can be used as an initial treatment to raise the FVIII level. Otherwise porcine FVIII concentrate should be considered as first-line treatment for most of the patients (Kessler & Ludlam 1993). Inhibitors react much less potently with porcine than with human FVIII, it does not transmit human pathogens therefore provides viral safety to those patients who have not previously exposed to blood products, and it is the only modality of treatment that allows the clinician to monitor plasma FVIII:C activity level as a predictor of clinical response. Adverse events observed with high purity polyelectrolyte fractionated porcine FVIII are very uncommon. Severe allergic reactions or anaphylaxis are possible, but rare (Erskine & Davidson 1981). Thrombocytopenia has been reported by some authors (Gringeri et al 1991, Gatti & Mannucci 1984) and the platelet count needs to be monitored during treatment with this product. If human FVIII concentrate is justified or selected for treatment, a concentrate that has been intensively virally inactivated or a recombinant type should be used as patients with acquired inhibitors often have not been transfused and exposed to human pathogens. Human FVIII’s other major problem is anamnesis. Recombinant FVIIa does not carry blood product risks, but is expensive, required in repeated doses because of its short half life and not licensed in many countries. APCCs have proven to be successful and are virally inactivated. However, frequent doses may be associated with the risk of thrombosis. Adjuvant therapy, such as antifibrinolytics, may also be used. When inhibitors levels are very high, they can be reduced with exchange plasmapheresis, with or without extracorporeal adsorption of the patients’ gamma globulin. Infusion of normal gamma globulins may also
reduce inhibitor levels (Sultan et al 1986).

The final goal of treatment is to accelerate the disappearance of FVIII inhibitors. This may be achieved by the use of immunosuppressive drugs as corticosteroids, cyclophosphamide, or azathioprine. In a large multi centre survey of 215 patients with acquired haemophilia, Green & Lechner 1981 found that FVIII inhibitor disappeared or declined in 58% of patients treated with an immunosuppressive drug, whereas the inhibitor disappeared or declined in only 38% of untreated patients. Due to the small risk of bone marrow depression and the increased risk of malignancies after long-term immunosuppressive treatment, the clinician has to decide, on an individual basis, the suitability of the treatment. However, major bleeding has been reported in 87% of patients with FVIII inhibitors and a fatal outcome in 22% (Green & Lechner 1981). These risks outweigh the small risk associated with immunosuppressive treatment.

There are conflicting evidences concerning the efficacy of corticosteroid therapy. It has been reported to be effective in post-partum inhibitors (Green & Lechner 1981). On the other hand, in a retrospective study of 51 cases with post-partum inhibitors conducted by Hauser et al 1995, steroid therapy appeared not to be superior to no treatment, but patients treated with immunosuppressive drugs (cyclophosphamide, azathioprine, 6-mercaptopurine) had a significantly shorter time to complete recovery i.e. absence of inhibitor and normalisation of FVIII activity. However, the results of these retrospective studies have to be regarded with caution. There has been only one prospective randomised trial (including only 31 patients) to determine the effectiveness of prednisone, cyclophosphamide, or a combination of both in
suppressing FVIII inhibitors (Green et al, 1993). The authors concluded from the results of their study and a review of the literature that immunosuppression with prednisone in a dose of 1 mg/kg should be started after diagnosis of FVIII inhibitors. The therapy may be continued beyond three weeks if the inhibitor titre is declining, otherwise, cyclophosphamide should be added and the prednisone tapered over the next 3-6 weeks. This regimen resulted in the disappearance of FVIII inhibitors in two thirds of their patients. They also concluded that patients with high inhibitor titres (more than 100 BU) are more likely to be resistant to this regimen and may require a combination of steroid and cyclophosphamide at higher doses, or even the addition of another immunosuppressive agent. Inclusion of larger number of patients into the randomised trial is necessary to confirm these conclusions.

The prognosis for subsequent pregnancies in patients with FVIII inhibitors is favourable especially in those achieving complete remission. A review of the literature by Coller et al 1981, revealed that there were no recurrences with second pregnancies in any of the eight patients with post-partum FVIII inhibitors, thus far reported, whose inhibitors had completely disappeared prior to delivery. A significant increase in FVIII inhibitor level during a second pregnancy has been reported (Vicente et al 1987) with no haemorrhagic complications during pregnancy, delivery and puerperium and the inhibitor level decreased progressively after delivery.
2.3 : FACTOR XI DEFICIENCY

2.3.1 - BIOCHEMISTRY AND THE ROLE OF FACTOR XI IN BLOOD COAGULATION

Factor XI (FXI) is a dimeric serine protease, each chain of 80 000 MW and composed of 607 amino acids. Activation by Factor XIIa results in cleavage at a single arginine\(^{369}\) - leucine\(^{370}\) bond, leading to a four-chain activation product with two light chains containing the active site and two heavy chains which contain binding sites for high molecular weight kininogen (HMWK) and calcium (Bouma & Griffin 1997, Asakai et al 1987). The heavy chain contains four tandem repeats (apple domains) and is 396 amino acids long. The overall sequence has considerable similarity to prekallikrein (58%) (Asakai et al 1987), especially in the light chain (81%). These two proteins compete for binding to HMWK, but have different substrates with little cross reaction.

The physiological role of FXI in blood coagulation is not fully understood. Surface-bound factor XIa activates FIX, but the importance of this \textit{in vivo} is not clear as there is no defect in FIX activation in FXI deficient patients (Bauer et al 1990). Although FXI deficient patients have a bleeding tendency, it is mild in comparison with FVIII and FIX deficiencies and individuals with undetectable FXII, HMWK or prekallikrein do not exhibit any bleeding tendency. This suggests that there may be an alternative activation mechanism for FXI. Platelet and endothelial cells can bind FXI and there is some evidence for an alternative platelet-mediated pathway for FXI.
activation, but the details and importance of this are not yet clear. Washed platelets contain FXI even in some severely FXI deficient patients (Walsh 1972).

Thrombin activation of factor XI is now generally accepted although some studies have not confirmed this (Schiffman & Lee 1974, Scott & Colman 1992, Brunnee et al 1993). This activation is independent of HMWK but is accelerated in the presence of thrombomodulin and an endogenous glycosaminoglycan, suggesting that it occurs on the endothelial and platelet surface. The biochemical cleavage is at the same site as by FXII, but the kinetic data suggest that thrombin is a better activator than FXII (Gailani & Broze 1991). These and other findings have resulted in a revised model of Coagulation (Figure 2.4). Coagulation is normally triggered in vivo via tissue factor (TF) and the extrinsic pathway. Back-activation of the intrinsic pathway could modulate coagulation activation without involvement of the rest of the “contact” system and provide a back-up coagulation pathway as FVIIa-TF is inactivated by the intrinsic pathway inhibitors (Broze & Gailani 1993).

Although FXI has been shown to activate fibrinolysis (Mandle & Kaplan 1979) and a fibrinolysis defect has been demonstrated in FXI deficient patients (Saito 1980), it is not clear whether this has any physiological significance. The main inhibitor of FXIa is alpha 1 antitrypsin which is responsible for about two-thirds of the inhibition. The rest of FXIa inhibition is by C1 esterase inhibitor, antithrombin III and alpha 2 antiplasmin (Scott et al 1982).
Figure 2.4: Revised coagulation pathway showing the role of thrombin in activation of factors V, VIII, and XI. (Pasi 1997)
2.3.2 - MOLECULAR GENETICS

The factor XI gene is located close to the gene for prekallikrein on chromosome 4 (Kato et al 1989). The gene is 23 Kilobases in length with 15 exons and 14 introns. The first two exons do not code for a functional part of the molecule, exons 3-10 code for four tandem repeats (apple domains), and exons 11-15 code for the carboxyterminal containing the active sites.

The first three FXI gene mutations were described in six severely affected Ashkenazi Jews (Asakai et al 1989). Several other mutations have since been described (Imanaka et al 1993, Peretz et al 1993, Pugh et al 1995). Those so far described are associated with failure or reduced production of the active protein, and not with production of dysfunctional molecules, unlike most coagulation factor deficiencies.

FXI deficiency in the Ashkenazi Jews is caused by a restricted number of mutation (types II and III) while most mutations remain to be defined in non-Jews. The type II (a stop condon in exon 5 with a change from GAA to TAA that leads to premature polypeptide termination) and type III (a single base change in exon 9 consisting of a change of TTC, coding for Phe$^{283}$ to CTC, coding for Leu) occur with equal frequency in the Ashkenazi population (Asakai et al 1991, Hancock et al 1991). Homozygous patients for type II mutation have very low FXI (< 1 iu/dl) levels, consistent with failure of production of any protein and are likely to bleed extensively from injuries and after surgery. The type III mutation leads to defective dimer formation and secretion (Meijers et al 1992). Homozygotes for this mutation produce a low factor XI (about 10 iu/dl) with a less severe bleeding tendency. Type II/III compound heterozygotes are intermediate in FXI level and clinical manifestation.
This combination is the commonest cause of severe FXI deficiency in Ashkenazi Jews. There is no evidence of a difference in clinical expression between heterozygotes for the type II and type III mutations. The restriction to two principal mutations and the history of the dispersion of Ashkenazi Jews is consistent with a founder effect in the population. Recently the type II mutation (but not the type III) has been found in Iraqi Jews but at a lower frequency (3.3%). This suggests that the type II mutation occurred before the partition of the Jews (the Ashkenazi population is considered to arise from Jews who left the original pool after destruction of the temple in Jerusalem in AD 70) (Shpilberg et al 1995).

2.2.3 - INHERITANCE OF FXI DEFICIENCY AND PRECONCEPTIONAL COUNSELLING

FXI deficiency was initially considered to be an autosomal dominant disorder with variable expression (Rosenthal et al 1955, Campbell et al 1957, Cavins & Wall 1960). However, the availability of the one-stage FXI assay (Rapaport et al 1961a) made distinction between severe (homozygous or compound heterozygous) and partial (heterozygous) FXI deficiency possible (Rapaport et al 1961b, Leiba et al 1965). It was, at that time, thought that heterozygotes have no bleeding tendency (Rapaport et al 1961b) and, therefore, autosomal recessive mode of inheritance was widely reported. However, doubt was cast on this mode of inheritance by the description of families who did not fit this pattern (Campbell et al 1957, Cavins & Wall 1960). The lack of an absolute relationship between FXI levels, bleeding tendency and the presence of bleeding symptoms was noticed by Leiba et al 1965. This was later confirmed by many others and between a third to a half of
heterozygote individuals were shown to bleed excessively (Ragni et al 1985, Bolton-Maggs et al 1988, Brenner et al 1995, Collins et al 1995). In a study of 164 individuals from 20 Jewish and 4 non-Jewish families by Bolton-Maggs et al 1988, the inheritance was confirmed to be autosomal with severe deficiency in homozygotes and partial deficiency in heterozygotes. The FXI level is < 15 iu/dl in homozygous or compound heterozygotes for FXI gene mutation (Bolton-Maggs et al 1988, Hancock et al 1991) and between 15-20 iu/dl and the lower limit of normal range in heterozygotes (Mammen 1983, Bolton-Maggs et al 1988). There is a clear demarcation in FXI levels between homozygotes and heterozygotes but a small overlap between the heterozygotes and normal (Figure 2.5) (Bolton-Maggs et al 1988). The probability of heterozygosity at different FXI levels has been constructed on a chart, from which the chance of an individual being a heterozygote can be calculated if the prior probability is assessed from pedigree (Figure 2.6) (Bolton-Maggs et al 1988). The risk of vertical transmission of severe FXI deficiency can thus be calculated for parents in FXI deficient families during their preconceptional counselling. Because FXI deficiency in the Ashkenazi Jews is caused by type II and type III mutations in most kindreds and these mutations are detected by polymerase chain reaction and restriction enzymes (Asakai et al 1991, Hancock et al 1991), carrier detection and prenatal diagnosis is possible in this population. However, the situation is different in non-Jews because of the larger number of mutations causing FXI deficiency in this group.
Figure 2.5: Derived frequency distribution: A homozygotes, B heterozygotes, C normal individuals. The probability of an individual with a factor XI level $x$ belonging to the heterozygote distribution (B) rather than the normal (C) is $h_1/h_2$. (Bolton-Maggs et al 1988).

![Figure 2.5](image)

Figure 2.6: Probability of heterozygosity at a different factor XI levels where the prior probability is 1:1. (Bolton-Maggs et al 1988).

![Figure 2.6](image)
2.3.4 - ETHNIC DISTRIBUTION AND FREQUENCY

FXI deficiency was originally described in a Jewish family in the USA and was called “haemophilia C” and distinguished from haemophilia A or B by its occurrence in either sex and the absence of spontaneous bleeding (Rosenthal et al 1953). In 1958 Biggs et al suggested that factor FXI deficiency might be more common in Jews than other populations, thereby accounting for the significantly different proportions of FXI deficient patients among all haemophilic individuals observed in series reported from European countries and United States (Rapaport et al 1961b). Several instances of vertical transmission of severe FXI deficiency in non-consanguineous Ashkenazi Jewish kindreds (due to homozygote-heterozygote mating) provided the first suggestion that the gene frequency in this population might be extremely high as was later proven (Seligsohn 1978). The frequency of the heterozygous state in the Ashkenazi population (i.e. those who originated from Eastern Europe and migrated away from Jerusalem to Poland and the Baltic states during the first century) is 8% (Seligsohn 1993) and it is therefore one of the commonest genetic disorders in this population. With this frequency it is relatively easy for two unrelated heterozygotes to marry and produce a severely deficient individual, or for vertical transmission of severe deficiency to occur if a severely deficient individual has marries a heterozygote (Bolton-Maggs et al 1988).

In the UK, FXI deficiency was responsible for 4.9% of all patients with bleeding disorders on the Haemophilia Centre Directors’ national register in 1997. A significant number of them have no known Jewish roots but its frequency in non-Jews is unknown. As patients with severe or partial FXI deficiency do not suffer from
spontaneous bleeding but may do so only after haemostatic challenge, this bleeding disorder is probably under-diagnosed.

2.3.5 - CLINICAL MANIFESTATIONS

FXI deficiency can be divided into “severe” and “partial” deficiency according to the FXI level. There is a variable bleeding tendency provoked by surgery and accidents which may be manifest in both severe and partial deficiency. Individuals with severe FXI deficiency (FXI less than 15-20 iu/dl) are homozygotes or compound heterozygotes for the FXI gene mutation. Partial deficient patients are heterozygotes and have FXI levels between 15-20 iu/dl to the lower limit of the normal range. There is no clear consensus in the UK concerning the lower limit of normal. Many hospitals quote 50 iu/dl, however, several recent studies have evidence that 70iu/dl is more representative (Bolton-Maggs et al 1988, Bolton-Maggs et al 1995). In the Royal Free Haemophilia Laboratory a cut-off of 70 iu/dl is used.

Patients with severe FXI deficiency are usually at risk of excessive bleeding after surgery and injury. Bleeding can be brisk at the time of injury or surgery and continue for hours or days unless treated. Alternatively, bleeding can begin several hours after injury and persist as oozing for many days (Seligsohn 1993). Paradoxically, however, some patients with severe deficiency do not have a bleeding tendency (Egeberg 1962, Edson et al 1967, Rimon et al 1976, Aghai et al 1984). In contrast to haemophilia A and B, spontaneous bleeding is not usually a feature, but can occur. Massive haemothorax (Campbell et al 1957), cerebral haemorrhage (Henry & Rosenthal 1956), subarachnoid haemorrhage (Slade & Rabiner 1973) and haematuria (Leiba et
al 1965) have been reported. Spontaneous haemarthroses are rare but have been reported, including individuals with partial deficiency (Bairey et al 1991). Menorrhagia has been shown to be associated with FXI deficiency (Cavin & Wall 1960, Leiba et al 1965, Philips et al 1965, Purcell & Nossel 1970, Hellstern et al 1985, Zacharski & French 1987, Bolton-Maggs et al 1988). Others have excluded menorrhagia as a relevant symptom (Ragni et al 1985, Litz et al 1998). However, assessment of menstrual loss and diagnosis of menorrhagia in all these studies were subjective.

Early reports (Leiba et al 1965) and recent studies (Bolton-Maggs et al 1995) have shown that some individuals with partial deficiency of FXI may be at risk of bleeding. While most Jewish kindreds will come to attention because of bleeding symptoms in severely deficient patients, the majority of non-Jewish families present in heterozygotes with bleeding. These bleeding symptoms include easy bruising, menorrhagia and bleeding following dental extraction, surgery and child birth.

2.3.6 - BLEEDING TENDENCY AND FXI LEVEL

It is well recognised that there is a poor correlation between the FXI level and bleeding tendency in patients with FXI deficiency (Leiba et al 1965, Ragni et al 1985, Bolton-Maggs et al 1988, Brenner et al 1995, Collins et al 1995). Some patients with severe deficiency may not bleed at all following trauma, while some heterozygotes have excessive bleeding after a challenge. The bleeding tendency may also vary in the same individual following haemostatic challenge (Seligsohn 1993). The reasons
for this unpredictable bleeding tendency are not fully understood but some possible factors are:

1. The patient’s genotype: Patients with genotype III/III have a less severe bleeding tendency than genotypes II/II or II/III (Seligsohn 1993).

2. The role of fibrinolysis at sites of the surgery/injury: surgical bleeding in factor FXI deficiency provoked by dental extraction, tonsillectomy, adenoidectomy and nasal and prostatic surgery. These are areas with increased fibrinolysis which clearly enhances the bleeding tendency (Sidi et al 1978, Zacharski & French 1978, Seligsohn 1993). However, severe bleeding has occurred after many other types of surgery, including appendectomy (Rosenthal et al 1955, Nossel et al 1966b, Purcell & Nossel 1970, Zacharskai & French 1978) and excision of breast lumps (Philips et al 1965, Nossel et al 1966b).

3. The presence of additional coagulation factor defects, most commonly vWD: several co-incidental cases of haemophilia A or von Willebrand disease have been reported with FXI deficiency (Bolton-Maggs et al 1995). A study from Israel suggested that most of the bleeding in partial FXI deficiency could be explained by the presence of associated vWD (Tavori et al 1990). In the UK, there is a significantly lower vWF level in Jewish and non-Jewish partially deficient patients with a bleeding history compared to non-bleeders, however, there is no increased incidence in vWD over that expected in the general population (Bolton-Maggs et al 1995).

4. Platelet defects and platelet FXI: platelet FXI can be detected in some severely FXI deficient patients who do not bleed, while in “bleeders” it was not detected (Walsh 1972). Alternative activation pathways for FXI involving platelets have also been proposed (Walsh et al 1993). These findings need to be confirmed and platelet FXI
quantified. Platelet defects have also been reported in some patients with FXI deficiency (Winter et al 1983, Peter et al 1995). In a study in the UK, no abnormal bleeding times were found in 63 patients with FXI deficiency (Bolton-Maggs et al 1995). However, a recent careful analysis of 27 patients from 18 kindreds led to the identification of 16 various platelet defects (Peter et al 1995).

5. Variant FXI molecules: Evidence for abnormal FXI molecules has been found by immunological assays (Rimon et al 1976, Ragni et al 1985, Saito et al 1985, Bolton-Maggs et al 1995) but discrepancy between FXI clotting activity and antigen is very rare and has been found in only three individuals (Ragni et al 1985, Mannhalter et al 1987).

2.3.7 - DIAGNOSIS

Excessive or prolonged injury-related bleeding or incidental finding of prolonged APTT usually leads to the diagnosis of FXI deficiency (Seligsohn 1978, Ragni et al 1985, Kitchens 1991). All patients with severe FXI deficiency (FXI < 15 iu/dl) have an APTT that is longer than two standard deviations above the normal mean (Seligsohn & Modan 1981). Type II homozygotes have the longest mean APTT values, type III homozygotes have the shortest and type II/III compound heterozygotes have intermediate mean APTT values (Asakai et al 1991). The APTT values of heterozygotes overlap substantially with the normal range (Seligsohn & Modan 1981), therefore the APTT assay is not a good screening test for identification of heterozygotes.
FXI coagulant activity levels in homozygotes or compound heterozygotes are less than 15 iu/dl and correspond very well with FXI antigen levels (Saito et al 1985). In view of the high and equal frequencies of type II and type III mutations in Ashkenazi Jews, the results of FXI activity in severely deficient patients might be helpful in predicting the genotype of a given patient. For example, a FXI activity of < 1 iu/dl would be consistent with type II homozygosity, and a value of 10-15 iu/dl with type III homozygosity. Mean FXI activity levels are significantly lower in heterozygotes than the normal population. However, there is a small overlap between heterozygotes and normal controls (Bolton-Maggs et al 1988), leading to problems in diagnosis of a small but significant proportion of heterozygote patients (Seligsohn 1978). Genotypic analysis of patients with FXI deficiency is possible for most of the mutations so far discovered by polymerase chain reaction and restriction enzymes and this can conclusively differentiate between heterozygotes and normal individuals.

2.3.8 - THERAPUTIC PRODUCTS AVAILABLE FOR THE MANAGEMENT OF FXI DEFICIENCY

FRESH FROZEN PLASMA (FFP)

FFP was the mainstay of treatment until the recent advent of FXI concentrates. The main disadvantages of FFP are the large volume required, the possibility of allergic reactions and the potential risk of transmission of infectious agents. FFP has now been made safer with the development of virally effective products, either by pooled solvent/detergent treated or single donor units treated with methylene blue. The half-life of the solvent/detergent treated product has been calculated to be 45 h, similar to standard FFP (Inbal et al 1993b). However, other studies have reported a rather
variable FXI content in this product with some batches containing only 35-50 iu/dl (Smith 1996). It is also possible to pasteurise pooled FFP (thawed at 30 °C then heated at 60 °C for 10 h) with preservation of 75-95% of FXI activity (Burnouf-Rdosevich et al 1993). FFP has been used for many years and is effective. In a study at the Royal Free hospital, FFP was used for 38 surgical procedures including 18 dental extractions with bleeding occurring in only one patient (Collins et al 1995). The patient had a FXI concentration of 11 iu/dl and bled on day 13 after dilatation and curettage in spite of receiving FFP for 11 days. Because of the reasonable efficacy of FFP and concerns about thrombogenicity in some patients receiving FXI concentrates, there is clearly a place for this product.

FACTOR XI CONCENTRATES

Three types of FXI concentrates have been developed and tried in FXI deficient patients. Two of these; (BPL, Bio Products Laboratory, Oxford) and (Hemoleven, manufactured at Lille, Laboratoire Francais du des Biotechnologies (LFP)) are currently available and provide good treatment for appropriately selected patients. Both products are haemostatically effective and virally safe, but have been associated with some thrombotic events usually in association with pre-existing vascular disease.

Since 1985, BPL has been available on a named patient basis. The BPL product is formulated with a high concentration of antithrombin III (mean 102 iu/ml) thought to protect against any residual XIa. The concentrate is dry heated at 80 °C for 72 hours to inactivate viruses. Bolton-Maggs et al 1992, reported its use in 30 patients aged 7-71 years (on 31 invasive procedures) and demonstrated good haemostatic efficacy
with no significant adverse effects. The mean FXI was 91% of the injected dose with a mean half-life of 52 hours. In vitro work demonstrated some evidence of thrombogenicity in the concentrate which was abolished by addition of heparin (10 iu/ml). The product was therefore modified in 1993 to include heparin. Subsequent to this, however, serious thrombotic events were reported with the use of the product (Bolton-Maggs et al 1994, Collins et al 1995, Biggs et al 1996). At the Royal Free Hospital two severe cardiac complications and an episode of pulmonary embolisation have been reported (Collins et al 1995). One of the patients with cardiac complication and the patient who suffered pulmonary embolus had other risk factors. Cramping calf pains immediately following FXI infusion were reported in another three cases. The LFP product contains 3-5 iu/ml heparin and 2-3 iu/ml of anti-thrombin III, both of these are less than BPL products (Burnouf-Radosevich & Banouf 1992). This product has been available and in use since 1993. Although in vitro testing showed no evidence of thrombogenicity, this concentrate is also associated with coagulation activation in laboratory tests (de Raucourt et al 1995) and clinical sequelae in some patients (Mannucci et al 1994a).

In the light of these concerns the UK Haemophilia Centre Directors' Organisation have issued guidelines for the use of FXI concentrate (UKHCDO 1994). Both LFP and BPL products are haemostatically effective and free from viral transmission, but should be used with caution especially in elderly patients, patients with pre-existing cardiovascular disease and individuals with pre-existing coagulation activation (e.g. pregnant patients, patients with malignant conditions). Care should be taken to avoid doses of more than 30 iu/kg and peak FXI levels in severely deficient patients of more than 50-70 iu/dl. Concurrent use of tranexamic acid or other antifibrinolytic
drugs should be avoided. Treatment with these products should only be carried out in centres experienced in the management of bleeding disorders. It may also be necessary to monitor thrombotic markers in some cases.

Normal pregnancy is associated with major changes in the coagulation and fibrinolytic system with an increased thrombotic potential which is marked around term and immediate post-partum period (Forbes & Greer 1992). This risk is further increased with increased maternal age (a 60 fold increase in risk over age 40 compared to age less 25), parity (Para 4 or more), obesity (> 80 Kg.), pregnancy complicated by preeclampsia, prolonged hospitalisation and immobility, presence of antiphospholipid antibodies, after prolonged labour, instrumental and Caesarean deliveries with greater increase after emergency Caesarean sections (Shaw et al 1993). Therefore, the potential risks and benefits of FFP or FXI concentrate should be carefully assessed individually when FXI replacement therapy is considered. In addition to the long-term complications, exposure to virally contaminated products during pregnancy also has the risk of vertical transmission and fetal infection. Therefore, in younger patients with no additional thrombotic risk factors, FXI concentrate may still be the treatment of choice for invasive procedures during pregnancy, labour and Caesarean sections. However, in patients with high risk of thrombosis treatment with FFP is recommended.

**FIBRIN GLUE**

Fibrin glue is applied through a pair of syringes, one containing calcium and thrombin, the other contain fibrinogen, factor XIII and aprotinin. It has been used successfully for dental extraction without the need for blood products (Rakocz et al
Its use alone or as an additional modality has also been recommended in other surgical procedures such as circumcision and hernia repairs.

**ANTIFIBRINOLYTIC DRUGS**

Antifibrinolytics are effective and important adjuvant in patients undergoing surgery in areas of the body prone to increased fibrinolysis such as the oral cavity, bladder and uterus. It has also been demonstrated that antifibrinolytics alone are sufficient to cover dental extractions in patients with FXI deficiency (Berline et al 1992).

**DDAVP**

As it has been suggested that some heterozygotic FXI deficient patients bleed because they also have mild vWD or have vWF levels towards the lower normal range, DDAVP might be effective. DDAVP has the advantages of safety and ease of use. Castaman et al 1996, have reported its successful use in two symptomatic FXI deficient patients before surgery. Bauduer et al 1998, also used DDAVP with success in another two FXI deficient patients for prophylaxis of surgical bleeding. However, to date, there is little experience with the use of DDAVP in FXI deficiency and more experience is required to establish its role in FXI deficiency.

**2.3.9 - FXI INHIBITORS**

There have been only few reports of antibodies to FXI (Schnall et al 1987, Hender 1990, Ginsberg et al 1993). FXI inhibitors are also a recognised complication of autoimmune disease. The reason of the infrequent presence of inhibitors in FXI deficiency may be that many FXI deficient patients never receive treatment with
plasma products. These inhibitors can cause significant clinical problems, therefore, it is important that prior to elective surgery where plasma products are used all patients are screened for inhibitors in the same way as in haemophilia A or B patients. Some of the inhibitors are associated with residual FXI activity and may be treated successfully with plasma products. Others cannot be overcome and have been treated successfully with products used in patients with inhibitors to FVIII or FIX i.e., prothrombin complex concentrates or recombinant FVIIa.
2.4 MENSTRUATION AND INHERITED BLEEDING DISORDERS

2.4.1 - DEFINITION OF MENORRHAGIA

Menstrual blood loss (MBL) in the general population has been assessed in relatively few large scale studies. In 1966a, Hallberg et al measured menstrual loss in 476 menstruating women, of various ages, in the town of Gothenberg in a cross sectional study. Of these 357 considered themselves as healthy and having a normal menstruation. The data from these women showed that menstrual loss has a positive skew distribution. The mean and median menstrual losses were 38.5 ml and 29.9 ml, respectively and in just over 11% the loss was 80 ml or more (Hallberg et al 1966a). Cole et al (1971) calculated the menstrual loss of 348 women in a Northumbrian mining village by determining the iron contents of their sanitary protections using an atomic absorption spectrophotometry method. A positive skew distribution was also shown in this study with 9.3% losing 80 ml or more blood per cycle. The mean and median menstrual losses were also very similar to the previous study (37.5 ml and 27.6 ml, respectively). In 20% of 421 Chinese women, Gao et al 1987, found menstrual loss in excess of 80 ml. A negative association between serum ferritin and haemoglobin levels with menstrual blood loss was also found.

Menorrhagia has been defined as an MBL of 80 ml or more per period (Hallberg et al 1966b). This was derived by determining the 95th percentile of menstrual loss in 183 women who considered their menstruation as normal with haemoglobin concentration of 12 gm/dl or more, plasma iron concentration of 80 μg/dl and/or mean corpuscular haemoglobin concentration (MCHC) of 30% or more (Hallberg et al 1966b) and this was found to be 76.4 ml. In the same study, the menstrual loss was
related to haemoglobin concentration, plasma iron concentration and MCHC. All haematological parameters decreased when the menstrual loss was between 60-80 ml per period and the decrease was even more evident when the loss exceeded 80 ml. The total iron binding capacity and the number of women without detectable iron stores increase with increasing blood loss. On the basis of these results, the authors calculated that the upper tolerance limit of menstrual blood loss for most women is 60-80 ml per period and a blood loss exceeding 80 ml must be considered pathological i.e. menorrhagia.

2.4.2 - ASSESSMENT OF MENSTRUAL BLOOD LOSS

In clinical practice, assessment of menstrual blood loss is usually subjective and relies on the description provided by the patient. Unfortunately, this is an inaccurate method of assessment as there is lack of correlation between patient’s impression and the objective measurement of actual volume of blood loss (Haynes et al 1977, Chimbria et al 1980, Fraser et al 1984). Therefore, objective assessment is of paramount importance. There are a variety of measurement techniques to quantify menstrual blood loss. As most of these methods require the use of laboratory facilities and technical support, they have never become established as part of routine practice.

The methods described for objective assessment of menstrual loss fall into the following categories:
1. **Weight estimates of sanitary protections:**

This is a simple method of determining the total menstrual fluid loss but is not, specific for blood. The contribution of blood to menstrual fluid has been shown to vary considerably, ranging from 1.6% to 82% (mean 36.1%) (Fraser et al 1985). Contamination with urine could also affect the results of this method (Rankin et al 1962). Therefore, this method is not reliable for assessing menstrual blood loss.

2. **Radioisotope methods:**

Radioisotopic markers e.g. iron (Fe$^{59}$) (Baldwon et al 1961) and chromium (Cr$^{51}$) (Rankin et al 1962) have been used to label venous red blood cells premenstrually, enabling the radioisotopic activity in the peripheral circulation to be measured at the commencement and cessation of the menses. The level of radioactivity is also counted in the collected sanitary protection used by the patient and the quantity of menstrual blood is then calculated from the mean venous and menstrual blood radioactivity. The problem with these radioisotope methods is that they are invasive and there is usually reticence to administer and to receive these compounds, in spite of the small doses involved.

3. **Iron determination:**

The determination of the quantity of iron in sanitary protections using wet-oxidation and dry ashing procedures (Thomas 1970) and atomic absorption spectrophotometry (Cheyne & Shepherd 1970) has been described to assess menstrual blood loss. However, these methods are time consuming and costly.
4. **Haemoglobin determination:**

The haemoglobin content of menstrual collections is converted to a coloured, haematin compound in alkaline solution (alkaline haematin method) and used to estimate menstrual blood loss. This colourimetric method was described by Hallberg & Nilsson in 1964 and is currently the most reliable, widely used technique of assessing menstrual loss and has been used in many studies. Sanitary towels and tampons used by the patient are collected and blood is extracted by soaking the tampons and pads for 48 hours in a measured volume of 5% of sodium hydroxide solution. An aliquot of sodium hydroxide solution is then centrifuged and the optical density at 546 nm measured by spectrophotometry and compared with that of a known concentration of a sample of patient’s venous blood collected at the end of the menstrual period. The total menstrual loss is expressed in terms of an equivalent volume of venous blood. The method has a number of disadvantages. Large volumes of sodium hydroxide solution are required to extract blood in a process that takes 20-24 hours. Sanitary protections require thorough squeezing and rubbing by hand till all coloured spots disappear and the process produces noxious odours requiring a fume cabinet and isolation from other laboratory personnel. The use of an automatic extractor for mechanical pummelling that shortens the extraction time to 30 minutes has been described by Newton et al 1977. Another method that uses a detergent to extract blood and sodium carbonate to convert haemoglobin to alkaline haematin has recently been described (Gannon et al 1996). The entire procedure can be carried out in less than 30 minutes.
6. **Pictorial blood assessment chart (PBAC):**

This chart (Appendix 2) was devised (Higham et al 1990) in an attempt to create a simple non laboratory method of assessing menstrual blood loss that is more accurate than other simple methods such as tampon and pad counting and the weighing of such blood-stained materials. In addition to recording the number of the towels and tampons used, the degree to which individual items are soiled is also taken into consideration. This factor is essential, as women use a widely varying quantity of sanitary protections to collect similar amounts of menstrual loss (Fraser et al 1984). Passage of clots and episode of flooding during the period are also recorded in this chart. A special scoring system (Appendix 2) is used and taking a score of 100 or more to be equivalent to blood loss of > 80 ml i.e. diagnostic of such menorrhagia, this chart was found to have a sensitivity of 86% and a specificity of 89%, using scores used by the women. The sensitivity and specificity were 86% and 81%, respectively, using the gynaecologist’s score (Higham et al 1990). The relation between the scores recorded by the gynaecologist and the measured MBL is shown in Figure 2.7 (Higham et al 1990). Good acceptance among the patients and simplicity of its use was also reported in the same study. Due to reasonable accuracy of this method and to avoid expensive and time consuming laboratory methods as well as the inconvenience to the patients of collecting menstrual pads and tampons, this chart was used in our studies for assessment of menorrhagia.
2.4.3 - MENSTRUATION AND BLEEDING DISORDERS

Haemostasis in the menstruating uterus is the result of a delicate balance between platelet aggregation, fibrin formation, vasoconstriction, and tissue regeneration on one hand, and prostaglandin induced platelet inhibition, vasodilatation, and fibrinolysis on the other (Christiaens et al 1982). Haemostasis in the endometrium differs from that in human skin by the relative scarcity of haemostatic plugs and by the complete intravascular location of these plugs. During the first 20 hours of menstrual bleeding, numerous thrombi are seen in the endometrium. After 20 hours of the onset of bleeding, most of the functional layer is shed and no more thrombi are seen (Christiaens et al 1980). It is therefore obvious that haemostatic plug formation plays an important role in uterine haemostasis during menstruation (Christiaens et al 1982) and not surprisingly there is an increased frequency of menorrhagia in patients with primary haemostatic disorders (e.g. vWD, thrombocytopenia). However, women
with these coagulation disorders bleed heavily during the whole menstruation and not only the first 20 hours when haemostatic plug is usually seen. A possible explanation is that thrombus formation itself promotes tissue shedding. Diminished plug formation could then lead to prolonged endometrial shedding and, as long as shedding is incomplete, further haemostatic plugs are required for shedding arrest (Christiaens et al 1982). This mechanism has also been described in menorrhagia associated with intra-uterine contraceptive devices (Christiaens et al 1981).

Activation of the coagulation cascade (secondary haemostasis) results in the stabilisation of the primary haemostatic plug by fibrin formation from fibrinogen by action of thrombin. Thrombin is formed from prothrombin through the activation of several coagulation factors in the coagulation cascade. Therefore, deficiencies of any of the coagulation factors in the cascade may also be associated with menorrhagia. Heavy menstruation has been reported in carriers of haemophilia A or B (Lusher & McMillan 1978, Greer et al 1991) and prothrombin, fibrinogen, factor V, factor VII, factor X (Silwer 1973, Mariani & Mazzucconi 1983, Roberts & Lozier 1991, Peyvandi et al 1997) and FXI deficient women (Bolton-Maggs et al 1995). Factor XIII is important in the final stage of haemostasis as it helps polymerisation and cross-linkage of fibrin and it has also been associated with menorrhagia (Roberts & Lozier 1991).

Menorrhagia is common in women suffering from bleeding disorders, particularly adolescent girls (Zimmerman & Ruggeri 1987), and can be the presenting symptom. Acute adolescent menorrhagia requiring urgent medical intervention has long been recognised to be associated with undiagnosed underlying bleeding disorders. A
primary coagulation disorder was found in almost 20% of 59 adolescents with such menorrhagia (Claessens & Cowell 1981a) and screening for vWD and platelet disorders has been recommended in these patients (Claessens & Cowell 1981b, Ward 1992). However, the prevalence of bleeding disorders in older women with menorrhagia seems to be under-estimated and has not been extensively investigated. There has been only one study to assess the frequency of vWD in these women (Edlund et al 1996). In this study (Edlund et al 1996), 6 of 30 (20%) women with objectively verified menorrhagia were found to have mild vWD and in 2 of them menorrhagia was the only bleeding symptom. In this thesis (Chapter 7) the prevalence of inherited bleeding disorders in a large number of patients with objectively confirmed menorrhagia is assessed.

The high prevalence of menorrhagia among women with vWD has been reported in several studies. Nilsson (1974) noted that 50% of women with mild vWD suffer from profuse menstrual blood flow. Evans (1971) also noted that among 6 patients with vWD admitted to a gynaecology department over a 5 year period, 5 were for management of menorrhagia and in 3 of them hysterectomy was required. Similarly, in a retrospective study of 8 patients with vWD, it was found that all of them had been referred to a gynaecologist at some time because of heavy menstruation (Greer et al 1991). In a survey of 99 patients with type 1 vWD from four haemophilia centres in the United States, 78% reported their periods to be heavy, 71% of whom required medical attention and 15% who required hysterectomy (Kouides et al 1997). In carriers of haemophilia, subjective menorrhagia has also been reported (Lusher et al 1978) although in the study by Mauser Bunschoten et al (1988) there was no significant difference in the percentage of carriers who considered their menstrual
loss to be greater than other women compared to a reference group. Women affected with FXI deficiency, including those with partial deficiency, are also more likely to have menorrhagia than their unaffected relatives (Bolton-Maggs et al 1995). In this study 19/46 (41%) of FXI deficient women reported symptoms usually indicative of menorrhagia compared with 6/33 (18%) of their non-deficient relatives. However, in all these studies the diagnosis of menorrhagia has been subjective and there has only been one study where objective assessment of menstrual blood loss was performed in patients with coagulation disorders (Fraser et al 1986), including only 2 patients with vWD and 2 carriers of haemophilia. In Chapter 8 of this thesis menstrual blood loss and the prevalence of menorrhagia are objectively assessed among women with inherited bleeding disorders.

Menstruation can be a cause of embarrassment and inconvenience to many women and has a major influence on women’s lifestyle and employment. As a consequence of changes in the pattern of family life, modern women experience approximately ten times the number of periods experienced by their ancestors (Short 1976). Women are also increasingly involved in activities outside the home, where episodes of excessive menstrual loss and flooding can be especially inconvenient. Therefore, in addition to its medical implications, excessive menstrual loss can be a debilitating social problem for women.

Women’s experience of menstruation has been assessed in a Swedish survey of 2200 women (Edlung et al 1994). Thirty percent of women included in this survey reported that they had to refrain from social activities because of their period ‘sometimes’, ‘quite often’ or ‘every month’. Some 30% of the women planned social activities
with their period in mind. The corresponding figures in the subgroup of women who
described their menstruation as excessive was 50% and 57%, respectively.
Therefore, menstruation may be a source of inconvenience to women in general, but
significantly more so for women with excessive blood loss. Excessive menstrual loss
may also have major financial implications. It has been estimated that excessive
menstrual loss can lead to more than 300 000 sick days per year in all women of
reproductive ages in Sweden (Edlung et al 1994). Women’s estimates of the expense
per menstruation is reported to be £6 and £8 in those with normal or excessive
menstruation, respectively (Edlung et al 1994).

To improve the quality of life and reduce the high number of surgical gynaecological
interventions among these women, increased awareness among caregivers of the high
frequency of menorrhagia, objective assessment using simple pictorial charts and the
available medical treatments is essential. In this thesis, quality of life during
menstruation in women with inherited bleeding disorders and its effect on their
employment and social activities are also assessed (Chapter 8).

2.4.4 - TREATMENT OF MENORRHAGIA IN WOMEN WITH INHERITED
BLEEDING DISORDERS

Menorrhagia in women with inherited bleeding disorders is usually due to their
clotting factor deficiency. However, each individual should be appropriately assessed
and local causes excluded, especially the possibility of malignancy in older women.
The treatment is then usually medical and the options are tranexamic acid, combined
oral contraceptive compounds, or more recently intra-nasal DDAVP spray. Cyclical
progesteragens are widely used in the treatment of dysfunctional uterine bleeding despite there being limited evidence to support this. They are only effective in women with anovulatory dysfunctional uterine bleeding. However, most women with dysfunctional uterine bleeding show no evidence of hormonal imbalance and have regular ovulatory cycles (Haynes et al 1977, Bonnar & Sheppard 1996). This treatment modality may be used as a second choice in patients with bleeding disorders not responding to the above treatments or when they are contraindicated. Other medical treatments including danazol and gonadotropin releasing hormone agonists are used with reasonable efficacy in the treatment of menorrhagia in general. However, experience regarding their effectiveness in women with inherited bleeding disorders is lacking in the literature. In addition, the side effects and risks associated with a long-term hypo-oestrogenic state make them unacceptable for long term usage.

Non-steroidal anti-inflammatory drugs are used successfully to reduce menstrual blood loss in women with primary menorrhagia i.e. dysfunctional uterine bleeding (Mäkäräinen & Ylikorkala 1986, Bonnar & Sheppard 1996). However, they are ineffective and may increase the menstrual blood loss in the treatment of menorrhagia in patients with underlying bleeding disorders (Mäkäräinen & Ylikorkala 1986). It has been demonstrated that although the production of prostacyclin (PGI₂, vasodilator and anti aggregatory agent) and thromboxane A₂ (vasoconstrictory and proaggregatory agent) are normal in the endometrium of patients with primary menorrhagia, the balance is shifted to a relative thromboxane deficiency (Mäkäräinen & Ylikorkala 1986). Non-steroidal anti-inflammatory drugs are non-selective i.e. suppress the production of both PGI₂ and thromboxane A₂. However, it has been shown that this may not necessarily be true in uterine
vasculature (Powell & Chan 1984). In addition, in normal individuals inhibition of prostaglandin production in the uterine vasculature outweighs the antiaggregatory effects of these drugs on platelet function. These features explain the otherwise paradoxical clinical success of non-steroidal anti-inflammatory drugs in the treatment of primary menorrhagia.

Anti-fibrinolytic agents have a beneficial role in the management of menorrhagia as fibrinolytic activity increases during menstruation (Cederblad et al 1977, Hahn et al 1976). Dockeray et al 1987 found a significant reduction in plasminogen activator activity and plasmin activity in the menstrual fluid with tranexamic acid. It has also been shown that tranexamic acid significantly reduces endometrial tissue plasminogen activator activity and antigen (Gleeson et al 1994) and reduces menstrual blood loss in patients with menorrhagia in general (Gleeson et al 1994, Bonnar & Sheppard 1996) as well as in patients with inherited bleeding disorders (Bonnar et al 1980). Bonnar & Sheppard (1996) randomised 76 women to one of three treatments: ethamsylate (a general haemostatic agent), mefenamic acid (a prostaglandin synthetase inhibitor) and the fibrinolytic agent, tranexamic acid at a dose of 1 g q 6 h hourly. Menstrual loss measured by the spectrophotometric method in three control menstrual periods and three menstrual periods during treatment showed that there was no reduction in menstrual blood loss with ethamsylate while there was a 20% reduction with mefenamic acid, and there was a 54% reduction with tranexamic acid. The bioavailability of tranexamic acid is only about 35% (Pilbrant et al 1981), which makes frequent administration of high doses (at least 1 g four times daily) necessary and this may reduce patient compliance. However, there is a recent report of the successful use of single high-dose antifibrinolytic therapy
(tranexamic acid at a dose of 4 g orally) in three type 2A and 2B vWD patients (Ong et al 1998). Anti-fibrinolytic therapy is safe and far less expensive than other treatment modalities and is certainly worth trying as a first line especially in DDAVP non-responsive patients with menorrhagia (i.e., type 2 and 3 vWD patients) prior to using a vWF-containing concentrate.

Combined oral contraceptives (OCPs) increase FVIII:Ac and vWF:Ac (Schiffman & Rapaport 1966, Glueck & Flessa 1972, Alperin 1982), as well as controlling the menstrual cycle. These are therefore effective, and are currently the most commonly used treatment of menorrhagia in patients with inherited bleeding disorders. However, the response has been reported to be variable and unpredictable in vWD patients (Alperin 1982, Mannucci 1997b). Interestingly, in a survey of type 2 and 3 vWD patients by Foster et al (1995), 88% of 25 women treated with OCPs stated that it was effective. On the other hand, in type I vWD patients, a standard dose of OCPs was effective only 24% of the time while high-dose oral contraceptive therapy was effective only 37% of the time (Kouides et al 1997). Occasionally, a girl with vWD will present at the menarche, or shortly afterwards, with marked, sometimes life-threatening, menorrhagia. In these cases, collaboration between the gynaecologist and haemophilia specialist is essential. Hormonal treatment such as norethisterone (10 mg three times a day) together with DDAVP and/or vWF-containing concentrates is usually required. Once control of bleeding is achieved, a combined oral contraceptive is prescribed.

Home therapy for menorrhagia with DDAVP is now possible with the advent of intranasal formulations (Rose & Aledort 1991, Lethagen & Ragnarson 1993,
Seremetis & Aledort 1997) and subcutaneous forms (Rodeghiero et al 1996, Mannucci 1997a). DDAVP administered intra nasally as a spray has been shown to increase plasma levels of FVIII and vWF in patients with mild haemophilia A or vWD type 1 (Lethagen et al 1990, Rose & Aledort 1991). It is effective when used prophylactically for minor procedures or for the treatment of bleeding episodes in these patients (Rose & Aledort 1991). Its use in the management of menorrhagia was described by Lethagen & Ragnarson in 1993. In 1997, a phase IV, multi-centre prospective trial of DDAVP nasal spray for treatment of menorrhagia was undertaken in 68 patients with type 1 vWD and 15 carriers of haemophilia A (Kobrinsky & Goldsmith 1997). The women used an interactive voice response technology system to register whether the treatment was effective (excellent or good response) or had minimal or no response. Among the 552 and 151 daily efficacy ratings, response was considered effective in 92% and 95% of the time in vWD patients and carriers of haemophilia, respectively. In the Italian multicentre study of home use of subcutaneous DDAVP, 14 vWD females used home therapy for a total of 43 episodes. In these patients, 65% of the responses were considered by the patient to be "very effective", 21% "effective" and 14% as "not effective". Interestingly, in that last subgroup, in-hospital treatment was not sought when the response was considered as "not effective" (Rodeghiero et al 1996).

Despite the apparent efficacy for home use of DDAVP, the assessment for response in these studies has been subjective. Objective correlation with menstrual blood loss had not been done. At present, there is no consensus regarding the optimal frequency and duration of treatment. Since 90% of all menstrual flow is in the first 3 days (Janssen et al 1995), it seems reasonable to give DDAVP nasal spray during the first
2-3 days, however, the frequency of administration is yet to be determined. In the phase IV intra-nasal DDAVP study, patients self-administered DDAVP on average for 1.8 days of each menstrual cycle (Kobrinsky & Goldsmith 1997). A study of objective assessment using pictorial menstrual chart in response to nasal DDAVP spray is presented in Chapter 9.

Hormone releasing intra-uterine systems, originally developed for use as a contraceptive, have now been shown to be highly effective in reducing menstrual loss in pre-menopausal women. The system currently licensed in the UK for contraception is the levonorgestrel intra-uterine system, Mirena (LNG IUS). In a study by Andersson & Rybo (1990), menstrual loss was significantly reduced in women with dysfunctional menorrhagia (menstrual blood loss of 80 ml or more per period). After 3 months usage of LNG IUS, there was an 85% reduction in menstrual loss and 97% after 12 months as measured by extraction of blood and there was also a significant increase in serum ferritin in the first year of use. This system is now increasingly used on a ‘named patient basis’ for non-contraceptive indications and the recent Government funded Effective Health Care Bulletin (The management of menorrhagia) stated that data from Scandinavia pointed to the effectiveness of this device as a first line treatment for menorrhagia. Data regarding their use and effectiveness in menorrhagia due to bleeding disorders are lacking from the literature. Due to the high local level of progestagens, the use of these hormone releasing systems is associated with suppression of endometrial growth and spiral arterioles as well as capillary thrombosis. In addition, it has also been shown that there is no effect on endometrial factor VIII activity which is reduced by ordinary coils (Zhu et al 1991). Therefore, it is reasonable to believe that they will be as effective in reducing
menstrual blood loss in women with bleeding disorders as in those with dysfunctional menorrhagia. This needs to be confirmed by appropriate studies.

Surgical intervention is sometimes required in patients unresponsive to medical treatment. Surgical procedures, even relatively minor operations such as hysteroscopy and/or diagnostic curettage, can be complicated by haemorrhage in patients with inherited bleeding disorders. Therefore, good liaison between the local haemophilia centre and the surgical/anaesthetic team is essential. Patients' factor levels should be checked preoperatively and adequate haemostatic cover provided with the aim of maintaining the clotting factors $> 50$ iu/dl and $> 30$ iu/dl for major and minor surgery, respectively, until healing is complete. The treatment may need to be continued post-operatively, sometimes for up to 10 days to reduce the development of secondary haematomas. Any surgical intervention should be carried out by a senior gynaecologist; a technique with least risk of bleeding should be chosen; bleeding vessels should be ligated and not cauterised as oozing can occur after surgery; and the use of surgical drains should be considered. Endometrial ablative techniques are increasingly used for management of menorrhagia not responding to medical treatment. These procedures, in particular endometrial resection, are associated with a risk of bleeding complications. It is therefore sensible to choose thermal or Laser ablation in these patients.

It is also important to remember that excessive bleeding may be surgical rather than a result of a failure of adequate replacement therapy. Monitoring post-operatively is continued depending on the nature of the operation and the patient's factor levels. In contrast, unexplained operative and post-operative bleeding that does not respond to
general measures should alert the gynaecologist to the possibility of bleeding
disorders as a causative factor.
2.5 DESMOPRESSIN (DDAVP) AND INHERITED BLEEDING DISORDERS

2.5.1 - HISTORY

DDAVP was first synthesised as an analogue of native hormone vasopressin by Zaoral et al 1967. The chemical structure of DDAVP (1-deamino-8-D-arginine vasopressin) differs from the natural hormone by deamination of homocystein at position 1 and substitution of D-arginine for L-arginine at position 8. (Figure 2.8). This results in greater potency and more prolonged duration of action of the antidiuretic effect and in marked reduction of pressor activity compared to vasopressin. Thus, DDAVP has virtually no vasoconstrictive effect and does not contract the uterus or gastrointestinal tract. DDAVP is metabolised more slowly than vasopressin because it is more resistant to enzymatic degradation (Shimizu et al 1980). The main two sites of metabolic inactivation are the liver and kidney (Walter 1976). This drug was primarily used for its antidiuretic properties in the treatment of conditions such as diabetes insipidus and enuresis.

Figure 2.8 : Structure of DDAVP
The haemostatic properties of DDAVP were discovered after a systematic search for haemostatically active pharmacological agent. In mid 1970s, two independent researchers (Cash et al 1974, Mannucci et al 1975) found that infusion of DDAVP increases plasma concentration of FVIII, vWF and tissue plasminogen activator (tPA) in normal volunteers. This was subsequently found to be dose-dependent and DDAVP was found to exert similar effects in patients with haemophilia A and vWD (Mannucci et al 1977, Nilsson et al 1980). DDAVP has also been shown to reduce bleeding time in a variety of disorders such as congenital and drug-induced platelet dysfunction, uraemia and liver cirrhosis, and it is now the standard drug for treatment of bleeding or as a cover for surgery in many of these conditions.

2.5.2 - MECHANISM OF ACTION

Despite the widespread clinical use of DDAVP for more than 20 years, its mechanisms of action are still not completely understood. There are two known classes of vasopressin receptors; V1 receptors that mediate smooth muscle contraction in the peripheral vasculature and glucose turnover in hepatocytes and V2 receptors that regulate water reabsorption in the collecting ducts of the kidney. DDAVP is a potent V2 receptor agonist with no effect on V1 receptors. The haemostatic effect of desmopressin seems to be mediated by the strong V2-receptor activity since patients with nephrogenic diabetes insipidus, who are unresponsive to V2 agonists, do not have increased FVIII and vWF levels after treatment with DDAVP (Kobrinsky et al 1985, Bichet et al 1988). Anephric patients respond normally (Mannucci et al 1975), indicating that the site of the receptors involved is not the kidney. It is possible that DDAVP-induced release of FVIII and vWF is
mediated by low-affinity, extra-renal V2-like receptors, though the precise location is not known. Proposed sites include endothelial cells, megakaryocytes, blood monocytes and mast cells.

It is most likely that the increase of plasma FVIII and vWF is caused by release from endogenous reservoirs rather than increased synthesis as these factors increase very rapidly, almost immediately, after treatment with desmopressin. The proposed site of release of FVIII is the sinusoid liver endothelial cells (Nachman 1975) and vWF from endothelial cells (Howard et al 1974, Nachman & Jaffe 1975). DDAVP does not appear to act directly on the endothelial cells, as addition of DDAVP to cultured endothelial cells does not result in release of vWF into the culture media (Tuddenham et al 1982). It may either act via a second messenger or require a cofactor that is not present in the cell culture. Another explanation is that the cells may have lost a receptor that is present in vivo. Hashemi et al (1990) found evidence suggesting that platelet-activating factor (PAF) released from monocytes to be responsible for the vWF-releasing effect of DDAVP.

DDAVP has no effect on platelet count or aggregation but enhances platelet adhesion to the vessel wall (Barnhart et al 1983, Sakariassen et al 1984). This effect on platelet function is probably the explanation to the effectiveness of DDAVP in bleeding disorders other than haemophilia and vWD and in patients with normal or even high levels of FVIII and vWF. The mechanism of action responsible for the marked increase of platelet adhesiveness is not fully understood. It has been speculated to be mediated by the quantitative increase of vWF (Sakariassen et al 1984). However, Lethagen & Nilsson (1992) found this DDAVP-induced platelet adhesiveness,
measured with a platelet retention test, to be unaffected by changes in plasma vWF concentrations, e.g. after intravenous infusion of vWF in patients with type 3 vWD. In addition, pregnant women with increased plasma concentration of vWF do not have increased platelet retention. Lethagen & Nilsson (1992) also found that the presence of platelet-vWF and normally functioning platelet receptor glycoprotein (GP) IIb/IIIa seem to be essential for the effect of DDAVP on the platelet. Other putative mechanisms or mediators have been proposed to explain the haemostatic efficacy of DDAVP; e.g. it induces erythrocyte adhesion to the endothelium (Tsai et al 1990) and decreases the endothelial production of 13-hydroxyoctadecadienoic (HODE) that inhibits platelet adhesion to the vessel wall (Setty et al 1992). However, the role of these mechanisms is uncertain and the search for additional or alternative mechanisms is still continuing.

Another effect of DDAVP is release of large amounts of tissue plasminogen activator into plasma (Cash et al 1974, Mannucci et al 1975). This is a short-lived effect and most of the plasmin that is generated by the plasminogen activator is complexed to α2-antiplasmin and does not produce fibrinolysis in circulating blood (Levi et al 1992). Hence, it is usually unnecessary to inhibit fibrinolysis when desmopressin is used for clinical indications.

2.5.3 - MODES OF ADMINISTRATION OF DDAVP

DDAVP can be administered parenterally (by intravenous or subcutaneous injection) or intranasally as a nasal drop or a nasal spray. The most commonly used mode of administration for haemostatic indications is intravenous injection (0.3 μg/kg diluted
in 50 ml of saline infused over 30 minutes). This dosage produces maximal effect on FVIII:C and vWF in healthy volunteers. The mean factor increase is usually about 3-5 times above the basal levels with peak levels after about 30-60 minutes (Mannucci et al 1981b). The response is not increased when DDAVP dosage is increased to 0.4 µg/kg, even though the plasma concentration of DDAVP is dose dependent. This maximal biological response is thought to be due to saturation at receptor sites (Lethagen et al 1987). The response to subcutaneous administration is comparable to intravenous administration with the exception that the plasma concentration of FVIII:C does not peak until about 1-2 hours (Kohler et al 1986).

Intranasal administration is of clinical interest because it is ideal for home use. Intranasal drops administered by rhinyle catheter or nasal pipette are not very effective in raising FVIII/vWF levels and produce poor and unpredictable results. However, intranasal spray formulation results in excellent nasal absorption and bioavailability and pharmacokinetic studies have shown that peak plasma concentration of DDAVP and of FVIII are significantly higher with this formulation than with drops (Harris et al 1988). Intranasal spray administration in a dose of 300 µg gives a slightly lower increase in factor levels (2-3 times above basal levels) in healthy volunteers, but its effect is comparable to an intravenous dose of 0.3 µg/kg for FVIII in patients with haemophilia A or for bleeding time response in those with vWD (Lethagen et al 1995). The reproducibility of the spray effect has been shown to be good with an intraindividual variation of 21% and an interindividual variation of 27%, which compares favourably with the interindividual variation of 23% after 0.3 µg/kg intravenous administration (Lethagen et al 1987).
2.5.4 - PHARMACOKINETICS

The plasma half-life of DDAVP is about 4-5 hours, irrespective of the mode of administration. The disappearance curve of this drug is probably of minor importance for its haemostatic effect as this effect occurs very rapidly and transiently (i.e. a 'hit and run' type of effect). However, it is important for its antidiuretic effect which is considered as a side effect when the drug is used for haemostatic indications.

The half-life of plasma FVIII after DDAVP administration has been found to be about 4-5 hours which is significantly shorter than its half-life after injection of FVIII concentrates. The reason for this short plasma FVIII half-life after DDAVP is not known. However, it is unlikely to be caused by increased proteolytic degradation, as DDAVP does not affect half-life of plasma FVIII in patients given both FVIII concentrates and DDAVP (McLellan et al 1985), but may reflect redistribution of FVIII from plasma to other compartments e.g. storage sites. Table 2.3 represents the recommended dosage, different routes of administration and pharmacokinetics of DDAVP-induced increases in FVIII and vWF.
Table 2.3 - DDAVP: Routes of administration, recommended dosage, pharmacokinetics of FVIII and vWF responses

| Dosage           | ■ Intravenous: 0.3 μg/kg diluted in 50 ml of saline infused over 30 minutes  
|                 | ■ Subcutaneous: 0.3 μg/kg (40μg/ml solution)  
|                 | ■ Intranasal spray: 300 μg (1.5mg/ml solution given with a precompression, metered dose spray pump delivering 100μl containing 150 μg of DDAVP to each nostril)  
| Increase in factor level above the baseline | ■ Mean: 3-5 times  
|                 | ■ Range: 1.5-20 times  
| Time to achieve peak level | ■ Intravenous: 30-60 minutes  
|                 | ■ Subcutaneous: 60-90 minutes  
|                 | ■ Intranasal spray: 90-120 minutes  
| Factor’s plasma half-life | ■ FVIII: 5-8 hours  
|                 | ■ vWF: 8-10 hours  

2.5.5 - DDAVP IN THE MANAGEMENT OF HAEMOPHILIA AND VWD

DDAVP is very useful in the management of haemophilia and vWD as it provides a form of autologous replacement therapy and the use of coagulation factor concentrates can be avoided. However, not all patients with haemophilia and vWD respond to DDAVP. DDAVP has no effect on FIX, therefore it is ineffective in the management of patients with haemophilia B. In haemophilia A and vWD, only patients with measurable levels of FVIII and vWF i.e. mild to moderate
haemophiliacs and type 1 vWD respond to DDAVP (Mannucci et al 1977, Warrier & Lusher 1983), whereas those with unmeasurable levels do not respond at all (Mannucci et al 1976). In haemophilia A, a baseline FVIII concentration of 10-15 iu/dl is generally required to achieve sufficiently high post-treatment FVIII to ensure haemostasis in connection of major surgery or heavy bleeding. As response to DDAVP is consistent in each individual patient, a test dose should be given to ensure a sufficient FVIII response.

In vWD, the majority of patients with type 1 disease respond to DDAVP with an increase in FVIII and vWF that is larger than that seen in haemophilic patients (Mannucci et al 1992). In addition, there is also shortening or normalisation of the bleeding time which is another determinant of clinical efficacy of DDAVP in these patients. In type 2 vWD, although DDAVP induces a quantitative release of vWF, it does not normalise the qualitative vWF defect and, in general, the bleeding time does not decrease significantly in these patients (Mannucci et al 1976, Ruggeri et al 1982). There are, however, a few patients with type 2A vWD in whom DDAVP does shorten the bleeding time (Gralnick et al 1986b). The reason for this different behaviour is not clear and a test dose is the only way to differentiate responders from nonresponders. In type 2B vWD, DDAVP can be potentially dangerous because it causes platelet aggregation and thrombocytopenia due to the release of an abnormal vWF which binds to the platelet receptor GP 1b (Holmberg et al 1983). Although there is some evidence that DDAVP is clinically effective in these patients (Castaman & Rodeghiero 1996) most haematologists would be reluctant to use it. Finally, patients with vWD type 3 do not produce vWF and, therefore, fail to respond to DDAVP.
DDAVP may also have a haemostatic effect and is used in the management of patients with acquired haemophilia and vWD. However, patients with high antibody titres and no measurable FVIII do not respond and no haemostatic effect is expected.

2.5.6 - OTHER THERAPEUTIC USES OF DDAVP AS A HAEMOSTATIC DRUG

DDAVP has been shown to shorten or normalise the bleeding time in a variety of disorders with prolonged bleeding time. Most forms of congenital, except Glanzmann's thrombasthenia (Lethagen & Nilsson 1992), and acquired (e.g. drug-induced, secondary to uraemia or liver cirrhosis) platelet dysfunction respond well to DDAVP (Kobrinsky et al 1984, DiMichele & Hathaway 1990, Lethagen & Nilsson 1992). Kim et al 1988 also found DDAVP to have a beneficial effect in patients with prolonged bleeding time of unknown aetiology. DDAVP has also been used in blood donors prior to blood collection to increase the yield of FVIII for the production of FVIII concentrates. Finally, DDAVP has been suggested to have a beneficial role as a blood saving agent during surgical operations in which blood loss is large and multiple blood transfusions are required e.g. open-heart surgery (Salzman et al 1986). However, this role of DDAVP at the moment appears doubtful as it has been debated by other investigators (Rocha et al 1988, Hackman et al 1989).

2.5.7 - TACHYPHYLAXIS

Repeated doses of DDAVP at short intervals has been reported to cause decreasing response (tachyphylaxis) in some patients with haemophilia A or vWD (Mannucci et
al 1992), perhaps because stores are exhausted. The average FVIII response obtained when desmopressin is repeated three to four times at 24 hour intervals are approximately 30% less than those obtained after the first dose (Mannucci et al 1992). The decreased FVIII response appears to be more common among haemophilic patients than those with vWD. The latter group may only show slight reduction in bleeding time's response (Mannucci et al 1992).

Tachyphylaxis is rarely a problem in the clinical management of haemophilia or vWD as only one or a few doses of DDAVP are required for most bleeds or surgical procedures. Even if prolonged DDAVP treatment is given, tachyphylaxis is seldom a problem. However, in such cases the treatment should be closely monitored and it may become necessary to use plasma-derived or recombinant factors, or to supplement DDAVP with them.

2.5.8 - ADVERSE EFFECTS AND CONTRAINDICATIONS

DDAVP has few side effects, the most common being facial flushing. Other less common side effects are headache (usually mild and transient), nausea and fatigue. A small reduction in systolic and diastolic blood pressure and an increase in heart rate are commonly seen. Because of its potent antidiuretic effect and the large doses needed for its haemostatic efficacy (15 times greater than for diabetes insipidus), DDAVP carries the risk of water retention. However, this is not a prominent clinical problem and very few cases of severe fluid overload have been reported. Nevertheless, there have been several case reports of hyponatraemia and seizures after treatment with DDAVP (Lowe et al 1977, Beach et al 1992, Humphries &
Most cases occurred in children, usually less than five years old, treated with repeated and frequent doses of DDAVP or in those receiving hypotonic intravenous fluids. Therefore, DDAVP should be used with caution in very small children and in patients with congestive heart failure with strict monitoring of fluid intake. The antidiuretic effect of a single dose lasts for 24 hours, with no difference in magnitude between intravenous or intranasal doses. The effect is prolonged as long as the doses are repeated. Serum sodium is marginally affected by single doses, but tends to decrease after four repeated doses at 12 hour intervals. Therefore, if DDAVP is repeated for a period of 48 hours or more, fluid intake should be restricted to 2 litres per day in adults (Lethagen et al 1998).

There are anecdotal reports of arterial thrombosis occurring in haemophilic (Bond & Bevan 1988) and uraemic (Byrnes et al 1988) patients treated with DDAVP, but no thrombotic episodes have been reported in patients with vWD treated with this drug. In connection with its use in surgery, a review of 31 clinical trials of DDAVP given to patients undergoing cardiac, orthopaedic or other major surgical interventions revealed no significant difference in the frequency of thrombosis between DDAVP-treated and placebo-treated patients (Mannucci et al 1994b). Thus, DDAVP would not seem to be associated with any increase in the risk of thrombosis, however it is recommended that this drug should be used with caution in elderly patients with atherosclerotic disease.

Thus DDAVP has an important role in the management of both congenital and acquired bleeding disorders. It is efficacious, relatively inexpensive compared to factor concentrates and is safe with very few contraindications. DDAVP is
contraindicated in cases of unstable angina or severe congestive heart disease due to its antidiuretic effect. In type 2B vWD DDAVP is contraindicated because it may cause platelet aggregation and thrombocytopenia.

2.5.9 - DDAVP IN WOMEN WITH BLEEDING DISORDERS

DDAVP has an important role in prophylaxis and the treatment of bleeding symptoms in carriers of haemophilia A, women with mild vWD and platelet dysfunction. These women often suffer from frequent spontaneous bleeding, which may have a detrimental effect on their daily life. Heavy and frequent nose bleedings or profuse menstrual bleeding may impede school attendance or impair work capacity. DDAVP nasal spray is an attractive treatment option for these women. They can treat themselves without delay and the effect is virtually immediate. The spray comes in handy size (2.5 ml) for taking along to school and work and can easily be administered. The optimal effect of DDAVP would be obtained by giving it immediately in the event of the bleeding episode, thus shortening its duration and avoiding patient’s absence from school or work to attend out-patient clinics for treatment (Lethagen & Ragnarson 1993). DDAVP in the management of menorrhagia is discussed in detail in Chapter 2.4 and in this thesis, its efficacy has been assessed in a prospective randomised placebo controlled trial (Chapter 9).

2.5.10 - DDAVP AND PREGNANCY

The use of desmopressin in pregnancy is controversial. Most haematologists and obstetricians are reluctant to use it during pregnancy because, theoretically, DDAVP
can cause uterine contractions and preterm labour. However, DDAVP is very specific to V2 receptors and has little effect on smooth muscle V1 receptors and consequently does not cause uterine contraction (Mannucci et al 1988). Theoretically, desmopressin may cross the placenta to the fetus which could cause neonatal hyponatraemia if given immediately before birth. However, this has not been seen in clinical practice. The other concern regarding antenatal use of this drug is decreasing blood flow from the placenta causing intra-uterine growth retardation. However, its vasopressor effect is very weak. Reproduction studies performed in rats and rabbits have revealed no evidence of harm to the fetus. There are also several publications on the management of diabetes insipidus (Burrow et al 1981, Linder et al 1986) and Ehlers-Danlos syndrome (Rochelson et al 1991) in pregnant women with no harm to the fetus. Recently, Ray (1998) reviewed 53 cases of antepartum use of DDAVP for the management of diabetes insipidus in 20 published articles and showed that it was not associated with prematurity, low birth weight or any serious adverse effect on maternal health or neonatal well being. However, the average daily dose of DDAVP used in these cases was approximately 29 µg intranasally (range 7.5-100 µg), which is smaller than the dose required for haemostatic purposes. While the theoretical risk of uterine contraction may be a contraindication to use during pregnancy this should not be a problem for a patient in labour, which is the usual time that DDAVP would be required.
CHAPTER 3

THE OBSTETRIC EXPERIENCE AND OUTCOME OF CARRIERS OF HAEMOPHILIA

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3.1 : INTRODUCTION

Haemophilia A and B are uncommon conditions with a prevalence in the general population of 1-2/10,000 and 1-2/100,000, respectively (Forbes 1984). The advent and appropriate use of clotting factor concentrates have dramatically improved the life expectancy and quality of life of patients with clotting factor deficiencies. This increased longevity and the obvious improvement in the general and reproductive fitness of haemophilic men over the last 40 years has resulted in an increase in the birth of daughters who are obligate carriers.

There are few studies addressing the haemorrhagic problems for haemophilia carriers and their fetuses in pregnancy and delivery. The Royal Free Hospital has a large, comprehensive care centre for haemophilia and many carrier women have their pregnancy care at this hospital. The aim of this study is to assess pregnancy outcome with particular regard to bleeding problems in both mother and fetus.
3.2 : PATIENTS AND METHODS

Thirty two haemophilia carriers (24 haemophilia A, 8 haemophilia B) registered at the Royal Free Hospital Haemophilia Centre who had obstetric care at this hospital over a 10 year period (1985-1995) were investigated. Twenty four were known to be obligate carriers, carrier status was suspected in 6 of the women during the antenatal period and was confirmed in the post-natal period, and diagnosis of carrier status was made in the remaining two after the birth of an affected male.

These women had a total of 82 pregnancies and both the Haemophilia Centre and maternity case notes were reviewed with particular emphasis on the obstetric experiences and coagulation status during pregnancy and the puerperium. The information recorded included:

1. Baseline (non-pregnant) clotting factor levels and any changes in pregnancy.
2. Uptake and results of prenatal diagnosis.
3. Occurrence of bleeding during pregnancy and post-partum period including primary post-partum haemorrhage (PPH) (blood loss in excess of 500 ml during the 24 hours after the birth of the infant) and secondary PPH (bleeding in excess of normal lochial loss after first 24 hours of delivery to 6 weeks).
4. Mode of delivery including indications for instrumental deliveries and Caesarean sections.
5. Neonatal outcome with special emphasis on haemorrhagic complications and impact of invasive monitoring techniques and mode of delivery on these complications.

Paired t-tests were performed to assess whether the changes in clotting factor levels during pregnancy were significantly different from zero.
3.3 - RESULTS

Prenatal diagnosis and antenatal care

Of the 82 pregnancies, 32 resulted in spontaneous abortions or termination for social reasons (Figure 3.1). Of the remaining 50, prenatal diagnosis was offered to the women in 48 pregnancies (in two pregnancies the carrier status was discovered post-natally). The women opted for prenatal diagnosis in only 17/48 (35%) pregnancies. Chorionic villus sampling was the method of prenatal diagnosis used in 10 cases which revealed four female, two unaffected male and four affected male fetuses. Termination of pregnancy was opted for in two of the four affected pregnancies. In the remaining seven, prenatal diagnosis was performed by second trimester fetal gender determination by ultrasound and cordocentesis for male fetuses. In this group, ultrasound revealed three female and four male fetuses. In the latter group, cordocentesis revealed only one affected pregnancy and the mother opted for termination of the pregnancy. There was one miscarriage following cordocentesis in one of the three remaining unaffected male fetuses. There was no miscarriages following Chorionic villus sampling. The methods prenatal diagnosis used and results are shown (Figure 3.1).

Among the 46 ongoing pregnancies, the gender of the fetus was determined in 13 from prenatal diagnosis, and was requested by the obstetrician in a further 14 pregnancies. The fetal genital area was visualised antenatally by ultrasound at 18-20 weeks' gestation in 12 of these 14 cases. In these 12 there was correct gender assignment. There was failure to visualise the genital area in two cases and no later attempts at gender assignment were made. In five other pregnancies, the mothers specifically did not want fetal sexing. In 14 pregnancies with known carrier status, no
gender diagnosis was offered. Therefore, in 21/46 (46%) pregnancies, fetal gender was not available to the attending obstetrician during labour.

Figure 3.1: Uptake and results of prenatal diagnosis in carriers of haemophilia
The non-pregnant mean factor VIII level in haemophilia A carriers was 52.5iu/dl (median 56iu/dl, range 18-96iu/dl) and non-pregnant mean factor IX level in carriers of haemophilia B was 57iu/dl (median 48iu/dl, range 16-80iu/dl). Factor levels were checked in nine and three pregnancies in the second and/or third trimesters in haemophilia A and B carriers, respectively (Figure 3.2). Most of the carriers of haemophilia A showed a significant increase in factor VIII levels (P = <0.001) but there was no significant increase in factor IX in haemophilia B carriers.

Figure 3.2: Changes in FVIII and FIX levels during pregnancy
Labour and mode of delivery

The mean gestation at delivery was 39.2 weeks (range 34-42 weeks). Fetal scalp electrodes (FSE) were used for fetal monitoring in labour in eight pregnancies where the fetal gender was not known. Two of these fetuses were subsequently found to be affected males. Fortunately there were no adverse affects from the use of FSE in these cases. On the other hand FSE was required for fetal monitoring in three cases but withheld due to lack of knowledge of the gender. Fetal blood sampling (FBS) was considered essential in nine pregnancies where gender was not known but was not performed in five of them because of this. In two of these five cases, emergency Caesarean sections were performed (Table 3.2; case no. 1, 3), another two had forceps delivery (Table 3.1; case no. 1, 3) and in the 5th case, the cardiotocogram (CTG) improved while preparing for Caesarean section and had a vaginal delivery. However, among the four cases where the procedure was performed, there was one affected male fetus who suffered no adverse effect.

The mode of delivery was normal vaginal delivery in 32 pregnancies (intact perineum in 14, vaginal tears in 9, episiotomy in 9). Low cavity forceps was used to deliver two affected male fetuses with no neonatal complications. However a severe cephalhaematoma developed subsequent to ventouse delivery in a fetus which did not have prenatal diagnosis or fetal gender determination. The fetus was later found to be an affected male, infant of a known carrier mother. Details of instrumental and Caesarean section deliveries are shown in Table 3.1 and 3.2, respectively. Of the eight caesarean section deliveries, knowledge of gender might have influenced management in four cases (Table 3.2; case no. 1, 2, 3, 6).
None of the Caesarean sections were performed under regional anaesthesia. However, epidural analgesia was the method of pain relief during labour in six of the total group. Factor levels were > 50% in five of these but was unknown in the 6th patient as her haemophilia carrier status was diagnosed post-natally. There were no reported complications from the procedure in any of the six cases.

Table 3.1 - Instrumental vaginal deliveries in carriers of haemophilia

<table>
<thead>
<tr>
<th>No.</th>
<th>Ges. age</th>
<th>Fetal sex if known in labour</th>
<th>MOD</th>
<th>Indication</th>
<th>Haem. Status of the bay</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>NK</td>
<td>FD</td>
<td>Fetal distress</td>
<td>Affected male</td>
<td>Outlet forceps, no neonatal complication</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>Female</td>
<td>FD</td>
<td>Prolonged 2nd stage</td>
<td>Female</td>
<td>Fetal sex was known to be female</td>
</tr>
<tr>
<td>3</td>
<td>38</td>
<td>NK</td>
<td>FD</td>
<td>Fetal distress</td>
<td>Female</td>
<td>Low mid cavity forceps</td>
</tr>
<tr>
<td>4</td>
<td>41</td>
<td>Male</td>
<td>FD</td>
<td>Prolonged 2nd stage</td>
<td>Affected male</td>
<td>Forceps considered less traumatic than LSCS, no neonatal problem</td>
</tr>
<tr>
<td>5</td>
<td>38</td>
<td>Female</td>
<td>VE</td>
<td>Prolonged 2nd stage</td>
<td>Female</td>
<td>Fetal sex was known to be female</td>
</tr>
<tr>
<td>6</td>
<td>38</td>
<td>NK</td>
<td>VE</td>
<td>Prolonged 2nd stage</td>
<td>Affected male</td>
<td>Huge cephalhaematoma fetal Hb dropped to 5.2g/dl</td>
</tr>
</tbody>
</table>

No, patient number; Ges. age, gestational age in weeks; MOD, mode of delivery; Haem., haemophilia; NK, not known; FD, forceps delivery; VE, vacuum extraction; LSCS, lower segment Caesarean section.
Table 3.2 - Caesarean sections in carriers of haemophilia

<table>
<thead>
<tr>
<th>No.</th>
<th>Ges. age</th>
<th>Fetal sex if known in labour</th>
<th>indication</th>
<th>Haem. Status of the bay</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>39</td>
<td>NK</td>
<td>Abnormal CTG</td>
<td>Affected male</td>
<td>FBS was not performed because fetal sex was NK</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>NK</td>
<td>Breech + fetal sex NK</td>
<td>Un-affected male</td>
<td>Unavailability of fetal sex influenced decision regarding mode of delivery</td>
</tr>
<tr>
<td>3</td>
<td>39</td>
<td>NK</td>
<td>Delay in 2nd stage + abnormal CTG</td>
<td>Female</td>
<td>FBS and mid cavity forceps were not performed because fetal sex was NK</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>Male</td>
<td>Previous LSCS</td>
<td>Un-affected male</td>
<td>Previous LSCS for CPD</td>
</tr>
<tr>
<td>5</td>
<td>39</td>
<td>Male</td>
<td>Delay in 2nd stage</td>
<td>Affected male</td>
<td>Instrumental delivery was not attempted because the fetus was known to be affected male. There was marked scalp bruising and cephalhaematoma. PPH of 1200 mls</td>
</tr>
<tr>
<td>6</td>
<td>34</td>
<td>NK</td>
<td>Breech + fetal sex NK</td>
<td>Female</td>
<td>Unavailability of fetal sex influenced decision regarding mode of delivery</td>
</tr>
<tr>
<td>7</td>
<td>39</td>
<td>Female</td>
<td>Face presentation</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>37</td>
<td>NK</td>
<td>Failed induction</td>
<td>Un-affected male</td>
<td></td>
</tr>
</tbody>
</table>

No., patient number; Ges. age, gestational age in weeks; Haem., haemophilia; NK, not known; CTG, cardiotocogram, FBS, fetal blood sampling; LSCS, lower segment Caesarean section; CPD, cephalopelvic disproportion
Maternal haemorrhagic complications

All maternal haemorrhagic complications were all post-partum. There were 10 primary PPH (Table 3.3), two of which were massive. Two Caesarean sections were followed by significant PPH. In spite of the fact that the factor levels were below 50% in six of these cases, prophylactic treatment was not arranged to cover delivery and the post-partum period. DDAVP was administered prophylactically immediately after delivery in only three women and in another who had a PPH within 30 minutes of delivery. This resulted in an increase in factor levels in these patients (mean factor level was 46iu/dl before and 84iu/dl after DDAVP administration, respectively).

Intravenous access in labour was established in 15 women but in only four of them was this because of the haemophilia carrier status. There were five secondary PPH (Table 3.4) and retained products of conception were obtained in only one. Blood products were used to control bleeding in two cases but none required blood transfusion.

Neonatal complications

Of the 46 infants born, 24 were male (8 were confirmed to have haemophilia) and 22 were female. The mean birth weight was 3.25 (range 2.34-4.26) kg. There were three neonatal problems in affected male fetuses as a result of haemophilia. One neonate suffered a huge cephalhaematoma after a ventouse delivery resulting in subsequent anaemia (Hb 5.2 g/dl) requiring transfusion but with no intraventricular haemorrhage. A second infant, already known to be an affected male, was delivered by Caesarean section after a 2 hour second stage and there was marked bruising and a cephalhaematoma but again no intraventricular haemorrhage. A third fetus was inadvertently given intramuscular vitamin K before the diagnosis of haemophilia was
made and developed extensive deep bruising around the site which settled with conservative management and did not require blood transfusion. Admission to the intensive care unit was required for six infants. In two, (Table 3.1; case 6 and Table 3.2; case 5) this was directly associated with bleeding complications. Preterm delivery, meconium aspiration and low Apgar scores were reasons for admission in the other four.
Table 3.3 - Primary post-partum haemorrhage in carriers of haemophilia

<table>
<thead>
<tr>
<th>No.</th>
<th>Factor level (gestation)</th>
<th>MOD</th>
<th>PPH mls</th>
<th>Blood products</th>
<th>Blood transf.</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 iu/dl (pre-preg)</td>
<td>LSCS</td>
<td>1500</td>
<td>FVIII conc.</td>
<td>4 units</td>
<td>Advice from haemophilia centre to give DDAVP after delivery, but ignored</td>
</tr>
<tr>
<td>2*</td>
<td>42 iu/dl (pre-preg)</td>
<td>VAD</td>
<td>1500</td>
<td>FFP</td>
<td>5 units</td>
<td>factor level checked 24 hours post-delivery, was 67 iu/dl</td>
</tr>
<tr>
<td>3</td>
<td>50 iu/dl (28 weeks)</td>
<td>LSCS</td>
<td>1200</td>
<td>FFP + FVIII conc.</td>
<td>3 units</td>
<td>Prolonged 2nd stage, lacerations of the lower segment during operation</td>
</tr>
<tr>
<td>4</td>
<td>18 iu/dl (pre-preg)</td>
<td>VAD</td>
<td>1000</td>
<td>FVIII conc.</td>
<td>2 units</td>
<td>Bleeding from extended episiotomy wound</td>
</tr>
<tr>
<td>5</td>
<td>52 iu/dl (28 weeks)</td>
<td>VAD</td>
<td>900</td>
<td>No</td>
<td>No</td>
<td>DDAVP given immediately after delivery. Bleeding controlled with IV oxytocin. Post-natal factor level was 71 iu/dl</td>
</tr>
<tr>
<td>6*</td>
<td>27 iu/dl (pre-preg)</td>
<td>VAD</td>
<td>900</td>
<td>FFP</td>
<td>No</td>
<td>Prolonged uterine atony required IV ergometrin and oxytocin infusion</td>
</tr>
<tr>
<td>7</td>
<td>38 iu/dl (34 weeks)</td>
<td>VAD</td>
<td>800</td>
<td>Recom. FVIII</td>
<td>No</td>
<td>Recom. FVIII given to cover labour. Retained placenta, manually removed. Post-partum factor level 55 iu/dl achieved</td>
</tr>
<tr>
<td>8</td>
<td>40 iu/dl (pre-preg)</td>
<td>VAD</td>
<td>750</td>
<td>FVIII conc.</td>
<td>No</td>
<td>Bleeding was from vaginal lacerations</td>
</tr>
<tr>
<td>9*</td>
<td>55 iu/dl (pre-preg)</td>
<td>VAD</td>
<td>700</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>60 iu/dl (pre-preg)</td>
<td>VE</td>
<td>600</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

*, haemophilia B; MOD, mode of delivery; trans., transfusion; Pre-preg, pre-pregnancy; LSCS, lower segment Caesarean section; VAD, vaginal delivery; VE, vacuum extraction; FVIII conc., FVIII concentrate; Recom, Recombinant
Table 3.4 - Secondary post-partum haemorrhage in carriers of haemophilia

<table>
<thead>
<tr>
<th>No.</th>
<th>Factor level (gestation)</th>
<th>MOD</th>
<th>Days from delivery</th>
<th>Blood products</th>
<th>Blood transf.</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16 iu/dl (postnatal)</td>
<td>VAD</td>
<td>11</td>
<td>FVIII conc.</td>
<td>No</td>
<td>D&amp;C was performed but revealed no RPOC</td>
</tr>
<tr>
<td>2</td>
<td>32 iu/dl (postnatal)</td>
<td>VAD</td>
<td>11</td>
<td>FFP</td>
<td>No</td>
<td>Required evacuation of RPOC</td>
</tr>
<tr>
<td>3</td>
<td>76 iu/dl (prenatal)</td>
<td>VAD</td>
<td>15</td>
<td>No</td>
<td>No</td>
<td>Endometritis treated by antibiotics</td>
</tr>
<tr>
<td>4</td>
<td>68 iu/dl (prenatal)</td>
<td>LSCS</td>
<td>intermittent</td>
<td>No</td>
<td>No</td>
<td>Controlled by combined O.C. pill</td>
</tr>
<tr>
<td>5</td>
<td>47 iu/dl (prenatal)</td>
<td>FD</td>
<td>intermittent</td>
<td>No</td>
<td>No</td>
<td>Controlled by combined O.C. pill</td>
</tr>
</tbody>
</table>

MOD, mode of delivery; trans., transfusion; LSCS lower segment Caesarean section; VAD, vaginal delivery; VE, vacuum extraction; FD, forceps delivery; FVIII conc., FVIII concentrate; RPOC, retained products of conception; D&C, dilatation and curettage, O.C., oral contraceptive.
3.4: DISCUSSION

There is little published information on the obstetric problems of haemophilia A and B carriers. This study represents the largest detailed assessment of the obstetric outcome of haemophilia carriers from one centre and illustrates the problems of pregnancy for both mother and fetus.

Recent advances in molecular genetic procedures have resulted in accurate DNA-based prenatal diagnosis and have therefore increased the available options for carrier women. However, there was a low uptake of prenatal diagnosis (35%) in this study and termination was chosen in only half of the affected pregnancies. Similarly, Varekamp et al (1990), evaluating the attitudes toward prenatal diagnosis among 549 non-pregnant potential and obligate carriers of haemophilia, found that only 31% of the study group would favour prenatal diagnosis, with the implication of a possible abortion in early pregnancy, and only half of them would choose this option even at 16-20 weeks. Most of the women who objected to prenatal diagnosis did so because they did not consider haemophilia to be a sufficiently serious disorder to justify an abortion.

Most carriers of haemophilia have factor VIII and IX levels within the normal range, although the reported range is wide (Rizza et al 1975). In this study group the non-pregnant mean value was 52.5iu/dl (range 18-86 iu/dl) and 57iu/dl (range 16-80 iu/dl) for factor VIII and IX, respectively. Pregnancy induces a rise in factor VIII levels in normal women (Rutherford et al 1964) and in carriers of haemophilia A (Kasper et al 1964). A significant increase in factor VIII concentration (average increase of 37iu/dl) was found among patients in this study who had their factor
levels checked during pregnancy (Figure 3.2). However, this rise is variable, unpredictable and inconsistent, and not all patients attain normal factor levels i.e. >50 iu/dl (Greer et al 1991). Factor IX levels, in contrast, often do not rise in pregnancy (Greer et al 1991, Briet et al 1982) and this trend was also observed in this study population (Figure 3.2).

Affected male fetuses are potentially at risk of scalp haemorrhage from FSE application and FBS but there is a lack of published data to support this. In this study these procedures were performed when fetal gender was unknown in some cases including three affected male fetuses without any apparent injury. While in some other cases these procedures were clinically indicated but were not performed due to the lack of knowledge of fetal gender. The inconsistencies in the use of both FBS and FSE may well be attributed to imprecise instructions within obstetric notes or poor understanding of haemophilia by the attending obstetricians. The lack of prior knowledge of fetal gender also influenced the method of delivery resulting in unnecessary Caesarean sections (Table 3.2). In pregnancies where karyotyping has not been performed, fetal gender can be assessed accurately by ultrasound in the mid-trimester (Plattner et al 1983). Indeed, with the advances in transvaginal sonography this can be determined as early as the first trimester (Bronshtein et al 1990). It may sometimes be difficult to visualise the fetal genital area due to fetal position and in these cases ultrasound examination should be repeated until a positive diagnosis of the gender is made. Although some patients would not wish to know, this information should be available to the attending obstetrician to help intra-partum care.
Affected male haemophilic fetuses are also at risk of serious scalp haemorrhage during labour and delivery from the process of birth or instrumental deliveries. The incidence of intracranial haemorrhage in haemophilic neonates has been reported to be 1-4% (Eyster et al 1978, Yoffe & Buchanan 1988). This risk is small in normal vaginal delivery and delivery of all affected fetuses by Caesarean section is not expected to eliminate this complication (Ljung et al 1994). However, traumatic and difficult instrumental deliveries, especially the use of vacuum extraction, have been shown to constitute a significant risk factor (Bray & Luban 1987, Ljung et al 1994, Kletzel et al 1989). In this study the one affected male neonate delivered by ventouse extraction developed a huge cephalhaematoma extending distally involving the fetal neck, necessitating blood transfusion and prolonged neonatal admission. Therefore, fetuses at risk of haemophilia should be delivered by the least traumatic method. Prolonged labour, and especially prolonged second stage of labour should be avoided and early recourse to Caesarean section should be considered. Although vacuum extraction should not be used, low forceps delivery may be considered less traumatic than Caesarean section when the head is deeply engaged in the pelvis and delivery can be achieved as an easy outlet procedure and performed by an experienced obstetrician. Mid cavity forceps and forceps involving the rotation of the head should not be used.

Epidural and spinal anaesthesia in the presence of a coagulation defect may cause an epidural haematoma if a blood vessel inside the spinal canal is punctured and may lead to permanent neurological damage (Moir & Thorburn 1986). However, it has been suggested that providing the coagulation screen is normal in patients with haemostatic disorder, there is no contraindication to insertion of epidural catheter
(Letsky 1991). In the Obstetric Unit at the Royal Free Hospital, regional block is used unless factor levels are below 50iu/dl. In the case of Caesarean section, spinal block is regarded as a safer option because a smaller needle is used and is less likely to injure a blood vessel (Milaskiewicz et al 1990).

This study also demonstrates an increased incidence of primary and secondary post-partum haemorrhage among carriers of haemophilia compared with the general obstetric population (22% versus 5%, and 11% versus 0.7%, respectively) (Cunningham et al 1989, Lee et al 1981). The tendency to bleed in haemophilia carriers has been explained by the low plasma levels of FVIII and FIX. This was also observed in this study as most of the significant PPH occurred in patients with factor levels below 50 iu/dl (Tables 3.3 and 3.4).

Even in the Obstetric Unit at the Royal Free Hospital where the help of the local Haemophilia Centre is easily available and haemophilia carriers are more commonly encountered and managed by obstetricians, there were many management inconsistencies. For most haemophilia carriers prenatal diagnosis is usually performed at tertiary referral centres where the expertise to obtain chorionic villi or fetal blood is available and where prompt evaluation of the fetal haemophilia status can be quickly assessed. Consequently, after prenatal diagnosis the remainder of the pregnancy may well be managed at a different hospital where expertise in haemophilia is not present and thus management inconsistencies are likely to be more extreme in these units. This clearly shows the importance of a protocol of management as well as active involvement of a local haemophilia centre in managing these patients. As a consequence of the results of this study and findings from other
investigators (Greer et al 1991) and the recommendations of Haemostasis and Thrombosis Task Force (Walker et al 1994), the following guidelines were laid down to aid consistent and appropriate management:

1- Pre-pregnancy counselling should be offered to discuss prenatal diagnosis and other aspects of pregnancy management. Women who may require blood product therapy should be immunised against hepatitis B. When there is any doubt about a woman's carrier status, genetic testing with DNA probes should be offered.

2- There should be a multidisciplinary approach to the prenatal diagnosis involving experts in the fields of fetal medicine, genetic counselling, haemophilia care and molecular genetics.

3- For couples that do not wish to have prenatal diagnosis, fetal gender determination is strongly recommend by ultrasound at 18 weeks' gestation when anomaly scan is performed. The importance of this should be emphasised to the couple. If they do not wish to know the sex of the baby, this information should be available to the obstetrician in charge and written in the notes.

4- The pregnancy should be managed in close liaison with a local haemophilia centre. The mother's factor level should be checked at booking, 28, 34 weeks' of gestation. This is specially important in patients with low pre-pregnancy levels (< 50 iu/dl) who would need prophylactic treatment for any invasive prenatal diagnostic procedures, spontaneous abortion, termination of pregnancy and during labour.

5- During labour maternal coagulation screen and appropriate factor assays should be checked. It may some times be difficult to assess factor levels in labour. In this situation, it is acceptable to rely on the third trimester factor levels to formulate a plan of management. When the factor level is less than 50iu/dl, an intravenous line
should be established and prophylactic treatment given. The risk of post-partum haemorrhage could be further reduced by minimising maternal genital and perineal trauma.

6- The use of invasive fetal monitoring techniques and instrumental deliveries, specially vacuum extraction, should be avoided in affected male fetuses or when fetal sex or coagulation status if male is unknown.

7- Providing the coagulation screen is normal and factor level is 50 iu/dl or more in patients with an inherited bleeding disorder, there is no contraindication to epidural analgesia.

8- A cord blood sample should be collected in a citrated tube and transferred to a haemophilia laboratory within two hours. Results of clotting factors should be conveyed to the parents by the person most involved in counselling, usually a staff member from the haemophilia centre.

9- Intramuscular injections must be avoided in affected male infants or when coagulation status is not known. Vitamin K should be given orally, and routine immunisations should be given carefully intradermally or subcutaneously.

10- Parents should be given follow-up counselling and haemophilic babies should be registered with and reviewed regularly by a haemophilia centre.
3.5: ACQUIRED POST-PARTUM HAEMOPHILIA

INTRODUCTION

Acquired haemophilia and, in particular, acquired post-partum haemophilia, is a rare bleeding disorder. However, it is an important condition as it can be associated with very severe bleeding symptoms and high rates of morbidity and mortality. Acquired post-partum haemophilia can be an unusual cause of post-partum haemorrhage that obstetricians may encounter only once in a lifetime. In the Haemophilia Centre at the Royal Free hospital, there were 28 patients with acquired inhibitors over a 27 year period (1970-1997). Four had acquired vWD and 24 had acquired haemophilia (i.e. developed FVIII inhibitors). Seventeen of the 28 patients were female and only four were acquired post-partum haemophilia. I was actively involved in the management of the last patient who presented as a severe secondary post-partum haemorrhage. This rare acquired condition is included this thesis to highlight the complicated and unusual presentation and to increase awareness among obstetricians so as to ensure early and appropriate management.
CASE REPORT

A 29-year old primigravid woman, previously fit and well, had an uncomplicated pregnancy, labour and a forceps delivery at 40 weeks gestation at another hospital. One week after the delivery she developed a brisk post partum haemorrhage requiring four units of blood. She proceeded to laparotomy for suspected internal bleeding but no cause of haemorrhage was found and the bleeding subsided. Six days later she developed a further episode of profuse vaginal bleeding associated with urinary retention. Catheterisation showed profuse fresh blood. Her haemoglobin was 6.4gm/dl, and she required further transfusion. Cystoscopy, for persisting haematuria, failed to reveal an active bleeding site. APTT was, at this point, prolonged at 40s (control 37s). Conservative approach was continued with a general improvement. One week later she again went into clot retention requiring return to theatre and insertion of a suprapubic catheter. Bilateral ureteroscopy was normal. Hysteroscopy and dilatation and curettage revealed no retained products of conception. Her haemoglobin remained at 9gm/dl despite transfusion, to this point, of 18 units of red cells. At this point her APTT was noted to be 70s (control 37s). Over the following days her APTT remained persistently elevated and increasingly prolonged. She was given repeated units of fresh frozen plasma which did not improve the APTT. Her haematuria slowly improved without further intervention, other than blood transfusion (total 22 units) and she was discharged with persisting mild haematuria.

Following discharge she was promptly readmitted on the same day with persistent right loin pain, increasing haematuria and high fever. Ultrasound and computerised tomography (CT), revealed a mass in the kidney which was biopsied. Histology revealed only normal renal medulla and clot. She continued to have gross haematuria.
In view of the persistent prolongation of the APTT and continuing bleeding, haematological advice was sought and the patient was transferred to Royal Free Hospital.

On admission, the patient was pale but stable haemodynamically. Although she had bruising at venepuncture sites and light blood stained urine there was no other overt bleeding. Investigations confirmed a prolongation of the APTT (>160s) which was not corrected with the addition of normal plasma. Her PT was normal. Factor VIII procoagulant activity (FVIII:C) was <1 iu/dl with a Bethesda assay confirming the presence of a high titre anti-human FVIII antibody. She was commenced on immunosuppression with Prednisolone 1mg/kg and intravenous immunoglobulin 1g/kg/24hr for 2 days. CT scan confirmed a large haematoma of the right kidney with no extension outside the capsule. The left kidney was normal and creatinine was within normal range.

Three days later she became acutely unwell with severe pain over the right loin, shocked, hypotensive and tachcardic. CT showed massive intraperitoneal haemorrhage. She was treated with porcine FVIII (100 iu/kg) to arrest bleeding. Emergency renal angiography revealed an extensive intraperitoneal and perinephric bleed from a renal arterial vessel. At angiography it was apparent that active bleeding had stopped.

Due to the massive haemorrhage and hypotension, the patient developed acute tubular necrosis requiring haemodialysis for 5 days. In addition, because of the substantial intraperitoneal haemorrhage leading to diaphragmatic splinting, she
required continuous positive pressure ventilatory support for 2 days. Additional complications included persistent diastolic hypertension and grand mal seizures.

Throughout she was maintained on high dose porcine factor VIII replacement therapy. However over the ensuing 10 days her response to porcine FVIII gradually tailed off, due to the development of an inhibitor to porcine FVIII. She was then commenced on FEIBA (Factor Eight Inhibitor bypassing activity) until all bleeding had ceased, approximately 4 weeks later. On discharge, 9 weeks after admission, her FVIII:C was 38 iu/dl. During follow up she was continued on high dose steroids which were slowly tailed off. Three months later she developed a traumatic haemarthrosis of her right knee. In view of the high dose of steroids required to maintain an adequate FVIII:C level she was commenced on low dose Cyclophosphamide. One year after initial presentation she remains on low dose steroids and Cyclophosphamide with an FVIII:C of around 50 iu/dl. Her dose of immunosuppressive drugs continues to slowly decline. Her Bethesda assay is negative. Her renal function is normal and she has no clinically significant sequelae from her acute illness.
DISCUSSION

This case presents an unusual and very rare cause of post-partum haemorrhage, however, with a complicated clinical course and associated with high morbidity and mortality rates. Therefore, there should be a high degree of suspicion for any circumstances suggesting this condition e.g. unexplained excessive and/or prolonged vaginal bleeding or large soft tissue haematomas, and specifically bleeding from multiple sites during the post-partum period. Acquired post-partum haemophilia is induced by antibodies to FVIII (inhibitors), that partially or completely suppress FVIII procoagulant activity, in patients who previously had normal levels of FVIII. The risk of developing FVIII inhibitors is highest after the first delivery, although appearance of inhibitors during pregnancy or after several deliveries has been reported. The bleeding symptoms usually become apparent within 3 months after delivery but it could be as late as 12 months post-delivery. Complete remission is achieved spontaneously in the vast majority of patients with post-partum acquired haemophilia, especially those with low level of inhibitors (Spero et al 1979, Green & Lechner 1981). The antibodies usually disappear within a few months but may persist for years. As FVIII inhibitors are primarily IgG, they may cross the placenta to the fetus (Broxson & Hathaway 1986, Vicente et al 1987), and persist for up to 3 months in the neonate, usually without any bleeding complications. However, Ries et al (1995) have reported a case of transplacental transfer of FVIII inhibitors causing intracranial haemorrhage in a neonate.

Acquired haemophilia is usually diagnosed in a general hospital. However, because of the rarity of the disorder, the need for strict monitoring with FVIII assays and inhibitor levels and complexity of the management, it is best treated in a haemophilia
centre by individuals specialised in these disorders. The aim of management is to control acute bleeding as well as to accelerate eradication of the inhibitors. Control of haemorrhage in patients with acquired FVIII inhibitors is usually achieved by raising the plasma FVIII level, if possible. There are a number therapeutic options. DDAVP infusion has few adverse effects and no risk of transmitting pathogens but its efficacy is limited and probably restricted to low titre inhibitors with a measurable FVIII. Porcine FVIII, human FVIII, recombinant FVIIa (rFVIIa) and activated prothrombin complex concentrates (APCC) are successful in most cases. The choice depends on the severity of the bleeding, the level of human and porcine FVIII inhibitor level, as well as the safety of each therapeutic method. The final goal of treatment is to accelerate the disappearance of FVIII inhibitors. This may be achieved by the use of immunosuppressive drugs as corticosteroids, cyclophosphamide, or azathioprine. Major bleeding has been reported in 87% of patients with FVIII inhibitors and fatal outcome in 22% (Green & Lechner 1981). These risks outweigh the small risk associated with immunosuppressive treatment. The prognosis for subsequent pregnancies in patients with FVIII inhibitors is favourable especially in those achieving complete remissions.
CHAPTER 4

ATTITUDES AND REPRODUCTIVE CHOICES
OF WOMEN IN FAMILIES WITH
HAEMOPHILIA

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4.1 : INTRODUCTION

Despite advances in the treatment of haemophilia, the condition is life long and incurable with potentially life threatening bleeds and chronic joint damage. Treatment is long-standing with potential side effects. In addition, modern developments in molecular biology have allowed precise carrier detection and prenatal diagnosis, expanding the reproductive choices for women in families with haemophilia. Decisions regarding reproduction and childbirth are often complex as there may be conflicts between the best interests of the family as a whole, the mother herself and the child. These decisions are affected by family experience with the disease as well as ethnic and cultural issues.

The aim of this study was to assess the attitudes and experiences of obligate and possible carriers of haemophilia towards starting a family, prenatal diagnosis, and termination of pregnancy with an affected male fetus and to describe factors that influence these decisions.
4.2 : PATIENTS AND METHODS

In 1996, there were 545 obligate and potential carriers of haemophilia (A and B), aged 14 - 60 years, registered with the Haemophilia Centre at the Royal Free Hospital. A questionnaire was sent to all of these carriers, of whom 197 (36%) completed and returned it. Reminders were not sent to women who did not respond because of the potentially sensitive nature of the subject. The questionnaire consisted of 10 main questions relating to the women’s experiences of pregnancy and their attitudes towards reproductive choices, in addition to questions regarding their demographic details (Appendix 1). To help the women complete the questionnaire easily, answers were precoded whenever appropriate, although the women were given the choice of providing additional answers when necessary. Each woman’s questionnaire was given a serial number in order that the data could be linked to clinical information within the patient notes. However, no names were included on the questionnaires in order to preserve confidentiality.

Clinical details including type of haemophilia (A or B), severity of the disease in the family, the carriers’ factor level and whether the carrier status was confirmed by DNA analysis were obtained from Haemophilia Centre records for all the women to whom a questionnaire had been sent. A comparison between the responders and non­-responders to the questionnaire was performed to evaluate whether there were any differences between the two groups of women which might have influenced or biased the results.
The questionnaire was split into three main sections describing:

i) The women’s attitudes to reproduction and decision making, including reasons for having a termination of pregnancy, if appropriate.

ii) The women’s decisions regarding when to start or extend their family.

iii) The women’s decisions not to have any / any more children.

The effect of a number of factors on these decisions were analysed using the Chi-squared test, Fisher’s exact test or Mann-Whitney U test, as appropriate. The factors considered were:

1. Severity of haemophilia in the family.
2. Carriership status (obligate or possible).
3. Results of DNA studies (confirmed carrier, confirmed non-carrier or inconclusive).
4. Type of haemophilia (A or B).
5. Religion (Catholic, other Christian, Muslim, Jewish, other religions and non-believer).
4.3: RESULTS

Comparison of the women who responded and those who did not is shown in Table 4.1. Women who responded to the questionnaire were more likely to be carriers (by DNA studies) and a greater proportion of them had a history of haemophilia B in the family, although these results were not statistically significant. Otherwise there were no large differences between the two groups.

Table 4.1 - Comparison of responders and non-responders

<table>
<thead>
<tr>
<th></th>
<th>Responders</th>
<th>Non-responders</th>
<th>P - Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of women</td>
<td>197</td>
<td>348</td>
<td></td>
</tr>
<tr>
<td>Age on 1/1/1998</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>36 (14-56)</td>
<td>37 (19-59)</td>
<td>0.81</td>
</tr>
<tr>
<td>Carrier status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Confirmed carrier</td>
<td>91 (55.8%)</td>
<td>118 (44.9%)</td>
<td></td>
</tr>
<tr>
<td>- Confirmed non-carrier</td>
<td>51 (31.3%)</td>
<td>102 (38.8%)</td>
<td></td>
</tr>
<tr>
<td>- Inconclusive</td>
<td>21 (12.9%)</td>
<td>43 (16.3%)</td>
<td>0.09*</td>
</tr>
<tr>
<td>Type of haemophilia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- A</td>
<td>146 (79.8%)</td>
<td>263 (86.5%)</td>
<td></td>
</tr>
<tr>
<td>- B</td>
<td>37 (20.2%)</td>
<td>41 (13.5%)</td>
<td>0.07*</td>
</tr>
<tr>
<td>Severity in the Family</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Median (range) of factor level</td>
<td>0.5 (0-90)</td>
<td>0.5 (0-33)</td>
<td>0.94</td>
</tr>
<tr>
<td>- Severe haemophilia (factor level &lt; 1%)</td>
<td>38%</td>
<td>62%</td>
<td>0.77</td>
</tr>
<tr>
<td>Carriers’ factor levels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>66 (9-153)</td>
<td>72 (13-175)</td>
<td>0.14</td>
</tr>
</tbody>
</table>

* statistically significant
The median age of women who responded was 36 (range 14-56) years. Of these women 138 (71%) were married or cohabiting with their partners and 56 (28%) were single, separated or divorced. 23 (12%), 119 (60%), 3 (2%), 10 (5%), 8 (4%) and 18 (9%) reported Catholic, other Christian, Muslim, Jewish, other religious beliefs and non-believer, respectively; information on religion was missing for the remaining 16 (8%) women. Information on the number of pregnancies was available for 194 women; of these, 34 (17%) of the women had never been pregnant, 29 (15%) had been pregnant on one occasion only and only 131(67%) had been pregnant more than once. Of the 160 women who had been pregnant at least once, 120 (75%) had sons and 101 (63%) had daughters. Seventy seven of the 120 women who had sons (64%) had at least one affected son. Twenty four (15%) of the women reported bleeding complications after one or more child birth; of these 11 required blood transfusion on at least one occasion.

Prenatal diagnostic tests:

Thirty six of the 160 women (22.5%) who had been pregnant had had a parental diagnostic test during at least one pregnancy, 112/160 (70%) did not have tests in any of the pregnancies and 12/160 (7.5%) women either could not remember or left the question unanswered.

Termination of pregnancy:

Twenty nine (18%) and 12 (8%) of the 160 women who had been pregnant had termination of one pregnancy or two or more pregnancies, respectively. These were mainly for social reasons (25/41 women, 61%). Haemophilia was the main reason for the termination of pregnancy in 11/41 (27%) cases and birth defects or medical
reasons other than haemophilia were mentioned by 5/41 (12%) women. As expected
the reason for having a termination of pregnancy was affected by religious beliefs:
whilst no Catholic women chose termination of pregnancy for social reasons, 10
(48%) of those with other Christian beliefs, two (50%) of those with Muslim, Jewish
or other religious beliefs and seven (88%) of those with no religious beliefs chose
termination of pregnancy for this reason (p = 0.03). In contrast, two (50%) Catholic
women and nine (43%) women with other Christian beliefs mentioned haemophilia
as a reason for termination of pregnancy compared to none of the women in the other
groups (p = 0.01).

Ten (43%) women who had been confirmed as carriers by DNA testing mentioned
haemophilia as a reason for termination of pregnancy, compared to none of those
who were either confirmed as a non-carrier or who had inconclusive results (p =
0.03).

**Reasons for becoming pregnant:**
The women were asked about the factors influencing their decision to become
pregnant for the first time (n=160) and for subsequent pregnancies (n=132) (Table
4.2). The main reason for starting or continuing a family was that the time was
thought to be right, either emotionally, socially or financially: 75 /160 (47%) and
58/132 (44%) first and subsequent pregnancies, respectively. The effect of maternal
age was particularly noticeable in subsequent pregnancies where 26 (45%) and 29
(41%) of those women born before 1955 and between 1955-1965 said that the time
was right, compared to only 3 (9%) of those born since 1965 (p = 0.002). Living
close to a haemophilia centre, with the availability of proper counselling and prenatal
diagnostic tests, influenced the decision in 22 (14%) and 13 (10%) first and subsequent pregnancies, respectively. In first pregnancies, this reason was more often mentioned in those with only mild haemophilia (14 [26%]) than those with severe haemophilia (5 [6%]) in the family (p = 0.002). In addition, for first pregnancies a greater proportion of women who were confirmed carriers (13 women, 17%) stated that living close to a haemophilia centre was a reason for becoming pregnant compared to confirmed non-carriers (one woman, 2%) and those whose carrier status was inconclusive (one woman, 6%) (p = 0.04), although no such relationships were noted with subsequent pregnancies. Pressure from the partner or the family and social/religious belief were mentioned in 7/160 (4.4%) first pregnancies and 3/132 (2.3%) subsequent pregnancies. Catholic and other Christian women (none and two (2%) women, respectively) were less likely to mention pressure from the family as a reason for having a first child compared to those with other (two women, 13%) or no (two women, 13%) religious beliefs (p = 0.03). Thirteen (8%) first and seven (5%) subsequent pregnancies were unplanned (Table 4.2).
Table 4.2 - Factors influencing the decision to start a family or have more children

<table>
<thead>
<tr>
<th></th>
<th>First child n = 160</th>
<th>Subsequent children n = 132</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right time</td>
<td>75 (47%)</td>
<td>58 (44%)</td>
</tr>
<tr>
<td>Availability of a haemophilia centre services</td>
<td>22 (14%)</td>
<td>13 (10%)</td>
</tr>
<tr>
<td>Unaware of being a carrier of haemophilia</td>
<td>15 (9%)</td>
<td>7 (5%)</td>
</tr>
<tr>
<td>Social pressures</td>
<td>7 (4%)</td>
<td>3 (2%)</td>
</tr>
<tr>
<td>Unplanned pregnancy</td>
<td>13 (8%)</td>
<td>7 (5%)</td>
</tr>
</tbody>
</table>

Decision not to have children:

The women were asked whether they had ever made a conscious decision not to have children/any more children of whom 106 (54%) had made this decision. Haemophilia was a major factor in this decision for a large proportion of women because:

1. They did not want to pass haemophilia on to a child (n = 47, 44%).
2. Their previous experience with haemophilia (in the family) or because they could not cope with another haemophilic child (n = 6, 6%).
3. The stress of going through prenatal tests or because they would not have termination of pregnancy even if the baby was affected (n = 7, 7%).

Of the 106 women, 36 (34%) had made this decision because they felt that their family was complete and 29 (27%) had felt constraints because of personal, social, financial and medical reasons. The impact of previous experience of haemophilia in
the family was most noticeable in potential carriers, of whom six (11%) quoted this a reason, compared to none of the obligate carriers (p = 0.04). The severity of the disease in the family, type of haemophilia, results of DNA studies, religion and year of birth had no effect on this decision.

Information about haemophilia:

Of 80 women who had received information from haemophilia centre staff, 75 (94%) found this information useful. This is in contrast to 23 (59%) of 39 women who had received information from their general practitioner, and 18 (47%) of 39 women who had received information from Haemophilia Societies.

Identification of fetal sex:

Ninety-five of 197 (48%) of women wished to know the sex of their baby during pregnancy, 86 of 197 (44%) did not wish to know and 16 of 197 (8%) did not have strong views about this.
4.4: DISCUSSION

Women in families with haemophilia and their partners face a multitude of anxieties and psychological considerations prior to making the decision of whether to have a child, and during the process of carrier detection, starting a pregnancy and prenatal diagnosis (Tedgård et al 1989). Anxieties arise from the risk of having an affected child with long-term morbidity, having potentially painful and invasive diagnostic tests, the risk of miscarriage due to these tests, receiving an abnormal result, making the decision whether to continue or have termination of pregnancy and undergoing the process of termination and its potential complications. In addition to these factors, their cultural and religious beliefs, previous experiences with haemophilia and pressure from partners and families may also influence their perceptions and attitudes towards reproductive choices and child-bearing. In this study, the experience and views of women in families with haemophilia towards these controversial issues are studied as well as factors that might affect their decision making.

The response rate in this study was only 36%, therefore the results may not represent an overview of experiences and attitudes of all women in families with haemophilia. Reminders were not sent as many of the women may have been tested for haemophilia some time ago, and may not have disclosed this to their partners. Therefore, it was felt that the decision not to respond should be respected. However, there were no significant differences in the age, carrier status, severity and type of haemophilia in the family between women who responded to the study and those
who did not. Therefore it is unlikely that the low response to the questionnaire has affected the results.

Haemophilia is, in general, considered as a severe disorder by families with severe and mild forms of haemophilia (Ranta et al 1994) because of its bleeding episodes, joint problems and its associated limitations in daily life. However, most women in these families do not consider haemophilia to be a sufficiently serious disorder to justify an abortion (Varekamp et al 1990) and therefore there is a low uptake of prenatal diagnosis and termination of affected pregnancies. In this study, 70% of women who had been pregnant did not have any prenatal diagnostic test in any of their pregnancies. Of the 41 pregnancy terminations performed, the majority were for social reasons and only 27% of women mentioned haemophilia as a direct reason for their choice to have a termination.

Whether haemophilia in the family or carriership of haemophilia influences women’s reproductive decisions has not been extensively studied. This study has shown that a significant proportion of women in families with haemophilia make a decision not to have a child or further children because of fear of passing on haemophilia, going through prenatal tests and termination of pregnancy and a perceived inability to cope with another haemophilic child. Living close to a haemophilia centre with access to proper counselling and the availability of prenatal diagnostic tests influences the decision to become pregnant. The severity of haemophilia in the family and results of DNA studies had some influence on women’s decisions when planning their first pregnancy, but not when planning subsequent pregnancies or when they had already made a decision not to have children.
Knowledge of fetal gender has important implications in prenatal diagnosis (Koerper 1990) and in the management of labour and delivery in carriers of haemophilia (Chapter 3). Fetal gender determination was previously achieved only by fetal karyotyping (i.e. invasive procedures). However, with better resolution of the newer ultrasound machines it is now possible to determine fetal gender accurately even during the first trimester of pregnancy (Bronshtein et al 1990). Among this study group, the women were equally divided between those who wished to know fetal gender during pregnancy and those who did not. It is likely that if the advantages of knowing fetal gender are discussed, a higher proportion of these women would agree to have fetal gender identified during pregnancy. In addition, the experience of clinicians at the Royal Free Hospital with carriers attending for genetic counselling suggests that those who do not wish to know fetal gender rarely object to the staff caring for them having access to this information.

Therefore it is clear that even in the late 1990s and in spite of accurate diagnosis, easier and more effective treatment of haemophilia and gene therapy “on the horizon”, counselling of potential/obligate carriers remains a central task of all health care providers. The final decision about having children must be left to women and their partners. However, it is the responsibility of the health care professionals to help carriers to make informed choices in the best interest of the individual and the family without losing sight of the best interests of the child. Individuals involved in providing information and genetic counselling for haemophilia should have proficiency in haemophilia care, molecular genetics, prenatal diagnosis and
interpersonal communication and counselling. Counselling should be readily available for women and their partners who should be motivated to attend.
CHAPTER 5

PREGNANCY IN WOMEN WITH VON WILLEBRAND’S DISEASE OR FACTOR XI DEFICIENCY

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5.3 - RESULTS 184
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5.1 : INTRODUCTION

Von Willebrand’s disease is now recognised as the commonest inherited bleeding abnormality with a prevalence of 0.8% (Rodeghiero et al 1987) to 1.3% (Werner et al 1993). It comprises a heterogeneous group of autosomally inherited bleeding disorders. The commonest form of the disease, accounting for approximately 70% of all cases, is type 1. This type is characterised by equally low plasma levels, usually between 5 and 40 iu/dl, of FVIII, vWF:Ag and vWF:Ac. The pathophysiological basis of type 2 is qualitative abnormalities of vWF. Type 2 comprises many different subtypes (2A, 2B, 2N, 2M) and is phenotypically very heterogeneous. Type 3 is the least common of all forms of vWD and is characterised by very low (or even undetectable) levels of plasma vWF and FVIII with severe bleeding manifestations. FXI deficiency is a rare bleeding disorder with an autosomal mode of inheritance. FXI levels are severely reduced (< 15iu/dl) in homozygotes and partially deficient or low normal in heterozygotes (Bolton-Maggs et al 1988). Although FXI deficiency is particularly common in Ashkenazi Jews, the disorder has been described in all racial groups.

Pregnancy in patients with vWD has been addressed in case reports and studies of small numbers of patients (Chediak et al 1986, Conti et al 1986, Greer et al 1991, Ramsahoye et al 1995). There has been one large multi-centre study by Foster in 1995 for the International Society of Thrombosis and Haemostasis including 69 pregnancies in 31 patients with vWD from 16 centres. However, only patients unresponsive to DDAVP (i.e. mainly type 2 and 3 vWD) were included in the study. Apart from a description of the risk of bleeding during delivery and the puerperium (Bolton-Maggs et al 1988), there is no published literature on the obstetric experience
in patients with FXI deficiency. In this chapter, the obstetric complications, management and outcome of women with vWD and patients with FXI deficiency registered with the Royal Free Hospital Haemophilia Centre, over a 17 year period is thoroughly reviewed and discussed.
5.2 : PATIENTS AND METHODS

Women with vWD (n=31) or with FXI deficiency (n=11) registered at the Royal Free Hospital Haemophilia Centre who had been pregnant within a 17 year period (1980-1996) were investigated. Details of the women and their bleeding disorder are given in Table 5.1. Each woman was interviewed and details of obstetric history were obtained. The Haemophilia Centre case notes of all the patients and maternity records for those who had delivered at the Royal Free Hospital (n = 36 [vWD], 21 [FXI deficiency]) were reviewed and documented evidence from correspondence with other hospitals was obtained for those who delivered elsewhere, with particular attention to the obstetric experience and coagulation status during pregnancy and the puerperium. There were 84 and 28 pregnancies in vWD and FXI deficiency, respectively. The diagnosis of bleeding disorder was not known in 11 pregnancies in six women with vWD and in one pregnancy in a FXI deficient patient. The diagnosis was made in two women (vWD) following post-abortal and post-partum bleeding complications (Table 5.2).

The following aspects were assessed:

1. Occurrence of bleeding during pregnancy.


3. Changes in clotting factor levels during pregnancy.

4. Mode of delivery including indications for instrumental deliveries and Caesarean sections.

5. Bleeding complications in the puerperium including primary post-partum haemorrhage (PPH) (blood loss in excess of 500 ml during the 24 hours after the
birth of the infant) and secondary PPH (bleeding in excess of normal lochial loss after first 24 hours of delivery to 6 weeks).

6. Neonatal outcome with special emphasis on haemorrhagic complications and the impact of mode of delivery on these complications.

Table 5.1 - Details of patients included in the study

<table>
<thead>
<tr>
<th>Bleeding disorder</th>
<th>vWD</th>
<th>FXI deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of women</td>
<td>31</td>
<td>11</td>
</tr>
<tr>
<td>Severity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>25</td>
<td>Mild/Moderate</td>
</tr>
<tr>
<td>Moderate</td>
<td>4</td>
<td>Severe</td>
</tr>
<tr>
<td>Severe</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type 1</td>
<td>27</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>Type 2A &amp; 2B</td>
<td>2</td>
<td>Homozygous</td>
</tr>
<tr>
<td>Type 3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>No. of pregnancies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>84</td>
<td>Total</td>
</tr>
<tr>
<td>Median/woman</td>
<td>3</td>
<td>Median/woman</td>
</tr>
<tr>
<td>Range/woman</td>
<td>1-8</td>
<td>Range/woman</td>
</tr>
<tr>
<td>PV bleeding ≤ 13 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>28</td>
<td>Total</td>
</tr>
<tr>
<td>Complete miscarriage</td>
<td>5</td>
<td>Complete miscarriage</td>
</tr>
<tr>
<td>Incomplete miscarriage</td>
<td>10</td>
<td>Incomplete miscarriage</td>
</tr>
<tr>
<td>Missed abortion</td>
<td>3</td>
<td>Missed abortion</td>
</tr>
<tr>
<td>Pregnancy continued</td>
<td>10</td>
<td>Pregnancy continued</td>
</tr>
<tr>
<td>Pregnancy outcome</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Live birth</td>
<td>55*</td>
<td>Live birth</td>
</tr>
<tr>
<td>Elective TOP</td>
<td>12</td>
<td>Elective TOP</td>
</tr>
<tr>
<td>Spontaneous miscarriage 18**</td>
<td></td>
<td>Spontaneous miscarriage 1</td>
</tr>
</tbody>
</table>

* Including a set of twins; TOP, termination of pregnancy; **, including a woman with 4 recurrent miscarriages
5.3 : RESULTS

Vaginal bleeding during pregnancy

Threatened miscarriage in the first trimester (≤13 weeks) was reported in 28/84 (33%) and 4/28 (14%) pregnancies with vWD and FXI deficiency, respectively. Of these, 18 pregnancies in women with vWD and one woman with FXI deficiency miscarried spontaneously (Table 5.1). In the 10 remaining pregnancies with vWD, there was intermittent bleeding from 6 to 18 weeks gestation in three. In two of these cases the bleeding stopped spontaneously. However, in the third case (type 3 vWD) the bleeding became continuous and heavy, requiring treatment with vWF rich clotting factor concentrate. The overall spontaneous miscarriage rate in women intending to continue the pregnancy was 14/68 (21%) in vWD, excluding a woman with 4 recurrent miscarriages, and 1/26 (4%) in FXI deficiency. All the women who miscarried reported some degree of bleeding, including the three women with missed abortions (see below).

Antepartum haemorrhage occurred in two pregnancies in women with vWD; in both cases no cause was found and the bleeding was moderate and stopped without treatment. Severe placental abruption requiring emergency Caesarean section occurred in one woman with FXI deficiency.

Post-abortal bleeding complications

In total, there were 30 abortions (spontaneous and elective) in women with vWD and three in women with FXI deficiency (Table 5.1). Twelve out of 30 pregnancies with vWD and two out of three with FXI deficiency were elective pregnancy terminations: 13 for social reasons and one because of chromosomal abnormality. The remaining
were complete (vWD n = 5), incomplete (vWD n = 10, FXI deficiency n = 1) or missed abortions (vWD n = 3). The median gestational age at miscarriage was 10 weeks (range 6-16) in vWD and eight weeks (range 8-12) in FXI deficiency. In vWD, excessive bleeding requiring blood transfusion was reported in three cases. In one of these cases vWD was diagnosed following this complication. Secondary haemorrhage requiring readmission to the hospital was reported in three cases, repeat curettage was performed in two, with no retained products of conception. Bleeding settled after administration of cryoprecipitate in two women and FVIII concentrate in one. Intermittent bleeding two weeks after miscarriage was reported in nine cases. Tranexamic acid to control the bleeding was prescribed for three individuals. Factor levels were measured prior to termination of pregnancy in only six cases and prophylactic treatment was administered in three, none of whom had any bleeding complication. In a woman with FXI deficiency, secondary haemorrhage occurred after termination of pregnancy. She received two units of blood and repeat curettage was performed revealing scanty amounts of retained pieces of conception. She continued to have intermittent bleeding for the following eight weeks.

Changes of clotting factor levels during pregnancy

The median FVIII, vWF:Ag, vWF:Ac levels in patients with vWD prior to pregnancy were 53 iu/dl (range 0.5-100), 43 iu/dl (range 0.5-72) iu/dl and 40 iu/dl (range 0.5-70), respectively. Median non-pregnant FXI level in patients with FXI deficiency was 23 iu/dl (range 2-50 iu/dl). Factor levels were checked in 22 pregnancies in the third trimester (28-36 weeks) in 18 women with vWD and in eight pregnancies in five women with FXI deficiency. Most of the women (apart from those with severe vWD) showed a significant increase in FVIII (P = 0.0001), vWF:Ag (P = 0.0001) and
vWF:Ac (P = 0.0001) levels (Figure 5.1) but there was no significant change in FXI (p = 0.16) in FXI deficient patients (Figure 5.2).
Figure 5.1: Changes in FVIII, vWF:Ag and vWF:Ac levels during pregnancy in women with vWD.
Labour and mode of delivery

There were 54 deliveries (55 births) including a pair of twins in women with vWD and 25 births in FXI deficient women. Of these deliveries 36 (67%) in vWD and 21 (84%) in FXI deficient women were at the Royal Free Hospital. The mean gestation at delivery was 40 (range 34-42) and 39 (range 37-42) weeks in vWD and FXI deficiency, respectively. Invasive monitoring techniques including fetal scalp electrodes (FSE) and/or fetal blood sampling (FBS) were used for fetal monitoring during labour in 14 pregnancies (vWD n = 11, FXI deficiency n = 3) with no bleeding complications. Recommendations to avoid these procedures were clearly written in the patients’ notes in 19 cases (15/19 were managed from 1990 onwards)
and these instructions were followed by the attending obstetrician during labour in all cases.

The mode of delivery was vaginal delivery with intact perineum in 16 vWD and eight FXI deficiency pregnancies, vaginal delivery with vaginal tears or episiotomy in 27 vWD and 13 FXI deficiency pregnancies. Forceps delivery was performed in six vWD pregnancies including one patient with severe vWD, and one with FXI deficiency. Caesarean section was the mode of delivery in five vWD pregnancies and three FXI deficiency pregnancies.

Regional anaesthesia for labour and delivery was used in eight vWD pregnancies, including one woman with moderate disease and two mild/moderate FXI deficiency pregnancies without any complications. Prophylactic treatment was given to cover the procedure only in the woman with moderate vWD. Apart from two women in whom the diagnosis of bleeding disorder was made after the childbirth, factor levels were 50 iu/dl in the remaining pregnancies. Intramuscular pethidine injection for pain relief in labour was used in 12 women with vWD and 10 with FXI deficiency. Extensive gluteal bruising was reported in two cases (vWD n = 1, FXI deficiency n = 1), both treated conservatively.

Post-partum haemorrhage

The incidence of primary PPH was 18.5% (10/54) and 16% (4/25) in vWD and FXI deficiency, respectively (Table 5.2). Blood transfusion was required in six pregnancies with vWD, including one woman who was transfused 12 units, and in two pregnancies with FXI deficiency (Table 5.2). The incidence of secondary PPH
was 20% (11/54) and 24% (6/25) in vWD and FXI deficiency, respectively (Table 5.3). Blood transfusion was required in three women with vWD. Extensive perineal bruising and haematoma was reported in three women with vWD, two were associated with forceps delivery and drainage was required in only one case. Ten of fourteen instances of primary post-partum haemorrhage occurred when maternal factor levels were < 50 iu/dl (Table 5.2), none of these women were given prophylactic treatment for labour.

In contrast, prophylactic treatment to cover labour and/or puerperium was given in 10 pregnancies with vWD, (DDAVP n = 3, cryoprecipitate n = 3, vWF rich concentrates n = 4). There were no bleeding complications in any of these apart from perineal bruising in one. In FXI deficient women, FXI concentrate was given in two Caesarean sections and a normal vaginal delivery and fresh frozen plasma for labour in two pregnancies; none had any bleeding complications.

**Neonatal complications**

The median birth weight was 3.6 (range 2.6 - 5) kg in vWD and 3.3 (range 2.8-3.9) kg in FXI deficiency. There were three (vWD n = 2, FXI deficiency n = 1) neonatal bleeding complications. One neonate suffered bleeding after intramuscular vitamin K injection. A second infant had bleeding from the umbilicus. A third fetus had bleeding requiring blood transfusion following circumcision which was performed soon after birth. All these infants were confirmed to be affected with a deficiency of vWF or FXI.
Table 5.2 - Primary post-partum haemorrhage in women with vWD or FXI deficiency

<table>
<thead>
<tr>
<th>No.</th>
<th>Factor level (timing)†</th>
<th>MOD</th>
<th>PPH mls</th>
<th>Blood product</th>
<th>BT. (units)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48 iu/dl (36 weeks)</td>
<td>LSCS</td>
<td>massive</td>
<td>vWF conc. + FFP</td>
<td>12</td>
<td>- Wound haematoma</td>
</tr>
</tbody>
</table>
| 2   | -                      | VAD  | 2000    | FFP           | 4           | - Admitted to ITU for 48 hours  
- Diagnosis of vWD was made following this complication |
| 3   | 38 iu/dl (32 weeks)    | FD   | 1500    | vWF conc. + FFP | 3           | - Extensive bruising at the episiotomy site |
| 4   | 18 iu/dl (pre-preg)    | FD   | 1000    | No            | 2           | - Extensive bruising at the episiotomy site |
| 5   | 31 iu/dl (pre-preg)    | VAD  | 1000    | No            | 2           | - Extensive bruising at the episiotomy site |
| 6   | 27 iu/dl (pre-preg)    | VAD  | 1000    | vWF conc.     | 2           | - Extensive bruising at the episiotomy site |
| 7   | 48 iu/dl (34 weeks)    | VAD  | 900     | No            | No          | - DDAVP infusion was given |
| 8   | 40 iu/dl (pre-preg)    | VAD  | 800     | vWF conc.     | No          | - Perineal bruising at episiotomy site |
| 9   | 55 iu/dl (pre-preg)    | VAD  | 800     | No            | No          | - Haematuria and mild perineal bruising  
- Secondary PPH (case 2, Table 5.3) |
| 10  | 60 iu/dl (pre-preg)    | VAD  | 750     | No            | No          | - Secondary PPH (case 2, Table 5.3) |
| 11* | 42 iu/dl (32 weeks)    | VAD  | 1500    | No            | 3           | - Placental abruption |
| 12* | 60 iu/dl (pre-preg)    | LSCS | 1000    | FFP           | 2           | - Placental abruption |
| 13* | 40 iu/dl (34 weeks)    | VAD  | 850     | FFP           | No          | |
| 14* | 58 iu/dl (pre-preg)    | VAD  | 750     | No            | No          | - Secondary PPH (case 15, Table 5.3) |

*, FXI deficiency; †, vWF:Ac in vWD and FXI in women with FXI deficiency; BT, blood transfusion VAD, vaginal delivery; FD, Forceps delivery; LSCS, Lower segment caesarean section; FFP, Fresh frozen plasma; vWF conc, vWF rich concentrate
Table 5.3 - Secondary post-partum haemorrhage in women with vWD or FXI deficiency

<table>
<thead>
<tr>
<th>No.</th>
<th>Factor level PN</th>
<th>MOD</th>
<th>Days post-delivery</th>
<th>BP</th>
<th>BT units</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16 iu/dl</td>
<td>VAD</td>
<td>4</td>
<td>vWF conc.</td>
<td>4</td>
<td>Scan revealed no RPOC</td>
</tr>
<tr>
<td>2</td>
<td>32 iu/dl</td>
<td>VAD</td>
<td>14</td>
<td>FFP</td>
<td>2</td>
<td>Scan revealed no RPOC</td>
</tr>
<tr>
<td>3</td>
<td>46 iu/dl</td>
<td>VAD</td>
<td>9</td>
<td>No</td>
<td>2</td>
<td>Small amount of PROC</td>
</tr>
<tr>
<td>4</td>
<td>NM</td>
<td>VAD</td>
<td>8</td>
<td>No</td>
<td>No</td>
<td>Required ERPC and vaginal packing. Diagnosis of vWD was not known then. Tranexamic acid was also given</td>
</tr>
<tr>
<td>5</td>
<td>46 iu/dl</td>
<td>FD</td>
<td>7</td>
<td>vWF conc.</td>
<td>No</td>
<td>Controlled by Tranexamic acid</td>
</tr>
<tr>
<td>6</td>
<td>NM</td>
<td>VAD</td>
<td>Intermittent</td>
<td>No</td>
<td>No</td>
<td>Controlled by Tranexamic acid</td>
</tr>
<tr>
<td>7</td>
<td>NM</td>
<td>VAD</td>
<td>Intermittent</td>
<td>No</td>
<td>No</td>
<td>Controlled by Tranexamic acid</td>
</tr>
<tr>
<td>8,9 &amp;10</td>
<td>NM</td>
<td>VAD</td>
<td>Intermittent</td>
<td>No</td>
<td>No</td>
<td>Following 3 consecutive pregnancies in the same patient, controlled by combined O.C.pill in all three cases</td>
</tr>
<tr>
<td>11</td>
<td>68 iu/dl</td>
<td>LSCS</td>
<td>Intermittent</td>
<td>No</td>
<td>No</td>
<td>Controlled with combined O.C.pill</td>
</tr>
<tr>
<td>12*</td>
<td>47 iu/dl</td>
<td>VAD</td>
<td>3</td>
<td>No</td>
<td>No</td>
<td>Readmitted few hours after she was discharged home. Conservative treatment</td>
</tr>
<tr>
<td>13* &amp; 14*</td>
<td>NM</td>
<td>VAD</td>
<td>Intermittent</td>
<td>No</td>
<td>No</td>
<td>Following 2 pregnancies in the same patient</td>
</tr>
<tr>
<td>15*</td>
<td>65 iu/dl</td>
<td>VAD</td>
<td>Intermittent</td>
<td>No</td>
<td>No</td>
<td>Controlled by Tranexamic acid</td>
</tr>
<tr>
<td>16*</td>
<td>NM</td>
<td>VAD</td>
<td>Intermittent</td>
<td>No</td>
<td>No</td>
<td>Controlled with ergometrine, diagnosis was not known then.</td>
</tr>
<tr>
<td>17*</td>
<td>4 iu/dl</td>
<td>VAD</td>
<td>Intermittent</td>
<td>FFP</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>

*, FXI deficiency; †, vWF:Ac in vWD and FXI in women with FXI deficiency; BP, blood products; BT, blood transfusion; NM, not measured; VAD, vaginal delivery; LSCS, Lower segment caesarean section; vWF conc, vWF rich concentrate; RPOC, Retained product of conception; ERPC, Evacuation of retained product of conception.
5.4: DISCUSSION

This is a large retrospective review of obstetric experience and outcome in women with vWD or FXI deficiency. It highlights the high incidence of post-abortal and post-partum bleeding complications as well as appropriate methods of management to minimise the risk of these complications. During the last 10 years, there has been better understanding and advances in the treatment and prophylaxis of bleeding complications of these inherited bleeding disorders and increased awareness and interest among obstetricians. Therefore, this study may not represent present day practice and may overestimate pregnancy-associated complications because of the retrospective nature of the study including pregnancies from as early as 1980. Most of the pregnancies from 1990 and onwards were managed in collaboration with the Haemophilia Centre, factor levels were monitored during pregnancy and prophylaxis was considered when appropriate.

Haemostatic response to pregnancy is variable in different types and subtypes of vWD. In type I vWD, there is usually a progressive increase in FVIII, vWF:Ag and vWF:Ac and correction of the bleeding time during pregnancy (Punnonen et al 1981, Hanna et al 1981, Greer et al 1991). However, failure of primary haemostasis to improve significantly (in particular vWF:Ac) in some cases, especially severely affected type 1 patients, has been reported (Adashi 1980, Ramsahoye et al 1995). In subtype 2A and 2B, the production of vWF may increase throughout the pregnancy. However, the abnormal multimetric pattern remains unchanged (Conti et al 1986) and this explains the lack of clinical improvement in pregnancy and the greater risk of post-partum haemorrhage in these women than in those with type 1 vWD (Ramsahoye et al 1995). In addition, women with subtype 2B vWD may develop
worsening thrombocytopenia during pregnancy (Rick et al 1987) due to increased production of the abnormal intermediate vWF multimers. These multimers bind to platelets and induce spontaneous platelet aggregation. Women with type 3 vWD show very little or no increase in their FVIII and vWF plasma levels. In this study, whilst factor levels showed a significant increase in general, they failed to increase in both of the women with type 3 vWD. Because of the great variability of haemostatic response of vWD to pregnancy between individuals and even in different pregnancies in the same individual (as seen in one the women in this study) regular monitoring of coagulation factors is required. vWF:Ac has been suggested (Lipton et al 1982) to be the best predictor for risk of bleeding in pregnant women with vWD and bleeding may still occur in women with normal FVIII. Therefore, it is essential to monitor both vWF:Ag and vWF:Ac levels, along with FVIII during pregnancy. The platelet count should also be monitored in type 2B women (Rick et al 1987). In those with factor levels < 50 iu/dl during the third trimester, appropriate prophylactic therapy for delivery requires correction of the quantitative and/or qualitative vWF defect in addition to elevation of FVIII levels and this means administration of vWF containing products and not pure FVIII preparations.

The change in FXI during pregnancy in FXI deficient women has not been studied, and controversial results have been obtained in pregnant women in general, one study showing an increase (Condie 1976) and another a fall in FXI levels (Nossel et al 1966a). In this study group, there was no significant change in FXI levels. Prospective studies are required to assess FXI changes during pregnancy in FXI deficient women.
Vaginal bleeding during the first trimester has been estimated to occur in 16% of all pregnant women (South & Naldrett 1973). In this study, 33% of pregnancies in women with vWD reported first trimester bleeding and the overall spontaneous miscarriage rate was 21%. Therefore, although there appears to be a higher incidence of vaginal bleeding in the first trimester in women with vWD, there is no increase in the miscarriage rate. It is possible that these women seek medical care for any vaginal bleeding, even if minor, more readily than other pregnant women. In FXI deficiency, the number of pregnancies complicated by vaginal bleeding that also miscarried were too small to draw any conclusions. The incidence of antepartum haemorrhage did not seem to be higher than the general population in both vWD and FXI deficiency. This study also highlights the high risk of bleeding complications associated with miscarriages. FVIII and vWF levels do not rise significantly until the second trimester by which stage many miscarriages have already occurred. Therefore, factor levels should be checked prior to termination of pregnancy and in women presenting with spontaneous miscarriage and prophylactic treatment arranged when factor levels are < 50 iu/dl.

Labour and delivery are critical periods for women with bleeding disorders and their affected fetuses. The latter are potentially at risk of haemorrhage from the process of birth, invasive monitoring techniques or instrumental deliveries. Therefore, it is recommended that delivery should be achieved by the least traumatic method. Prolonged labour (especially prolonged second stage of labour) and difficult instrumental deliveries (especially vacuum extraction) should be avoided and early recourse to Caesarean section should be considered. However, low forceps delivery may be considered less traumatic than Caesarean section when the head is deeply
engaged in the pelvis and delivery is performed by an experienced obstetrician. An elective Caesarean section has been recommended for delivery in women with type 2 and type 3 vWD (Chediak et al 1986) to avoid bleeding complications in the newborn infant. This study, included two vaginal deliveries in a woman with type 2B vWD and a vaginal delivery and a low forceps in two women with type 3 vWD, with no neonatal complications. Three of these babies were later confirmed to have mild vWD.

Affected babies are also at risk of bleeding complications from postnatal invasive procedures including intramuscular injections, immunisation and surgical interventions. Therefore vitamin K should be given orally to the neonate and immunisation by intradermal route and hepatitis B immunisation should be considered (Walker et al 1994). Intramuscular injections should be avoided and the parents should be advised to postpone circumcision until the diagnosis of bleeding disorders has been confirmed or refuted in the neonate. One of the newborns of FXI deficient mothers in this study had bleeding after circumcision requiring blood transfusion. When assessing the neonatal factor levels it should be appreciated that these are low at birth and correlate with gestational age (Andrew et al 1981) and reach adult levels at the age of 6 months (Andrew et al 1987). Thus, although severe forms of these disorders can be diagnosed at the time of birth, mild forms are not always readily diagnosed, and the child should be screened later during the first year of life.

Successful epidural analgesia/anaesthesia for labour and delivery in women with type 1 vWD and normal factor levels has been reported (Cohen et al 1989, Milaskiewicz
et al 1990). Recently, Jones et al (1999) also reported an uncomplicated epidural analgesia in a woman with type 2A vWD after normalisation of coagulation status with vWF concentrate. However, most anaesthetists are reluctant to perform spinal or epidural anaesthesia in these women. In this series, regional analgesia/anaesthesia for labour or Caesarean section was used in 8 vWD and 2 FXI deficient pregnancies with no complication. Therefore, these women should not be denied the benefits of regional blocks. Each case should be assessed individually and, provided that clotting factors are >50 iu/dl during the third trimester and the coagulation screen is normal when the women presents in labour, these procedures could be made available.

The high incidence of primary PPH in vWD, in particular in women with variant vWD, is well documented (Greer et al 1991, Ramsahoye et al 1995, Foster 1995). This study shows the risk of primary PPH associated with vWD to be 18.5% and the majority of cases occurred when maternal clotting factor was <50 iu/dl with no prophylactic treatment for labour. As maternal coagulation factor activity falls rapidly after delivery the risk of secondary PPH is even higher with a reported incidence of 25% (Ramsahoye et al 1995) to 28% (Greer et al 1991). In this study group, the incidence of secondary PPH was 20%. None of the women with vWD deficiency who received prophylactic treatment for labour and puerperium had any significant bleeding complication. DDAVP can be used in women with type 1 vWD and some of women with type 2A disease as they respond favourably to this treatment. However, women with type 3 vWD do not respond to DDAVP and it is contraindicated in women with type 2B disease as it can precipitate thrombocytopenia in these patients (Holmberg et al 1983). Prophylactic therapy with vWF containing blood products has been advocated for all women with type 2 and 3 vWD (Greer et al 1991, Foster
The treatment should start at the onset of labour aiming to raise FVIII and vWF:Ac to above 50 iu/dl and maintaining this for 3-4 days after vaginal delivery and 4-5 days after Caesarean section (Walker et al 1994).

The incidence of primary and secondary post-partum haemorrhage has not been determined in women with FXI. However, it has been reported that childbirth in FXI deficient patients is accompanied by relatively few problems (Bolton-Maggs et al 1988). The results of this study contrast with this as the incidence of primary and secondary post-partum haemorrhage was 16% and 24%, respectively. Due to the unpredictable nature of bleeding tendency and its poor relation to FXI level (Bolton-Maggs et al 1988) and because of limited experience of pregnancy management in women with FXI deficiency, labour and delivery should be managed with caution, in a centre where appropriate treatment with blood products can be given promptly if there is any bleeding complication. Prophylaxis to cover Caesarean section should be considered especially in homozygous patients. Fresh frozen plasma (FFP) provides adequate haemostatic cover for operative procedures but carries the risk of viral transmission. Because of this risk, FXI concentrate was introduced and its efficacy at preventing bleeding has been confirmed (Collins et al 1995). Recent data have shown that FXI concentrate is associated with thrombosis (Bolton-Maggs et al 1994, Collins et al 1995) and this should be considered when used in pregnancy or the post-partum period. In this study FXI concentrate was used prophylactically for an amniocentesis, a Caesarean section and a normal vaginal delivery in 2 homozygous patients without any complications.
This chapter concludes that pregnancy, labour and puerperium are associated with particular problems in women with vWD or FXI deficiency and their offspring. Increased awareness and better understanding of these disorders by the obstetricians and close collaboration with the local haemophilia centre is essential to achieve optimal outcome and to minimise maternal and neonatal complications. Management guidelines should be available and strictly followed by all the staff involved in the patients’ management.
CHAPTER 6

VARIATIONS IN COAGULATION FACTORS IN WOMEN

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6.1 : INTRODUCTION

A wide range of values for coagulation screen and clotting factors has been reported in both normal individuals and patients with bleeding disorders, with a small but considerable overlap between patients with mild disorders and normal subjects. In addition, a wide intraindividual variability has been reported. These variations have been speculated to be the effect of different physiological and pathological factors, including age, sex, blood group, stress, exercise, smoking, drugs (especially oestrogen containing contraceptives), and hormonal changes during the menstrual cycle in women. These variations can have important clinical implications in the diagnosis and management of coagulation disorders.

The aim of this study to is to investigate possible fluctuations in surface induced coagulation time (activated partial thromboplastin time, APTT) and coagulation factors; FVIII, vWF:Ag, vWF:Ac, FXI and fibrinogen during the normal menstrual cycle, and to monitor possible changes induced by the new low dose oestrogen combined oral contraceptive pills. In addition, to illustrate the benefits of longitudinal data over cross-sectional data when investigating time trends in these laboratory variables. The effect of women's characteristics and behaviour (weight, blood group, ethnic background, alcohol consumption and smoking) on the levels of the coagulation factors were also assessed.
6.2 : SUBJECTS, MATERIALS AND METHODS

Cross-sectional study

As part of a cross-sectional study on the frequency of inherited bleeding disorders in women with menorrhagia, information on APTT, FVIII, vWF:Ag, vWF:Ac and FXI levels were collected in 150 women. These women aged 16-50 years, were referred to the gynaecology out-patient clinics at the Royal Free Hospital complaining of heavy regular (23-39 day cycle) periods with no history of recent (within the last 2 months) ingestion of anticoagulants, antifibrinolytics, non-steroidal anti-inflammatory medications, combined oral contraceptives and progestagens. For the purposes of this analysis, only those in whom no bleeding disorder (n = 123) was diagnosed were included in the study. Levels of the five laboratory markers were compared in those of different ethnic origins, in those with different blood groups, and in women on different days of their menstrual cycle.

Longitudinal study

Forty healthy Caucasian women with 28-30 day regular menstrual cycles participated in this part of the study. Of these, 20 had been on a low dose oestrogen monophasic combined oral contraceptive pill (≤ 30 μg) for at least 4 months and the remaining 20 had not used any hormonal contraception for at least 4 months. One of the latter group was found to have mild vWD and was excluded from further analysis. Details of the 39 women included in the study are presented in Table 6.1. Blood samples were collected on four occasions in all 39 women enrolled in the study. Samples were collected on days 2 (range 1-4), 8 (range 6-11), 15 (range 10-18) and 21 (range
20-25) in these individuals. A further sample was available from 15 of the women on day 28 (range 24-30). In order to obtain more detailed information about changes in the measurements over time, samples were collected from two of the women not using oral contraception at two-day intervals. All samples were collected between 9-11 a.m. The individuals were asked to rest for at least 15 minutes prior to blood sampling. The samples were obtained by direct venepuncture and collected into citrated tubes and analysed at the Haemophilia Centre within 2 hours of collection.

Table 6.1: Details of 39 women included in longitudinal study

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Pill users</th>
<th>Non pill users</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of women</td>
<td>39</td>
<td>20 (51%)</td>
<td>19 (49%)</td>
</tr>
<tr>
<td>Mean (sd) age</td>
<td>26 (5.5) years</td>
<td>26.0 (4.4)</td>
<td>27.5 (6.5)</td>
</tr>
<tr>
<td>Mean (sd) weight</td>
<td>62.6 (9.18) kg</td>
<td>65 (8.0)</td>
<td>61.6 (10.3)</td>
</tr>
<tr>
<td>Smoker</td>
<td>10 (26%)</td>
<td>6 (30%)</td>
<td>4 (21%)</td>
</tr>
<tr>
<td>Alcohol consumption</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 units/week</td>
<td>12 (%)</td>
<td>3 (15%)</td>
<td>9 (47%)</td>
</tr>
<tr>
<td>1-7 units/week</td>
<td>14 (36%)</td>
<td>7 (35%)</td>
<td>7 (37%)</td>
</tr>
<tr>
<td>8-14 units/week</td>
<td>10 (26%)</td>
<td>7 (35%)</td>
<td>3 (16%)</td>
</tr>
<tr>
<td>&gt;14 units/week</td>
<td>3 (8%)</td>
<td>3 (15%)</td>
<td>0</td>
</tr>
</tbody>
</table>

ds, standard deviation
STATISTICAL ANALYSIS

Levels of the five markers obtained from the cross-sectional study were compared according to ethnicity, blood group and day of the cycle using analysis of variance (ANOVA). Multivariate comparisons of the cross-sectional data were performed using linear regression methods.

In order to illustrate the longitudinal data, means and standard deviations of all values were calculated at each time point. Initial analyses used repeated measures ANOVA to investigate whether there were differences in each value over time. This method of analysis is an extension of the standard AVONA method in which time is considered as a factor in the analysis. The primary aim of this analysis was to identify whether there were any differences in the overall average values according to the day of the cycle. The method does not, however, make full use of the fact that measurements within an individual are correlated and provides a fairly inefficient method of studying the patterns of change within individuals over a month.

In order to study these patterns of change in more detail, multilevel modelling methods were used. These methods were originally developed in educational research and are specifically designed for situations where the data is hierarchical in nature, as in this study. For example, APTT levels within an individual are linked by some biological mechanism which controls both the absolute level and changes in these levels over time. If a woman has a low APTT level on one day of the monthly cycle, relative to other women on the same day of the cycle, then it is likely that she will also have a relatively low level on other days. Measurements on two different women
are not likely to be related, although they may share common features, such as a general tendency towards a monthly ‘cycle’ in their values. Thus, this is a two-level hierarchy where the APTT value at any time point is dependent on individual characteristics, but may also share characteristics at a population level with APTT values in other women. Thus, some information about changes in a marker over time in one woman can be obtained from the trends seen in the other women in the population. Because of the use of this shared information, these methods provide a powerful means for testing the effect of covariates on the pattern over time.

A multi-level model of this sort is usually made up of two parts: a fixed part which describes how the overall mean laboratory value changes over time in the group of women studied, and a random part, which allows each woman’s individual values to deviate from this population mean.

A visual inspection of the changes in the markers over time and the knowledge that these variables must return to baseline levels by the end of the monthly cycle, suggested that simple linear or polynomial relationships with time were not suitable for these data and therefore a model was fitted which allowed the data to take a cyclic form by the inclusion of sine and cosine terms. Each individual is assumed to follow a common cyclic pattern over the month, although the baseline level for each individual is allowed to vary, allowing for natural variation between individuals. The models were fitted separately for women who were users of the oral contraceptive pill, and those who were not. Simpler models, including linear and quadrate models, were assessed but found not to provide a good fit of the data.
The impact of age (as a continuous variable), weight (above/below median), blood group (A/others), alcohol use (yes/no) and smoking status (yes/no) on the pattern of these laboratory markers were investigated in these models. All analyses were carried out using the Statistical Analysis System (SAS) and M1n software packages.

LABORATORY METHODS

Activated Partial Thromboplastin Time (APTT)

APTTs were performed in duplicate on the FUTURA Coagulometer (Instrumentation Laboratories (IL UK) Ltd, Kelvin Close, Birchwood Science Park, Warrington, Cheshire). 50μl of platelet poor plasma (PPP) was incubated with 50μl of a cephalin and micronised silica (APTT Lyophilised Silica, IL) for five minutes at 37°C, before initiation of clotting with 50μl of 25mM CaCl₂.

Fibrinogen

Fibrinogen estimations were performed using the Clauss method, adapted to use on the FUTURA (Clauss1957). When a high concentration of thrombin is added to diluted plasma, the thrombin clotting time is inversely proportional to the fibrinogen concentration. Automatic recording of the thrombin time is performed on the diluted test plasma, and fibrinogen concentration read from a standard curve prepared from a known fibrinogen standard (1st International Reference Plasma for Blood Coagulation Factors, National Institute for Biological Standards and Control (NIBSC), Blanche Lane, South Mimms, Potters Bar, Herts). The FUTURA was programmed to calibrate a standard curve and to dilute test and control plasmas in
Owrens Buffered Saline (OBS) before the addition of bovine thrombin 90u/ml (Baxter Diagnostics Inc. Distributed by Gamidor Ltd, 67 Miton Pk, Abingdon, Oxfordshire, OX14 4RX).

**Factor VIII and XI Assays**

Three point APTT based, one-stage assays were performed on the ACL1000 Coagulometer (IL). Patient PPP was diluted 1:10, 1:20 and 1:40 in OBS and compared to a three-point standard curve (log linear) derived from pooled normal plasma (calibrated against the 19th British Standard for FVIII, NIBSC). The method for both assays was identical except for the type of deficient plasma used. Factor VIII deficient plasma was obtained from Diagen (Diagnostic Reagents Ltd, Thame, Oxon) and factor XI from Immuno Ltd (UK), (Arctic House, Rye Lane, Dunton Green, Kent TN14 5HB).

**ELISA for von Willebrand Factor Antigen (vWF:Ag)**

Polyclonal anti-vWF:Ag (Dako LTD, 16 Manor Courtyard, Hughenden Ave, High Wycombe, Bucks) was diluted 1/1000 in bicarbonate buffer (1.59gms Na$_2$CO$_3$, 2.93gms NaHCO$_3$ in 1 litre distilled water, pH 9.6). 100µl of diluted coat antibody was added to each well of a microtitre plate (Nunc polysorb microtitre plates, Life Technologies Ltd, PO Box 35, Trident House, Washington Rd, Paisley, Scotland). The plate was sealed and left at 4°C overnight. A seven-point standard curve (125 to 6.25 iu/dl) was prepared using calibrated twenty normal pool (20NP) (6th British Standard NIBSC), initially diluted 1/80 (125u/dl). Test plasmas and controls (20NP and Baxter Dade abnormal control plasma "trol P") were diluted to give a vWF:Ag concentration within the linear range of the standard curve (1/100 and 1/200). The
coated plate was washed five times with a high salt wash buffer (HSBT: 95g NaH$_2$PO$_4$·2H$_2$O, 141.1gms NaCl, 13.4gms Na$_2$HPO$_4$·12H$_2$O, 10ml Tween 20 in 5 litres of distilled water, pH 7.4) using a plate washer (Well Wash 5, Stacking Microtitre Plate Washer, Denley, Natts Lane, Billinghamurst, Sussex) then inverted and blotted gently on absorbent paper. Test, control or standard curve dilutions were added in 100μl volumes in duplicate to the plate. The plate was sealed and incubated on a plate shaker (400-500 oscillations per minute) (Amersham International plc, Lincoln Place, Green End, Aylesbury, Bucks) for one hour at room temperature. At the end of incubation the plate was washed a further five times, after which 100μl of diluted (1/8000) horseradish peroxidase conjugated anti-vWF antibody (Dako) was added to each well of the plate. The plate was again incubated for one hour on a plate shaker at room temperature. Just before the end of incubation the substrate solution was prepared by dissolving one 10mg ortho-phenyline-diamine (OPD) tablet (Sigma Chemical Company Ltd, Fancy Rd, Poole, Dorset) in 15ml substrate buffer (7.3gms citric acid, 23.87gms Na$_2$HPO$_4$·12H$_2$O in 1 litre of distilled water, pH 5.0). The plate was then washed a final five times. Immediately before the next step, 7μl of 30% hydrogen peroxide was added to the substrate solution. 100μl of substrate solution was then added to each well at timed intervals (approximately one second). After ten minutes the reaction was stopped by the addition of 100μl 1.5M sulphuric acid to each well at the same interval. The absorbance of each well of the plate was then read within 30 minutes at 492nm by a Titertek plate reader' (ICN, Flow Biomedicals LTD, Eagle House, Peregrine Business Park, Gomm Rd, High Wycombe, Bucks). The plate reader software calibrated a vWF:Ag standard curve (optical density against vWF:Ag concentration (iu/dl) on a linear scale) and then calculated the mean test and control results from the curve (Cejka 1982).
ELISA for von Willebrand Factor Activity (vWF:Ac)

The assay method is essentially the same as described above but patient and control samples were diluted 1:25 and 1:50, and the standard stock of 125iu/dl, diluted 1:20 (Murdock et al 1997).
6.1: RESULTS

Cross-sectional study

The age of women included in this part of the study ranged from 15 to 50 years (median age 39 years) at the time of the study. 49 (39.8%) were blood group A, 48 (39.1%) were blood group O, 17 (13.8%) were blood group B and the remaining 9 (7.3%) were blood group AB. The women were predominantly of Caucasian origin (72 women, 58.5%), although a significant majority were black (19 women, 15.4%) or of Mediterranean origin (14 women, 11.4%). The remaining 18 women were from other ethnic backgrounds. Women attended the hospital on a wide range of days of the monthly cycle (median day 14, range 1-33).

APTT, FVIII, vWF:Ag, vWF:Ac and FXI levels were available for all 123 women included in the study, and means and standard deviations are shown in Table 6.2. Figure 6.1 (a & b) shows these laboratory values split according to blood group. There were significant differences in the APTT values according to blood group, with women of blood groups B or O having longer APTT than those of groups A or AB (p=0.01, ANOVA). There were no differences in any of the other laboratory values according to blood group. Figure 6.2 (a & b) shows the laboratory values split according to ethnic group. Whilst there were no differences in either APTT or FXI levels in the four groups of women, levels of FVIII, vWF:Ag and vWF:Ac were significantly higher in black women than in those of other ethnic origins. These relationships remained after adjusting for blood group differences between the groups (p < 0.001 in each case). In contrast after adjusting for differences in the blood group
of women of different ethnicities, APTT levels were slightly lower in black women (p = 0.01). APTT levels remained higher in those blood group A (P = 0.008) in multivariate analyses.

In order to assess whether the levels of any of these markers differed over the menstrual cycle, values were grouped into those which were taken in the early follicular stage (days 1-6, 32 women, 26.0%), the late follicular stage (days 7-11, 25 women, 20.4%), during ovulation (days 12-16, 21 women, 17.1%) and in the luteal stage (after day 16, 45 women, 36.6%). Figure 6.3 (a & b) shows the values of each laboratory marker obtained in the different stages. From this cross-sectional analysis, there were no significant differences in the levels of any marker according to the stage of menstrual cycle.

Table 6.2: Laboratory markers in 123 women included in cross-sectional study

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Standard deviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTT (seconds)</td>
<td>31.3</td>
<td>3.4</td>
</tr>
<tr>
<td>FXI (iu/dl)</td>
<td>106.2</td>
<td>23.4</td>
</tr>
<tr>
<td>FVIII (iu/dl)</td>
<td>126.9</td>
<td>40.0</td>
</tr>
<tr>
<td>vWF:Ag (iu/dl)</td>
<td>113.4</td>
<td>37.5</td>
</tr>
<tr>
<td>vWF:Ac (iu/dl)</td>
<td>106.8</td>
<td>33.7</td>
</tr>
</tbody>
</table>
Figure 6.1 (a) - Values of APTT, FVIII and vWF:Ag according to blood group

- APTT (secs): p=0.01
- FVIII (u/dl): p=0.86
- vWF:Ag (u/dl): p=0.29
Figure 6.1 (b) - Values of vWF:Ac and FXI according to blood group

- vWF:Ac (u/dl)
  - A
  - B
  - AB
  - O
  - p=0.09

- FXI (u/dl)
  - A
  - B
  - AB
  - O
  - p=0.74
Figure 6.2 (a) - Values of APTT, FVIII and vWF:Ag according to ethnic origin.
Figure 6.2 (b) - Values of vWF:Ac and FXI according to ethnic origin

![Graph showing distribution of vWF:Ac and FXI levels by ethnic origin with p-values for comparison between groups.]

- **vWF:Ac (u/dl)**
  - Cauc.: Lower values, <100 u/dl
  - Medit.: Mid-range values, 100-150 u/dl
  - Black: Higher values, >150 u/dl
  - Others: Mixed values, varying across range

- **FXI (u/dl)**
  - Cauc.: Lower values, <100 u/dl
  - Medit.: Mid-range values, 100-150 u/dl
  - Black: Higher values, >150 u/dl
  - Others: Mixed values, varying across range

- **p-values:**
  - p=0.003 for vWF:Ac
  - p=0.29 for FXI

The graphs illustrate the significant difference in vWF:Ac levels across ethnic groups, with a p-value of 0.003 indicating statistical significance. The p-value for FXI levels is 0.29, suggesting no significant difference among groups.
Figure 6.3 (a) - Values of APTT, FVIII, and vWF:Ag according to the day of menstrual cycle

- APTT (secs)
  - 1-6
  - 7-11
  - 12-16
  - >16
  - p=0.95

- FVIII (u/dl)
  - 1-6
  - 7-11
  - 12-16
  - >16
  - p=0.68

- vWF:Ag (u/dl)
  - 1-6
  - 7-11
  - 12-16
  - >16
  - p=0.69
Figure 6.3 (b) - Values of vWF:Ac and FXI according to the day of menstrual cycle

\[
p=0.85
\]

\[
p=0.59
\]
Longitudinal study

Laboratory values from the two women who had blood sampling at two daily intervals are shown in Figure 6.4 (a & b). All measurements showed a high level of variation both within each individual and between the two individuals over the monthly cycle. vWF:Ac and fibrinogen levels appear to show the most consistent patterns between the two women, with both measures dropping slightly throughout the first half of the month before returning to baseline by day 28 of the cycle.

The means and standard deviations of the six measurements (APTT, FVIII, vWF:Ag, vWF:Ac, FXI and fibrinogen) at each of the five time points in pill users and non-pill users are shown in Figure 6.5 (a & b). Overall, repeated measures ANOVA identified significant time effects for the levels of vWF:Ag (p=0.03) and vWF:Ac (p=0.01). In each case, levels in both groups of women dropped over the first week of the cycle before increasing to peak levels at the end of the third week. As expected, levels of both measurements returned to baseline levels by day 28. The change over the monthly cycle was more pronounced in non-pill users, although the interaction between oral contraceptive pill use and the time effect was only significant for vWF activity levels (p=0.01). Fibrinogen levels were also found to vary significantly over time (p=0.01) although the magnitude of the effect was small. Again, this effect appeared to be more pronounced in non-pill users, although the interaction between pill use and the time effect was not significant. Repeated measures ANOVA of APTT, FVIII and FXI levels revealed no significant time effects.
Figure 6.4 (a) - Longitudinal measurements (APTT, FVIII and vWF:Ag) in two individuals over a one month cycle.
Figure 6.4 (b) - Longitudinal measurements (vWF:Ac, FXI and Fibrinogen) in two individuals over a one month cycle.
Figure 6.5 (a) - Means and standard deviations of APTT, FVII and vWF:Ag values on days 1, 7, 14, 21 and 28 of the menstrual cycle separately for pill users and non-pill users.
Figure 6.5 (b) - Means and standard deviations of vWF:Ac, FXI and fibrinogen values on days 1, 7, 14, 21 and 28 of the menstrual cycle separately for pill users and non-pill users.
Multi-level modelling results:

**APTT**

Over the month, the mean APTT level was 32.9 seconds in all women, with most women having values between 28.4 and 37.4 seconds. APTT levels showed no evidence of any cyclic pattern over the monthly period in non-pill and pill users ($p = 0.23$ and $p = 0.97$, respectively, for inclusion of cyclic pattern). There were no significant associations between APTT levels and either age, weight, blood group, alcohol consumption or smoking status.

**FVIII**

In pill users, FVIII levels were found to display a cyclic pattern ($p=0.05$). On day one of the monthly cycle, average FVIII levels were found to be 106.0 iu/dl, with 95% of individuals having a value between 68.1 and 143.9 iu/dl. These levels decreased to a mean value of 103.4 iu/dl on day 5, before rising to a peak level of 113.4 iu/dl on day 20. Levels then fell to baseline over the remainder of the month. There was some evidence that the four women with blood group A had different FVIII patterns to the other women in the group, although this was marginally non-significant ($p=0.07$). However, there were no significant relationships between FVIII levels and either age, weight, alcohol consumption or smoking status.

Among non-pill users, there was no evidence of any cyclic pattern to FVIII levels over time ($p=0.36$). The mean FVIII level was 106.5 iu/dl, with the majority of
women having values in the range 60.3 to 152.7 iu/dl. There were no relationships between these values and the other factors studied in the model. Figure 6.6 shows the fitted patterns of FVIII levels for pill users and non-pill users.

Figure 6.6 - Fitted multilevel models showing FVIII levels over a 28 day cycle, separately for oral contraceptive pill users and non-users.
vWF:Ag and vWF:Ac

In users of the oral contraceptive pill, vWF:Ag levels showed a strong cyclic pattern over the month (p=0.01). On day one, levels ranged from 43.8 to 136.8 iu/dl in the majority of women (mean value 90.3 iu/dl). These levels dropped to a mean value of 86.0 iu/dl on day 6 before rising to a peak value of 100.8 iu/dl by day 20. Levels then dropped towards baseline. A similar pattern was noted for vWF:Ac levels (p=0.003), with levels dropping from 80.9 iu/dl on day one to 78.7 iu/dl on day 5. Values then rose to peak levels of 92.3 iu/dl on day 19, before falling again over the remainder of the month. A similar spread of values was seen, with most women having vWF:Ac levels between 39.4 and 122.4 iu/dl on day one. Again, neither age, weight, blood group, alcohol consumption or smoking status were associated with either vWF antigen or activity levels.

In women who did not use the pill, even stronger cyclic patterns in both markers were reported (p=0.003 and p=0.0002 for vWF:Ag and vWF:Ac respectively). vWF:Ag dropped from a mean value of 107.8 iu/dl on day one to a mean of 94.8 iu/dl on day 9. Levels then rose to a peak value of 118.0 iu/dl on day 23, before dropping towards baseline levels. The wide variability in baseline values was evident in this group of women, with levels on day one ranging from 39.0 to 176.6 iu/dl. vWF:Ac fell from a mean baseline level of 103.3 iu/dl to a mean value of 84.3 iu/dl on day 10. Levels then rose to a peak value of 112.1 iu/dl on day 24 before falling again. Of interest, in this group of women there were also strong associations between the levels of both laboratory markers and age, with levels rising by 16.6 and 15.1 iu/dl respectively for each 10 year increase in age. This age effect was
significant for both markers (p=0.02 and p=0.04 for vWF:Ag and vWF:Ac respectively). Figures 6.7 and 6.8 show the fitted values of vWF antigen and activity in pill users and non-pill users.

**Figure 6.7 - Fitted multilevel models showing vWF antigen levels over a 28 day cycle, separately for oral contraceptive pill users and non-users.**
Figure 6.8 - Fitted multilevel models showing vWF activity levels over a 28 day cycle, separately for oral contraceptive pill users and non-users.

FXI levels

FXI levels showed no cyclic relationship over the monthly period in either pill users (p=0.17) or non-users (p=0.32). Mean levels of FXI were 94.0 iu/dl (with most values in the range 68.4 to 119.6 iu/dl) in pill users and 92.0 iu/dl (range 63.1 to 121.0 iu/dl) in non-pill users. There were no significant relationships between FXI levels and age, weight, alcohol consumption, smoking status or blood group, in either of the groups of women studied.
Fibrinogen

Mean fibrinogen levels were 2.69 g/l (range 1.89 to 3.49 g/l) in pill users with no evidence of a cyclic pattern over the month \( (p=0.62) \). None of the cofactors studied were significantly associated with these levels. Amongst non-pill users, however, there was a strong and significant cyclic effect over the month \( (p=0.00003) \). On day one, levels ranged from 2.14 to 3.42 g/l (mean value 2.78 g/l). This level dropped to 2.48 g/l by day 11 before rising to a peak value of 2.85 g/l towards the end of the month. There was no relationship between these values and either age, weight, alcohol consumption, smoking status or blood group. Figure 6.9 shows the fitted values of fibrinogen in pill users and non-pill users.

Figure 6.9 - Fitted multilevel models showing fibrinogen levels over a 28 day cycle, separately for oral contraceptive pill users and non-users.
Table 6.3 summarises the results of multi-level modelling analyses.

Table 6.3 - Menstrual cycle variation (multi-modelling analysis)

<table>
<thead>
<tr>
<th></th>
<th>Non OC Users</th>
<th>P value</th>
<th>OC users</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>APTT</td>
<td>No</td>
<td>0.97</td>
<td>No</td>
<td>0.23</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>Yes</td>
<td>0.0003*</td>
<td>No</td>
<td>0.62</td>
</tr>
<tr>
<td>FVIII</td>
<td>No</td>
<td>0.36</td>
<td>Yes</td>
<td>0.05*</td>
</tr>
<tr>
<td>vWF:Ag</td>
<td>Yes</td>
<td>0.01*</td>
<td>Yes</td>
<td>0.003*</td>
</tr>
<tr>
<td>vWF:Ac</td>
<td>Yes</td>
<td>0.0002*</td>
<td>Yes</td>
<td>0.003*</td>
</tr>
<tr>
<td>FXI</td>
<td>No</td>
<td>0.32</td>
<td>No</td>
<td>0.17</td>
</tr>
</tbody>
</table>

OC, oral contraceptives; *, statistically significant
6.4 : DISCUSSION

In the cross-sectional part of this study the influence of certain physiological parameters on the haemostatic balance in women was assessed. The effect of ABO blood type is well known and FVIII:C and vWF:Ag have been shown to be significantly lower in carriers of haemophilia and normal women with type O blood group (Wahlberg et al 1980, Graham et al 1986). In this study, APTT was significantly longer in women with blood group B or O in comparison to those with group A or AB. There was also a tendency for vWF:Ac, vWF:Ag and FVIII:C to be lower in women with blood group O but the difference was not statistically significant. It is possible that the additive effect of these 3 factors together led to prolongation of APTT.

FVIII:C, vWF:Ag, vWF:Ac were significantly higher in black women than in those of other ethnic origins (Figure 6.2). Werner et al (1993) showed lower vWF:Ac and vWF:Ag in a population with blood group O but did not report any significant differences by ethnicity. However, Werner et al (1993) did not exclude patients with vWD from their series and it is possible that the low levels from these patients skewed their results. In addition, the inclusion of both males and female in that study may have also affected their results as women are known to have lower vWF:Ag and vWF:Ac levels than men (Werner et al 1993).

As vWF tends to increase with age, bleeding manifestations become milder and less common. It has been reported that individuals with type 1 vWD may become phenotypically normal as they grow older (Zimmerman & Ruggeri 1987). This was
confirmed in this study with a rise of 0.17 and 0.15 U/ml for each 10 year increase of age for vWF:Ag and vWF:Ac, respectively.

In the cross-sectional part of this study, no significant differences were found in the levels of haemostatic markers according to the stage of the menstrual cycle. However, large interindividual variations in the levels of these clotting factors are documented (Abildgaard et al 1980, Blombäck et al 1992). Hence, it was decided to perform a longitudinal study to assess this further.

Previous longitudinal studies on the variation of coagulation factors during the normal menstrual cycle have produced conflicting results (Beller et al 1964, Cederblad et al 1977, Manadalaki et al 1980, Jespersen 1983, Siegbahn et al 1989, Lebech & Kjaer 1989) possibly due to the relatively small numbers of women who have been studied and the statistical methodology used to analyse such data. The wide inter- and intra-individual biological variation in these factor levels means that real differences between the different phases of the menstrual cycle may be missed unless large groups of women are studied using approaches which explicitly take account of the wide variation between individuals. In this longitudinal study, changes over time were studied using multi-level modelling methods. The results of these analyses revealed some interesting patterns in the coagulation markers over the 28 day period. These include a lack of cyclic variation for APTT and FXI in both non-pill and pill users. Fibrinogen, vWF:Ag and vWF:Ac, however, showed a strong cyclic variation with peak values in the luteal phase. This pattern, although still present, was dampened for vWF:Ag and vWF:Ac but completely disappeared for
fibrinogen with the use of combined oral contraceptives. Surprisingly, there was a cyclical pattern for FVIII:C in pill users, which was not evident in non-pill users.

Awareness of the intraindividual variation of coagulation markers and the factors that affect this has important clinical implications. Intraindividual variability is greater in patients with type 1 vWD (Abildgaard et al 1980) making the diagnosis of mild forms of vWD difficult. As most female hormones are at their baseline in the early follicular phase (no later than day 7 of the cycle), blood sampling for assessment of clotting factors during this phase is recommended. Edlund et al (1996) reported a very low variation when restricting sampling to days 5-7 of the cycle. In addition, diagnosis should be based on several samples especially when the mild form of vWD is being considered. FVIII:C, vWF:Ag and vWF:Ac were at their nadir during the early follicular phase in this study population and this has also been shown by others (Blombäck et al 1992, Edlund et al 1996). Therefore, it is advisable to avoid any planned surgical intervention during this phase in patients with mild vWD.

The variability of FXI if any, during the menstrual cycle has not been assessed in any study in the past. The results of this study show that that FXI has no cyclical variation with the menstrual cycle and there was no significant difference between the levels in pill or non-pill users. It seems that FXI is not affected by female sex hormones as there is no significant change in FXI levels during pregnancy (as shown in Chapter 5 of this thesis) or by the use of combined oral contraceptives (Egeberg & Owren 1963). This study has also demonstrated that there is no significant relationship between FXI level and blood group, ethnicity, age, weight, alcohol consumption or smoking status.
A review of the literature on the effect of combined oral contraceptives on coagulation factors reveals large variations of study designs and results. Whilst the majority of the studies demonstrated an increase in fibrinogen, prothrombin, FVII, FVIII and vWF, other factors (FV, FIX, FXI and FXIII) do not seem to be affected. The effect is dose dependent and related to oestrogens and appreciable above a dose of 0.5 μg of ethynylestradiol (Beller & Ebert 1985). Smaller doses of oestrogen (i.e. < 0.5 μg) are less effective and may not be significant. However, a lack of any significant oestrogen dose effect on venous thromboembolic risk has been shown (WHO Collaborative Study of Cardiovascular Disease and Steroid Hormone Contraception 1995). The present study found no significant differences between the mean values of APTT, fibrinogen, FVIII:C, vWF:Ag, vWF:Ac and FXI in pill and non-pill users, but the women in the study were using low dose 0.3 μg oestrogens. The use of combined contraceptives, however, does affect the menstrual cycle variations of some of these factors, especially FVIII:C and vWF. This effect was inconsistent as the cyclical variation was stronger for FVIII:C, a finding which cannot be explained, but dampened for vWF:Ag and vWF:Ac. Assuming an effect of the menstrual cycle on the levels of these parameters, it can be speculated that users of the pill may, if anything, experience less pronounced variation in their cycles. Thus, studies with more frequent measurements of coagulation factors and sex hormones are required to confirm these changes.

There are some limitations in this study. Firstly, measurements were only available at four or five time points in the cycle for the majority of the women. With this limited number it is only possible to fit smooth, simple curves which can be
described by a small number of parameters. It is possible that in this case a number of
different models may fit the data equally well, and it is hard to judge which is the
preferred model. In particular, the possibility of short term peaks in the levels of
these parameters which occur between the measurement times cannot be eliminated
without more frequent measurements. More frequent samples in two individuals were
studied, however, the extreme levels of variability meant that it was difficult to
identify any such peaks in the data. Secondly, plasma levels of oestradiol and
progesterone were not determined in women included in this study. Therefore, for
non-pill users, there was no confirmatory evidence that the cycle studied was
ovulatory. In addition, it would have been possible to demonstrate the correlation
between various coagulation markers and sex hormones, if plasma oestradiol and
progesterone levels had been assessed at the same time as measuring coagulation
factors.
CHAPTER 7

INHERITED BLEEDING DISORDERS IN
PATIENTS PRESENTING WITH
MENORRHAGIA

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7.1 : INTRODUCTION

Excessive menstrual bleeding is a common condition and about 5% of women aged 30-49 years consult their General Practitioner with this problem (Royal College of General Practitioners 1986). It also accounts for 12% of all gynaecology referrals (Bradlow et al 1992). Menorrhagia has been attributed to a number of local or systemic disorders, but in more than half of the women no organic pathology is found (Rees 1987). Profuse menstrual blood loss has been reported in patients with von Willebrand's disease (Evans 1971, Fraser et al 1986, Greer et al 1991), carriers of haemophilia (Lusher & McMillan 1978), and FXI deficiency (Bolton-Maggs et al 1995). However, testing for these disorders is not part of the routine investigation for patients with menorrhagia and there is little published data on this subject.

The aim of this study was to assess the prevalence of inherited bleeding disorders in patients presenting with menorrhagia and to determine whether patients' history might be predictive of these disorders. It was decided to measure APTT as well as FVIII:C, vWF:Ac and vWF:Ag as vWD is the commonest inherited bleeding disorder. FXI was also measured for two reasons; first, the Royal Free Hospital is located in an area with a high Jewish population and therefore a high incidence of FXI deficiency. Second, heterozygote patients have a partial deficiency of FXI and their bleeding symptoms are usually trivial and only revealed after a haemostatic challenge; therefore, they may be under-diagnosed.
7.2: PATIENTS AND METHODS

Patients, aged 16-50 years, referred consecutively (October 1995- June 1997) to the gynaecological clinics at the Royal Free Hospital complaining of heavy regular (23-39 day cycles) periods with no known bleeding or endocrine disorders, no history of recent (within the last 2 months) use of any intra-uterine device or ingestion of anticoagulants, antifibrinolytics, non-steroidal anti-inflammatory medications, combined oral contraceptives, and progestagens were recruited for this study. All patients underwent gynaecological examination and pelvic ultrasound and patients were excluded if any pelvic pathology that might be associated with menorrhagia (any submucous uterine fibroids, other fibroids more than 2 cm in diameter, uterine polyps and ovarian tumours including endometriomas) was detected. The patients were interviewed and after obtaining an informed consent, a thorough history was taken including menstrual history, history of other bleeding symptoms including easy bruising, nose bleeding, gum bleeding, post-operative and post-partum bleeding, bleeding after tooth extraction as well as family history of inherited bleeding disorders. Patients were then taught to use and complete a pictorial blood assessment chart (PBAC, Appendix 2) during their next menstrual period. The patients were also instructed to use Kotex Maxi Super towels and/or Tampax Super tampons during the period. The pictorial chart was returned and the charts were scored using the same scoring system described by Higham et al (1990). A score of 100 or more was used as confirmatory evidence of menorrhagia. Of 208 patients who completed their charts, 58 (28%) had scores of <100 and were excluded from the study.

A venous blood sample was obtained from the remaining 150 patients for blood grouping, full blood count, APTT and FVIII:C, FXI, vWF:Ag and vWF:Ac assays.
The blood was analysed at the Haemophilia Centre and Haemostasis Unit, the Royal Free Hospital, within two hours of collection. Normal ranges used in this laboratory are APTT = 28-38 seconds, vWF:Ac = 50-150 iu/dl, vWF:Ag = 50-150 iu/dl, FVIII:C = 50-150 iu/dl, and FXI = 70-130 iu/dl.

Patients with abnormal (vWF:Ag, vWF:Ac and FVIII < 50 iu/dl and FXI < 70 iu/dl) results (n = 25) or borderline (vWF:Ag, vWF:Ac and FVIII = 50-60 iu/dl and FXI = 70-80 iu/dl) results with another bleeding symptom in addition to menorrhagia (excluding easy bruising) (n = 15) were referred to the Haemophilia Centre for further investigation and assessment. Factor assays were then repeated on two further occasions and the patient was diagnosed to have vWD or FXI deficiency if vWF:Ac was < 50 iu/dl and FXI was < 70 iu/dl, respectively, in two of the three occasions. When FVIII was < 50 iu/dl with normal vWF levels, carriership of haemophilia was suspected and diagnosis was confirmed by family study and genotype analysis. To avoid inconvenience to the patients the first sampling was performed during their attendance at the Gynaecology clinic. However, for those who had repeated testing, the second and third samples were obtained early in the menstrual cycle (no later than day 7 in the cycle).

**Laboratory methods:**

Please refer to Chapter 6.

**Statistical methods:**

Four groups of women were identified; group A - no bleeding disorder (n = 123), group B - vWD (n = 20, including two patients with combined disorders), group C -
FXI deficiency (n = 6, including two with combined disorders), group D - other bleeding disorders (n = 3). For the purpose of analyses, two sets of comparisons were performed: (i) group B versus group A and (ii) group C versus group A.

For each woman, the number of other bleeding symptoms in addition to heavy menstruation (including easy bruising, nose bleeding, gum bleeding, post-operative bleeding, bleeding following tooth extraction, post-partum bleeding) were recorded.

Comparison of quantitative variables between these groups were performed using the Wilcoxon Mann-Whitney U test. Qualitative variables were compared using either Chi-squared tests or Fisher's exact test, where appropriate. All analyses were performed using the Statistical Analysis System (SAS) software package.
7.3 : RESULTS

Of 150 patients tested, 25 patients with abnormal results and 15 with borderline results were referred to the Haemophilia Centre and of these inherited bleeding disorders were confirmed in 21 and five, respectively. In total, inherited bleeding disorders were diagnosed in 26 patients (17%) including 18 with von Willebrand’s disease alone (three with moderate and 15 with mild severity), four with mild FXI deficiency alone, one with mild vWD/FXI deficiency, one with vWD/FXI/FX deficiency, one carrier of haemophilia A, and one with platelet dysfunction. Factor XII deficiency (an inherited clotting factor deficiency not associated with bleeding tendency) was also diagnosed in one patient. This makes the prevalence of von Willebrand’s disease 13% (95% confidence interval 7.9-18.8%) compared to the prevalence of 1.3% in the general population (Werner et al 1993), and FXI deficiency 4% (95% confidence interval 1.5-8.5%) compared to the estimated prevalence of 1 in 100,000 (Smith 1996) in the general population. Of the 150 patients screened in this study there were four women of known Jewish origin and two of them were diagnosed to have combined mild clotting factor deficiencies (vWD/ FXI deficiency and vWD/FXI/FX deficiency).

Demographic and details of menstrual periods of groups A, B and C are shown in Table 7.1. There were no significant differences in the women’s age at the time of interview, blood group distribution, family history of inherited bleeding disorders, past history of blood transfusion, or presence of anaemia (haemoglobin < 11 gm/dl). Sixty-five percent (13/20) and 66.7% (4/6) of patients in group B and C, respectively, had menorrhagia since menarche in comparison to only 8.9% (11/123) in group A (p
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PBAC scores were significantly higher in patients with vWD or FXI deficiency (P < 0.001, p = 0.007, respectively). However, the duration of menstruation, passage of clots and episodes of flooding were not significantly different.

Other bleeding symptoms suffered by the women including easy bruising, nose bleeding, gum bleeding, post-operative and post-partum bleeding and bleeding after tooth extraction were recorded (Table 7.2). On average, women reported one additional bleeding symptom with menorrhagia (range 0-5). The number of bleeding symptoms were significantly higher (p < 0.001) in patients with vWD (group B) compared to women with no bleeding disorder (group A), however, the difference was not significant in patients with FXI deficiency (group C) (p = 0.41). Women with vWD were significantly more likely to bruise easily (p = 0.05), and bleed following tooth extraction (p = 0.001), post-operatively (p < 0.001), and post-partum (p = 0.005) than women in group A. However, three (15%) of them had no other bleeding symptom apart from easy bruising. Among women with FXI deficiency, the incidence of bruising, gum bleeding and nose bleeding and bleeding following tooth extraction (only three had tooth extraction) were not significantly different and two (33.3%) patients had no other bleeding symptom apart from bruising. However, the incidence of post-operative and post-partum bleeding were significantly higher than that in group A.
Table 7.1 - Demographic features and details of menstrual history in patients included in the study

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Group (A) No bleeding disorder</th>
<th>Group (B) vWD</th>
<th>Group (C) FXI deficiency</th>
<th>p value*</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>150</td>
<td>123</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age Median (range)</td>
<td>39(15-50)</td>
<td>39(15-50)</td>
<td>40(27-50)</td>
<td></td>
<td>0.83</td>
<td>0.14</td>
</tr>
<tr>
<td>Blood group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>58(38.7%)</td>
<td>49(39.8%)</td>
<td>6(30.0%)</td>
<td></td>
<td>3(50.0%)</td>
<td>0.40</td>
</tr>
<tr>
<td>O</td>
<td>62(41.3%)</td>
<td>48(39.0%)</td>
<td>11(55.0%)</td>
<td></td>
<td>3(50.0%)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>30(20.0%)</td>
<td>26(21.1%)</td>
<td>3(15.0%)</td>
<td></td>
<td>0</td>
<td>0.65</td>
</tr>
<tr>
<td>FH of bleeding disorder</td>
<td>4(2.7%)</td>
<td>2(1.6%)</td>
<td>1(5.0%)</td>
<td></td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>PH of BT</td>
<td>17(11.3%)</td>
<td>10(8.1%)</td>
<td>3(15.0%)</td>
<td></td>
<td>2(33.3%)</td>
<td>0.10</td>
</tr>
<tr>
<td>Anaemia Hb&lt;11gm/dl</td>
<td>22(14.7)</td>
<td>19(15.5)</td>
<td>2(10.0%)</td>
<td></td>
<td>2(33.3%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Duration of menorrhagia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 24 months</td>
<td>61(40.7%)</td>
<td>57(46.3%)</td>
<td>3(15.0%)</td>
<td></td>
<td>0</td>
<td>2(33.3%)</td>
</tr>
<tr>
<td>&gt; 24 months</td>
<td>62(41.3%)</td>
<td>55(44.7%)</td>
<td>4(20.0%)</td>
<td></td>
<td>4(66.7%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Since menarche</td>
<td>27(18.0%)</td>
<td>11(8.9%)</td>
<td>13(65.0%)</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Duration of menstruation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>median(range) (days)</td>
<td>6.5(3-13)</td>
<td>6.5(3-13)</td>
<td>6.5(5-12)</td>
<td></td>
<td>9.5(6-12)</td>
<td>0.04</td>
</tr>
<tr>
<td>Passage of clots</td>
<td>131(87.9%)</td>
<td>107(87.7%)</td>
<td>17(85.0%)</td>
<td></td>
<td>6(100%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Episode of flooding</td>
<td>106(70.7%)</td>
<td>84(68.3%)</td>
<td>16(80.0%)</td>
<td></td>
<td>4(66.7%)</td>
<td>1.00</td>
</tr>
<tr>
<td>PBAC score median(range)</td>
<td>184(100-1036)</td>
<td>172(100-667)</td>
<td>297(122-800)</td>
<td></td>
<td>344(183-1036)</td>
<td>0.007</td>
</tr>
</tbody>
</table>

* Comparison between groups A and B; † Comparison between groups A and C; FH, family history; PH of BT, past history of blood transfusion.
Table 7.2 - Other bleeding symptoms and symptom scores in women included in the study

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Group (A)</th>
<th>Group (B) vWD</th>
<th>p value*</th>
<th>Group (C) FXI deficiency</th>
<th>p value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>150</td>
<td>123</td>
<td>20</td>
<td></td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Bruising</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>88 (58.7%)</td>
<td>66 (53.7%)</td>
<td>16 (80%)</td>
<td>0.05</td>
<td>4 (66.7%)</td>
</tr>
<tr>
<td>Nose bleeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>22 (14.7%)</td>
<td>17 (13.8%)</td>
<td>5 (25%)</td>
<td>0.20</td>
<td>0</td>
</tr>
<tr>
<td>Gum bleeding</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>54 (36%)</td>
<td>41 (33.3%)</td>
<td>9 (45%)</td>
<td>0.45</td>
<td>1 (16.7%)</td>
</tr>
<tr>
<td>Bleeding after tooth extraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>13/98 (13.3%)</td>
<td>6/81 (7.4%)</td>
<td>6/13 (46.2%)</td>
<td>0.001</td>
<td>1/3 (33.3%)</td>
</tr>
<tr>
<td>Post-op. Bleeding</td>
<td></td>
<td>18/109 (16.5%)</td>
<td>7/90 (7.8%)</td>
<td>8/13 (61.5%)</td>
<td>&lt;0.001</td>
<td>3/5 (60%)</td>
</tr>
<tr>
<td>Post-partum bleeding</td>
<td></td>
<td>29/97 (29.9%)</td>
<td>17/80 (21.3%)</td>
<td>8/13 (61.5%)</td>
<td>0.005</td>
<td>3/3 (100%)</td>
</tr>
<tr>
<td>Symptom scores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td></td>
<td>1 (0-5)</td>
<td>1 (0-5)</td>
<td>2 (1-5)</td>
<td>&lt;0.001</td>
<td>1.5 (0-4)</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>40 (26.7%)</td>
<td>39 (31.7%)</td>
<td>0</td>
<td></td>
<td>1 (16.7%)</td>
</tr>
<tr>
<td>1-2</td>
<td></td>
<td>78 (52%)</td>
<td>65 (52.9%)</td>
<td>11 (55%)</td>
<td>3 (50%)</td>
<td>2 (33.3%)</td>
</tr>
<tr>
<td>3-4</td>
<td></td>
<td>28 (18.7%)</td>
<td>17 (13.8%)</td>
<td>7 (35%)</td>
<td>2 (30%)</td>
<td></td>
</tr>
<tr>
<td>5-6</td>
<td></td>
<td>4 (2.7%)</td>
<td>2 (1.6%)</td>
<td>2 (10%)</td>
<td>&lt;0.001</td>
<td>0</td>
</tr>
</tbody>
</table>

* Comparison between groups A and B; † Comparison between groups A and C; Post-op., post-operative
The laboratory results obtained from the first blood sample are plotted in Figure 7.1 (a & b). Women with vWD had significantly longer APTT (p < 0.001), and significantly lower levels of FVIII:C, vWF:Ag and vWF:Ac (p < 0.001 for all three parameters) than women in group A. Women with FXI deficiency had significantly higher levels of APTT (p = 0.001) and significantly lower levels of FXI (p < 0.001), FVIII:C (p = 0.03), vWF:Ag (p = 0.02) and vWF:Ac (p = 0.03) than women in group A. There were no significant differences in platelet counts between the 3 groups.
Figure 7.1 (a) - Scatter plots of Coagulation markers in women with menorrhagia. Individual values for APTT, FXI and FVIII:C are plotted for patients with no bleeding disorders, vWD and FXI deficiency.
Figure 7.1 (b) - Scatter plots of Coagulation markers in women with menorrhagia. Individual values for vWF:Ag, vWF:Ac and platelet count are plotted for patients with no bleeding disorders, vWD and FXI deficiency.
This study has demonstrated that undiagnosed inherited bleeding disorders, especially in the mild form, can be the underlying cause in a significant number of patients presenting with menorrhagia. Therefore, it is recommended that testing for these disorders, especially von Willebrand’s disease, should be part of the routine work-up in patients with menorrhagia without any obvious pelvic pathology before embarking on any invasive procedures. Diagnosis of inherited bleeding disorders in these patients has several medical implications. First, it enhances rapid and effective treatment of menorrhagia. Second, if any surgical intervention were to become necessary, the risk of bleeding complications can be prevented by appropriate pre-operative assessment and prophylactic treatment when indicated. Lastly, it has genetic ramifications and important implications for the management of any future pregnancies. However, the subjects included in this study may not be representative of women in the general population complaining of heavy periods as not all women presenting to their General Practitioners with such a problem are referred to the hospital. Unfortunately data on referral rates in the geographical area covered by the Royal Free Hospital was not available but as Gynaecology Clinics at this hospital are intended for general referrals there is no reason to believe that the referral rates are different from elsewhere. Therefore, it is reasonable to assume that this study population is representative of women who are referred to the hospital with menorrhagia for further assessment.

Inaccuracy of subjective diagnosis of menorrhagia and the need for an objective measure of menstrual blood loss are well documented (Hallberg et al 1966a, Haynes et al 1977, Fraser et al 1984). A pictorial blood assessment chart, using a score of
100 or more as equivalent to a menstrual loss of > 80 ml (Higham et al 1990) was used in this study for objective diagnosis of menorrhagia. This simple non-laboratory method has been compared with the alkaline haematin method (Hallberg & Nilsson 1964) and was shown to have a reasonable accuracy with a sensitivity of 86% and a specificity of 89% (Higham et al 1990). Alkaline haematin and other laboratory methods are specialised, time-consuming techniques and inconvenient to the patients.

von Willebrand’s disease is now recognised as the most common hereditary haemorrhagic disorder. Type 1 is the most common form, accounting for approximately 70% of all cases. Most patients with type 1 and 2 vWD have a relatively mild bleeding tendency. Type 3 vWD usually has severe manifestations and is the least common of all forms of vWD. Severe forms of vWD are often easily suspected and diagnosed on the basis of clinical symptoms and pattern of inheritance. In contrast, the milder forms can go undiagnosed as they can be asymptomatic until subjected to a haemostatic challenge such as major trauma or invasive surgical procedure. However, menorrhagia is not uncommon and can be the presenting symptom. Although the prevalence of bleeding disorders in older women with menorrhagia seems to be under-estimated and has not been extensively investigated, acute adolescent menorrhagia requiring urgent medical intervention has long been recognised to be associated with undiagnosed underlying bleeding disorders. A primary coagulation disorder was found in almost 20% of 59 adolescents with such menorrhagia (Claessens & Cowell 1981a) and screening for vWD and platelet disorders has been recommended in these patients (Claessens & Cowell 1981b, Ward 1992). One small study showed that menorrhagia can be a valuable predictor for
bleeding disorders in women of reproductive age group and can be a guideline when looking for mild forms of these disorders (Edlund et al 1996). The results of this study show that 13% of all patients with objectively confirmed menorrhagia and normal pelvic examination have vWD.

In the absence of genetic analysis, the diagnosis of vWD is made on a clinical basis and on laboratory results of coagulation screen and clotting factor (vWF:Ag, vWF:Ac, FVIII:C) assays. However, the clinical expression of the disease is variable and the laboratory data may overlap with the normal range and fluctuate with time in a given individual (Abildgaard et al 1980) making the diagnosis, especially of mild forms, difficult and complex (Zhang et al 1995). Bleeding time and APTT are usually prolonged in patients with vWD. However, these tests may be normal in most of the patients with mild disease and may therefore not be sensitive enough to be used for screening and diagnosis of this disorder. The APTT reflects deficiencies of factors VIII, IX, XI and XII but when the concentration of these factors are over 30% of normal, the APTT will not be prolonged (Lusher 1996). In patients with mild vWD, FVIII:C, vWF:Ag, vWF:Ac levels are variable and may be normal, but on repeated testing at least one abnormal value is usually observed. vWF:Ac assay has been shown to be the single most sensitive assay for screening for most forms of vWD (Werner et al 1992, Rodeghiero et al 1990). Because of the variability of laboratory findings in vWD, repeated testing to establish the diagnosis of mild vWD has been suggested (Nilsson 1977, Abildgaard et al 1980). A large intraindividual variation in vWF and FVIII has been described (Mandalaki et al 1980, Edlund et al 1996) and it
has been suggested that restricting sampling to cycle days 5-7 will minimise this variation (Edlund et al 1996).

FXI deficiency predominantly affects Ashkenazi Jewish kindreds. In these communities the heterozygous frequency has been estimated to be 4.3% (Seligsohn 1978) to 11% (Seligsohn & Modan 1981). The frequency in non-Jews is unknown, but a significant number of patients with FXI deficiency on the Haemophilia Centre Directors’ national register have no known Jewish origin (Bolton-Maggs 1995). The Royal Free Hospital serves an area with a high Jewish population and therefore a potentially high prevalence of FXI deficiency. However, of the six patients diagnosed to have FXI deficiency only two had known Jewish origin. It is well recognised that the correlation between FXI levels and bleeding symptoms is poor (Bolton-Maggs et al 1988). Spontaneous bleeding is rare and bleeding usually occurs after haemostatic challenge (e.g. trauma or surgery) especially when the exposure of the operative site to fibrinolysis is high e.g. dental extraction (Bolton-Maggs et al 1988). Affected women, including those with partial deficiency, are more likely to have menorrhagia than their unaffected relatives (Bolton-Maggs et al 1995). As with vWD, APTT is not a good screening test for identification of FXI deficiency since values of heterozygotes with partial deficiency substantially overlap with normal ranges (Seligsohn & Modan 1981). The lower limit of the normal range for FXI is controversial. In the literature, this varies from 50 iu/dl (Seligsohn 1979) to 72 iu/dl (Bolton-Maggs et al 1988). At the Royal Free Hospital, a cut-off of 70 iu/dl is used, as it has been shown that 18% (Bolton-Maggs et al 1988) to 19% (Leiba et al 1965) of bleeders in families transmitting this deficiency have levels greater than 50 iu/dl.
Combined deficiencies of clotting factors have been reported in several cases (Lian et al 1976, Chediak et al 1980, Tavori et al 1990) and bleeding in partial FXI deficient patients has been explained by additional vWD (Tavori et al 1990). In this study, vWF:Ag, vWF:Ac and FVIII:C were significantly lower in patients with FXI deficiency compared to those with no clotting factor defect, and two patients had combined clotting factor deficiencies.

This study also emphasises the importance of obtaining a careful medical history, as certain predictive factors suggest an increased risk for bleeding disorder. These factors include a history of long-standing menorrhagia, specifically menorrhagia since menarche, and history of bleeding after tooth extraction, post-operatively or post-partum. Easy bruising and mucosal bleeding, particularly epistaxis and gingival bleeding are also common symptoms of vWD, less common with FXI deficiency and carriers of haemophilia. Therefore, there should be a high index of suspicion of a bleeding disorder in women with long-standing menorrhagia and history of bleeding after haemostatic challenge. However, if only women with more than 2 additional bleeding symptoms had been tested in this study, 55% and 66.7% of vWD and FXI deficiency, respectively, would have been missed (Table 7.2). Among this study population, 15%(3/20) and 33.3%(2/6) of women diagnosed to have vWD or FXI deficiency, respectively, suffered no other bleeding symptoms apart from easy bruising. Thus, the clinical severity of inherited bleeding disorders varies considerably, and menorrhagia may be the only clinical manifestation (Edlund et al 1996). Hence, it is important to test all patients with menorrhagia.
In conclusion, menorrhagia may reflect an underlying defect in haemostasis, the commonest of these being vWD. The role of mild inherited bleeding disorders in the aetiology of menorrhagia has been so far under-estimated. Testing for vWD should be performed in every patient with menorrhagia in whom no organic cause can be identified on pelvic examination and pelvic ultrasound, and before embarking on any invasive procedures. Screening for FXI deficiency should also be considered especially in high risk populations e.g. women of Ashkenazi descent.
# CHAPTER 8

MENSTRUATION AND INHERITED BLEEDING DISORDERS

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**8.1: INTRODUCTION**

Haemostasis in the menstruating uterus is the result of a delicate balance between platelet aggregation, fibrin formation, vasoconstriction, and tissue regeneration on one hand, and prostaglandin induced platelet inhibition, vasodilatation, and fibrinolysis on the other (Christiaens et al 1982). As the haemostatic plug formation plays an important role in uterine haemostasis during menstruation (Christiaens et al 1982), it is not surprising that there is an increased frequency of menorrhagia in patients with coagulation disorders. Many clinical reports in the literature link disorders of blood coagulation with subjectively reported menorrhagia (Vinazzer 1966, Nilsson 1974). However, no data have been published that provide an objective assessment of menstrual blood loss or assess the prevalence of menorrhagia in women with these conditions.

Menstruation can be a cause of embarrassment and inconvenience to many women and has a major influence on women’s lifestyle and employment. As a consequence of changes in the pattern of family life, modern women experience approximately ten times the number of periods experienced by their ancestors (Short 1976). Women are also increasingly involved in activities outside the home, where episodes of excessive menstrual loss and flooding can be especially inconvenient. Therefore, in addition to its medical implications, excessive menstrual loss can be a debilitating social problem for women.

In this study, objective assessments of menstrual blood loss, quality of life during menstruation and the frequency of menorrhagia in patients with vWD, carriers of haemophilia A or B and women with FXI deficiency are made in comparison with an
age matched control group. Other gynaecological complications in these patients are also reported.
8.2 : PATIENTS AND METHODS

All female patients (n = 276), aged 15-50 years, with vWD (n = 118), FXI deficiency (n = 43) and carriers of haemophilia A (n = 88) or B (n = 27), registered with the Royal Free Haemophilia Centre and living in London or its outskirts were invited for an interview by a letter sent to their home address. The patients were informed that the study was aimed at assessing several aspects of bleeding disorders in women without specifying assessment of menstrual loss or gynaecological problems to avoid bias and a higher response rate in those with these problems. During their attendance, the patients were interviewed and a structured questionnaire assessing their gynaecological and menstrual histories was completed. The nature of their bleeding disorder, type and severity was obtained from the Haemophilia Centre case records. Each patient was then given a pictorial menstrual blood assessment chart (PBAC) (Appendix 2) and a detailed questionnaire which assessed quality of life during menstruation (Appendix 3) for completion with their next period. A verbal explanation of how the PBAC should be used, and a written instruction leaflet were also provided. The patients were also given a stamped addressed envelope to return their completed PBAC and the quality of life questionnaire. Those who did not attend the first appointment were contacted and offered another appointment at their convenience and if they still did not attend, they were sent the two questionnaires (i.e. gynaecology and menstrual assessment and quality of life questionnaire), PBAC and information regarding the use of PBAC. They were also provided with a telephone number contact if they had any queries about the questionnaires or the PBAC. For comparison, PBAC and the quality of life questionnaire were also given to 100 female staff in the hospital, aged 15-50 years with no known bleeding or endocrine disorders, no history of recent (within the last 2 months) ingestion of
anticoagulants, for completion with their next period. The charts were scored using the scoring system described by Higham et al 1990 and a score of 100 or more was taken as diagnostic for menorrhagia.

The quality of life questionnaire consisted of four main sections:

(i) **General health**: Women were asked whether they considered their general health as excellent (scored as 5), very good (4), good (3), fair (2) or poor (1).

(ii) **Health and daily activities**: Women were given a list of ten activities (vigorous activities, such as running, lifting heavy objects, participating in strenuous sports; moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf; lifting or carrying groceries; climbing several flights of stairs; climbing one flight of stairs; bending, kneeling or stooping; walking more than a mile; walking more than half a mile; walking 100 yards; bathing and dressing) and were asked whether their menstruation limited them a lot (scored as 2), a little (1) or not at all (0) in each of these activities. Each individual’s scores for the ten activities were then summed to give a total score (health and daily activity score A) which could range between 0 (no limitation in any activity) to 20 (limited a lot in each activity). Women were also asked whether they (i) had to cut down the amount of time spent on work and other activities, (ii) had accomplished less than they would like, (iii) were limited in the kind of work they could do, or (iv) had difficulty performing the work or other activities as a result of the menstrual period. This part was scored separately (health and daily activity score B) with the scores ranging from 0 (for negative answers to the four questions) to 4 (for positive responses to each question).
(iii) **Dysmenorrhoea**: Women were asked how much bodily pain they had experienced during their last menstrual period (scored from 1 [none] to 6 [very severe]) and how much this pain had interfered with their normal work during their last period (scored from 1 [not at all] to 5 [extremely]).

(iv) **Quality of life during menstruation**: Women were finally asked how much of the time during their period they felt: (i) full of life, (ii) nervous+, (iii) down in the dumps+, (iv) calm and peaceful, (v) full of energy, (vi) downhearted and low+, (vii) worn out+, (viii) happy, (ix) tired+, or (x) that their health limited their social activities+. All answers were scored on a six-point scale ranging from 1 (none of the time) to 6 (all of the time), except those marked with a + which were scored in the reverse direction. Each individual’s scores were summed to give a total “Quality of life score” which could range from 10 (poor quality of life) to 60 (good quality of life).

In total 116/276 (42%) patients responded: vWD 66/118 (60%), carriers of haemophilia A or B 30/115 (26%), FXI deficiency 20/43 (47%); including 103 patients who were personally interviewed and 13 patients who returned the postal package. Details of the patients are given in Table 8.1. Of these, 95 (57 vWD; 14 carrier of haemophilia A; 7 carriers of haemophilia B; 17 FXI deficiency) and 99 (57 vWD; 17 carrier of haemophilia A; 7 carriers of haemophilia B; 18 FXI deficiency) completed and returned the PBAC and the quality of life questionnaire, respectively. Of the 99 patients who completed the quality of life questionnaire, 87 had also completed the PBAC during the same menstrual period. In these cases, the relationship between the quality of life score and menstrual scores were studied. Of
the control group 69 (69%) women completed and returned the PBAC and the quality of life questionnaire.

Table 8.1 - Details of patients included in the study

<table>
<thead>
<tr>
<th>Haem. Carriers (n=30)</th>
<th>vWD (n=66)</th>
<th>FXI Deficiency (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemophilia A</td>
<td>22</td>
<td>Type I 59</td>
</tr>
<tr>
<td>Haemophilia B</td>
<td>8</td>
<td>Type II B 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type IID 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Type III 3</td>
</tr>
<tr>
<td>Severity</td>
<td></td>
<td>Mild* 51</td>
</tr>
<tr>
<td>FVIII&gt;30iu/dl</td>
<td>19</td>
<td>Moderate** 12</td>
</tr>
<tr>
<td>FVIII ≤ 30iu/dl</td>
<td>11</td>
<td>Severe*** 3</td>
</tr>
<tr>
<td>Race/Religion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Caucasian</td>
<td>23</td>
<td>Mild/Moderate 14</td>
</tr>
<tr>
<td>- Jewish origin</td>
<td>1</td>
<td>Severe 6</td>
</tr>
<tr>
<td>- Others</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Blood group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- O</td>
<td>13</td>
<td>47</td>
</tr>
<tr>
<td>- A</td>
<td>15</td>
<td>16</td>
</tr>
<tr>
<td>- B</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>- AB</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Age at interview</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Median (range)</td>
<td>35(15-50)</td>
<td>31(15-50)</td>
</tr>
</tbody>
</table>

Haem., haemophilia; * vWF:Ac < 5 iu/dl; ** vWF:Ac 5-30 iu/dl; *** vWF:Ac > 30iu/dl; t FXI < 20 iu/dl, tt FXI > 20iu/dl

**Statistical methods**

Comparisons of the PBAC scores between different groups of patients and the control group were made using either the chi-squared test, Wilcoxon test, Kruskal-Wallis test or Mann-Whitney U test, where appropriate. The percentages of women experiencing menorrhagia (PBAC score ≥ 100) in different groups were compared.
using the Chi-squared test. Spearman's rank correlation coefficient was used to study the relationship between the PBAC score and factor levels within each group.

When analysing the quality of life questionnaire, comparisons between the patients and controls were made using either Chi-squared tests or Wilcoxon Mann-Whitney U tests, where appropriate. Relationships between the questionnaire responses and the clinical factors of interest were tested for significance using non-parametric methods (Wilcoxon Mann-Whitney U test or Kruskal-Wallis test, where appropriate) due to the expected non-normality of the data.
8.3: RESULTS

**Menstrual History:**

The length of menstrual cycle was significantly shorter ($p = 0.0007$) and the duration of menstrual bleeding was significantly longer ($P = 0.001$) in patients with inherited bleeding disorders compared to the control group (Table 8.2). There were episodes of flooding in 54% and 17% ($p = 0.001$) and passage of clots in 68% and 59% ($p = 0.31$) of patients and women in the control group, respectively (Table 8.2). On asking the patients whether they considered their periods to be heavy, 66% (77/116) answered yes and in 73% (56/77) of these the history of heavy periods was since menarche. Emergency admission to the hospital and blood transfusion for severe haemorrhage during menstruation was required in six cases. Four of them were ≤ 20 years of age and the other two were 32 and 39 years respectively. The latter required a 17 unit blood transfusion; however, the heavy bleeding failed to respond to medical treatment and radiation of the ovaries to induce premature menopause was performed to control the bleeding.

Twenty eight percent (32/116) of patients had been anaemic because of heavy periods at some time and had been prescribed iron treatment. Forty seven percent (54/116) of the patients had a consultation with their general practitioner or a gynaecologist because of heavy periods. Thirty six percent (42/116) had medical treatment for menorrhagia. At the time of the interview 28% (32/116) of the women were on combined oral contraceptives; in 44% (14/32) the main reason was to control heavy periods. Diagnostic curettage and/or hysteroscopy for menorrhagia was performed in 24% (28/116) of patients including two during their teenage years. The procedure was repeated more than once in five patients, including one patient who had this
procedure four times. Excessive bleeding after this operation occurred in four patients including two cases of vWD and one carrier of haemophilia A; only one (vWD) required blood transfusion. The fourth patient was a woman with FXI deficiency who bled despite receiving fresh frozen plasma to cover the operation and the post-operative period. Hysterectomy was performed in 10 patients. The median age at hysterectomy was 38 years (range 29-46). Details of the patients who had hysterectomy and their bleeding complications are presented in Table 8.3.

Table 8.2 - Menstrual history: comparison of between patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>35 (15-50)</td>
<td>33 (15-48)</td>
<td>0.36</td>
</tr>
<tr>
<td><strong>Length of the cycle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>27 (17-70)</td>
<td>28 (21-40)</td>
<td>0.0007*</td>
</tr>
<tr>
<td><strong>Duration of menstruation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-4 days</td>
<td>16 (16.8%)</td>
<td>21 (30.4%)</td>
<td></td>
</tr>
<tr>
<td>5-7 days</td>
<td>55 (57.9%)</td>
<td>45 (65.2%)</td>
<td></td>
</tr>
<tr>
<td>8-9 days</td>
<td>18 (19.0%)</td>
<td>3 (4.4%)</td>
<td></td>
</tr>
<tr>
<td>10+ days</td>
<td>6 (6.3%)</td>
<td>-</td>
<td>0.001*</td>
</tr>
<tr>
<td><strong>Menstrual scores</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>122 (38-482)</td>
<td>73 (9-310)</td>
<td>0.0001*</td>
</tr>
<tr>
<td><strong>PBAC score</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 100</td>
<td>64 (67.4%)</td>
<td>20 (29.0%)</td>
<td></td>
</tr>
<tr>
<td>&lt; 100</td>
<td>35 (32.6%)</td>
<td>49 (71.0%)</td>
<td>0.001*</td>
</tr>
<tr>
<td><strong>Episodes of flooding</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>54 (56.8%)</td>
<td>12 (17.4%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>45 (43.2%)</td>
<td>57 (82.6%)</td>
<td>0.001*</td>
</tr>
<tr>
<td><strong>Passage of clots</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>65 (68.4%)</td>
<td>41 (59.4%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>30 (31.6%)</td>
<td>28 (40.6%)</td>
<td>0.31</td>
</tr>
</tbody>
</table>

* Statistically significant
Table 8.3 - Details of patients who had hysterectomy

<table>
<thead>
<tr>
<th>No.</th>
<th>Bleeding disorder</th>
<th>Indication</th>
<th>Age</th>
<th>Route</th>
<th>Prophylaxis</th>
<th>Bleeding complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>vWD type 1</td>
<td>Menorrhagia*</td>
<td>44</td>
<td>Abd.</td>
<td>None</td>
<td>-Vault haematoma - conservative management</td>
</tr>
<tr>
<td>2</td>
<td>vWD type 1</td>
<td>CIN + Menorrhagia</td>
<td>39</td>
<td>Abd.</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>vWD type 1</td>
<td>Ovarian cyst + Menorrhagia</td>
<td>40</td>
<td>Abd.</td>
<td>DDAVP infusion</td>
<td>-Wound haematoma - surgical drainage</td>
</tr>
<tr>
<td>4</td>
<td>vWD type 3</td>
<td>Menorrhagia*</td>
<td>29</td>
<td>Abd.</td>
<td>vWF conc.</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>vWD type 3</td>
<td>Menorrhagia*</td>
<td>34</td>
<td>Abd.</td>
<td>vWF conc.</td>
<td>-Post-operative haematuria. -Secondary haemorrhage (day 9) - 4 units blood transfusion</td>
</tr>
<tr>
<td>6</td>
<td>Haem. A carrier</td>
<td>Menorrhagia*</td>
<td>46</td>
<td>Vag.</td>
<td>None</td>
<td>-Vault haematoma - conservative management</td>
</tr>
<tr>
<td>7</td>
<td>Haem. A carrier</td>
<td>Menorrhagia*</td>
<td>37</td>
<td>Abd.</td>
<td>None</td>
<td>-per-operative bleeding - surgeon resorted to subtotal hysterectomy -2 units blood transfusion</td>
</tr>
<tr>
<td>8</td>
<td>Haem. A carrier</td>
<td>Menorrhagia*</td>
<td>43</td>
<td>Abd.</td>
<td>DDAVP infusion</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>FXI def.</td>
<td>Menorrhagia*</td>
<td>33</td>
<td>Abd.</td>
<td>FXI conc.</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>FXI def.</td>
<td>Menorrhagia*</td>
<td>34</td>
<td>Abd.</td>
<td>FXI conc.</td>
<td></td>
</tr>
</tbody>
</table>

Age, age at the time of hysterectomy; * Menorrhagia not responding to medical treatment; Haem., haemophilia; FXI def.; FXI deficiency; Abd, abdominal; Vag., vaginal; vWF conc., vWF rich concentrates; FXI conc., FXI concentrates; TCRE, transcervical resection of endometrium.
Menstrual blood loss according to PBAC:

PBAC was completed by 95 patients (details of menstrual scores in these patients are presented in Table 8.4). The reasons for not completing the chart in the remaining 21 were: amenorrhoea due to hysterectomy (10), ovarian radiation (1), breast feeding (1) and difficulty in understanding the chart (9). The median PBAC score was 122 (range 38-482) and 73 (range 9-310) (p = 0.0001) and the score was 100 or more in 67% (64/95) and 29% (20/69) (p = 0.001) of women with inherited bleeding disorders and the control group, respectively (Table 8.4). The individual values and median scores in haemophilia A or B carriers, vWD, FXI deficient patients and controls are presented in Figure 8.1. The scores tend to be higher in vWD patients with vWF:Ac $\leq$ 30iu/dl in comparison to those with higher vWF:Ac levels, however, the difference was not statistically significant (p = 0.07, Mann-Whitney test). There was no significant difference in the scores between mild/moderate and severe FXI deficient patients (p = 0.23, Mann-Whitney test). In carriers of haemophilia, there was also no significant difference in the score between women with factor VIII or IX levels of $\leq$ 30iu/dl compared to those with higher levels (p = 0.62, Mann-Whitney test); however, the number of women with factor levels of $\leq$ 30iu/dl with menstrual scores were very small.

A strong relation between the duration of menstruation and the menstrual score was found; the longer the duration of the period the higher the score (p= 0.0009, Kruskal-Wallis test) and the greater likelihood of a menstrual score of $\geq$ 100 (p=0.003, Chi-squared test) (Figure 8.2). The scores were also higher in those women experiencing
flooding (p = 0.0001, Mann-Whitney test), passage of clots (p = 0.0003, Mann-Whitney test) and those who had been anaemic (p = 0.02, Mann-Whitney test).

Table 8.4 - Menstrual scores in women with inherited bleeding disorders and in the control group

<table>
<thead>
<tr>
<th></th>
<th>Median score</th>
<th>Range</th>
<th>Women with score ≥ 100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carriers of Haemophilia A (n = 14)</td>
<td>111</td>
<td>50-482</td>
<td>8 (57.1%)</td>
</tr>
<tr>
<td>Carriers of Haemophilia B (n = 7)</td>
<td>115</td>
<td>53-200</td>
<td>4 (57.1%)</td>
</tr>
<tr>
<td>vWD (n = 57)</td>
<td>139</td>
<td>55-456</td>
<td>42 (73.7%)</td>
</tr>
<tr>
<td>FXI deficiency (n = 17)</td>
<td>108</td>
<td>38-424</td>
<td>10 (58.8%)</td>
</tr>
<tr>
<td>Total (n = 95)</td>
<td>122</td>
<td>38-482</td>
<td>64 (67.4%)*</td>
</tr>
<tr>
<td>Control (n = 69)</td>
<td>73</td>
<td>9-310</td>
<td>20 (29.0%)*</td>
</tr>
</tbody>
</table>

n, number of women completed PBAC, * statistically significant difference (p = 0.001)

Other gynaecological problems:
Intra-peritoneal bleeding secondary to a ruptured haemorrhagic corpus luteum occurred in one of the carriers of haemophilia B who required laparotomy and salpingo-oophorectomy under FIX concentrate cover. Laparoscopic sterilisation was performed in nine cases. In one (vWD type 1), bleeding from a small tear in the fallopian tube required mini-laparotomy and unilateral salpingectomy. The procedure
was complicated by wound haematoma in another patient (vWD type 1). A diagnostic laparoscopy was performed in five and bleeding from the wound requiring resuturing complicated the procedure in a carrier of haemophilia A. Five patients had a cone biopsy. Severe secondary post-operative bleeding following the procedure occurred in a patient with mild vWD. This was managed by a blood transfusion (five units), cauterisation of the bleeding sites and vaginal packing.

Figure 8.1 - Menstrual scores in carriers of haemophilia A, B, patients with vWD, FXI deficiency and controls. Horizontal lines represent median values.
Assessment of quality of life:

General health

General health scores were significantly worse in patients with inherited bleeding disorders than the control group (Table 8.5). Patients felt that their health, in general, was very good (median score: 4, range 1 to 5). Fifty-seven percent (56/99) of patients felt that their health was very good or excellent and only 2% (2/99) felt that they had poor general health. Among the patients with bleeding disorders, there were no differences in general health scores according to the type of disorder (p=0.27), or according to whether the women experienced passage of clots during their period.
(p=0.10). However, general health was significantly worse in those who experienced flooding (p=0.04) and in those with a PBAC score ≥ 100 (p=0.003). There was no relationship between the general health score and the number of days of bleeding (p=0.19).

Health and daily activities

For most activities listed, less than 10% of women reported being “limited a lot” during menstruation. However, for vigorous activities, such as running and lifting heavy objects, and for walking more than a mile, a relatively high proportion of women felt that they were limited a lot in their activities (27/99 and 15/99, respectively). The median total health and daily activity score (A) was 2 (range 0 to 19) indicating that whilst few women felt limited by their menstrual period, a minority did experience some limitation over the whole range of activities (Table 8.5). This score was worse than in the control group (median 1, range 0-11) (p = 0.07). Health and daily activity score (B) was also significantly worse in patients with inherited bleeding disorders compared to the control group (p=0.004) (Table 8.5) with 39/99 (39%) of the patients having to cut down on the amount of time spent on work and other activities as a result of their menstrual period. Forty-seven women (47%) felt that they accomplished less than they would like during the period and 38 (38%) felt that they were limited in the kind of work and other activities that they could do. Forty women (40%) found that it took extra effort to perform their work during the menstrual period. As before, whilst there was no effect of the type of bleeding disorder on the Health and Daily Activity score (p=0.25), women who experienced passage of clots (p=0.0001) or flooding (p=0.0001) were more limited in their daily activities, as were those with a PBAC score ≥ 100 (p=0.007).
Furthermore, there was a strong relationship between the Health and Daily Activity score and the number of days of bleeding (p=0.003), indicating that the longer a woman’s menstrual period, the more limited she felt in her daily life.

**Dysmenorrhoea**

Dysmenorrhoea (p=0.001) and its interference on daily work (p=0.001) was significantly greater in patients with inherited bleeding disorders than in women in the control group (Table 8.5). Of the 99 women with bleeding disorders who responded, 50 (51%) experienced moderate, severe or very severe pain during their period. In general, this pain only interfered “a little” with their normal work, although a quarter of women (25/99) felt that it interfered “quite a bit”, or “extremely”. Dysmenorrhoea and work interference as a result of this pain were worse in women with vWD than in those with other bleeding disorders (p=0.007 and p=0.06 for dysmenorrhoea and work interference, respectively). Pain and interference were significantly worse in those women who experienced passage of clots (p=0.01 and 0.007, respectively) and flooding (p=0.0001 and 0.0001 respectively), in those with longer bleeding periods (p=0.0002 and 0.0008, respectively) and in those with PBAC scores ≥ 100 (p=0.01 and 0.02, respectively).

**Quality of life during menstruation**

Total Quality of Life scores in patients with inherited bleeding disorders are shown in Figure 8.3. Overall, these women had a median quality of life score of 40 (range 15 to 60) compared to a score of 47 (range 27 to 60) in women in the control group (p=0.0001) (Table 8.5). Quality of life scores were significantly lower in those with vWD (p=0.03) than in other women. Quality of life was also significantly worse in those women who experienced passage of clots (p=0.001) and flooding (p=0.0001),
and in those with a PBAC score $\geq 100$ ($p=0.0002$). In addition, there was a direct relationship between quality of life and number of days of bleeding, with a poorer quality of life in those who experienced the longest bleeding periods ($p=0.0002$).
Table 8.5 - Quality of life during menstruation: comparison of between patients with inherited bleeding disorders and control group

<table>
<thead>
<tr>
<th></th>
<th>Patients (N = 99)</th>
<th>Control (n = 69)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Median (range)</td>
<td>35 (15-50)</td>
<td>33 (15-48)</td>
<td>0.36</td>
</tr>
<tr>
<td>General health</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Poor</td>
<td>2 (2.0%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>- Fair</td>
<td>16 (16.2%)</td>
<td>2 (2.9%)</td>
<td></td>
</tr>
<tr>
<td>- Good</td>
<td>25 (25.3%)</td>
<td>14 (20.6%)</td>
<td></td>
</tr>
<tr>
<td>- Very good</td>
<td>47 (47.5%)</td>
<td>37 (54.4%)</td>
<td></td>
</tr>
<tr>
<td>- Excellent</td>
<td>9 (9.1%)</td>
<td>15 (22.6%)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Health and daily activity score A Median (range)</td>
<td>2 (0-19)</td>
<td>1 (0-11)</td>
<td>0.07*</td>
</tr>
<tr>
<td>Health and daily activity score B - Score 0</td>
<td>44 (44.4%)</td>
<td>30 (43.5%)</td>
<td></td>
</tr>
<tr>
<td>- Score 1</td>
<td>6 (6.1%)</td>
<td>15 (21.7%)</td>
<td></td>
</tr>
<tr>
<td>- Score 2</td>
<td>14 (14.1%)</td>
<td>14 (20.3%)</td>
<td></td>
</tr>
<tr>
<td>- Score 3</td>
<td>10 (10.1%)</td>
<td>3 (4.4%)</td>
<td></td>
</tr>
<tr>
<td>- Score 4</td>
<td>25 (25.3%)</td>
<td>7 (10.1%)</td>
<td>0.004*</td>
</tr>
<tr>
<td>Dysmenorrhoea</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- None</td>
<td>16 (16.7%)</td>
<td>6 (8.8%)</td>
<td></td>
</tr>
<tr>
<td>- Very mild</td>
<td>20 (20.8%)</td>
<td>22 (32.6%)</td>
<td></td>
</tr>
<tr>
<td>- Mild</td>
<td>10 (10.4%)</td>
<td>21 (30.9%)</td>
<td></td>
</tr>
<tr>
<td>- Moderate</td>
<td>27 (28.1%)</td>
<td>16 (23.5%)</td>
<td></td>
</tr>
<tr>
<td>- Severe</td>
<td>15 (15.6%)</td>
<td>3 (4.4%)</td>
<td></td>
</tr>
<tr>
<td>- Very severe</td>
<td>8 (8.3%)</td>
<td>0</td>
<td>0.001*</td>
</tr>
<tr>
<td>Interference of dysmenorrhoea with daily work - Not at all</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- A little bit</td>
<td>39 (41.1%)</td>
<td>23 (33.3%)</td>
<td></td>
</tr>
<tr>
<td>- Moderately</td>
<td>21 (22.1%)</td>
<td>31 (44.9%)</td>
<td></td>
</tr>
<tr>
<td>- Quite a bit</td>
<td>10 (10.5%)</td>
<td>11 (15.9%)</td>
<td></td>
</tr>
<tr>
<td>- Extremely</td>
<td>20 (21.1%)</td>
<td>4 (5.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5 (5.3%)</td>
<td>0</td>
<td>0.001*</td>
</tr>
<tr>
<td>Quality of life score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Median (range)</td>
<td>40 (15-60)</td>
<td>47 (27-60)</td>
<td>0.0001*</td>
</tr>
</tbody>
</table>

* Statistically significant
Figure 8.3 - Relationship between quality of life score and menstrual score, duration of menstrual period, passage of clots, episode of flooding and type of inherited bleeding disorder. Horizontal lines represent median values.
8.4: DISCUSSION

This is the first large study which objectively assesses menstrual blood loss, quality of life and gynaecological problems in patients with inherited bleeding disorders. There has only been one other study where an objective assessment of menstrual blood loss was performed in patients with coagulation disorders (Fraser et al 1986), including only two patients with vWD and two carriers of haemophilia. The results of the present study show that objectively confirmed menorrhagia is significantly higher in patients with vWD, carriers of haemophilia and FXI deficiency (74%, 57% and 59%, respectively) that in an age-matched control group of women (29%). It is also clear that patients with inherited bleeding disorders not only suffer from heavy menstruation but prolonged menstrual periods with more episodes of flooding. Among the study group, menstrual bleeding was ≥ 5 days and ≥ 8 days in 83% and 25%, respectively, compared to 48% and 4% in the control group. However, the response rate in the study group was only 60%, 47% and 26% in vWD, FXI deficiency and carriers of haemophilia, respectively, compared to 69% in the control group. Therefore, these figures may represent an overestimate, especially in carriers of haemophilia, as the large proportion of non-responders might have had normal menstruation. The low response rate in carriers of haemophilia may be due to the fact that they suffer less problems from their bleeding disorder compared to patients with vWD or FXI deficiency.

Menorrhagia has been reported to be a frequent symptom in sufferers of vWD, particularly in adolescent girls (Evans 1971, Nilsson 1974, Claessens & Cowell 1981a &b, Greer et al 1991). However, all these studies included a small number of women and diagnosis of menorrhagia has been subjective as assessment of menstrual
blood loss was not performed. Subjective menorrhagia has also been reported in carriers of haemophilia (Lusher et al 1978) although in a study by Mauser Bunschoten et al (1988) there was no significant difference in the percentage of carriers who considered their menstrual loss to be greater than other women compared to a reference group. In contrast, this study has demonstrated that a significant number of carriers of haemophilia A or B have objectively verified menorrhagia. In FXI deficiency, affected women, including those with partial deficiency, are more likely to have heavy menstruation than their unaffected relatives (Bolton-Maggs et al 1995). In this study, 59% of women with FXI deficiency were shown to have objectively verified menorrhagia and there was no significant difference in the incidence of menorrhagia between heterozygous and homozygous women. This finding seems to be in agreement with the lack of correlation between FXI levels and bleeding tendency in FXI deficient patients (Bolton-Maggs et al 1988).

The inaccuracy of subjective assessment and the need for an objective method for measuring menstrual blood loss is well documented (Cole et al 1971, Haynes et al 1977, Rees et al 1987). In a population study 41% of women with actual menstrual loss of > 80 ml. considered their period as moderate or scanty and 14% of women with actual loss of < 20 ml. considered their period as heavy (Hallberg et al 1966a). Several laboratory techniques have been described for measurement of menstrual blood loss (Hallberg & Nilsson 1964, Newton et al 1977, Vasilenko et al 1988, Gannon et al 1996, described in Chapter 2.4). However, all these techniques are specialised, time consuming, and involve collection and saving of sanitary material by women. To avoid these problems the PBAC (Higham et al 1990) was used during
a single period for objective assessment of the menstrual blood loss. Some authors advocate repeated measurements of menstrual blood loss for the accurate diagnosis of menorrhagia (Haynes et al 1977). However, only 18% of women have a difference of greater than 20 ml. in volume between two periods (Cole et al 1971). Hallberg & Nilsson (1964) found that the correlation between two measurements of menstrual blood loss was high. Gannon et al (1996) also reported that a single measurement is usually sufficient for the diagnosis of menorrhagia. No relationship has been found between the number of days of menstrual bleeding and the total menstrual loss in the general population (Haynes et al 1977, Chimbria et al 1980). In contrast, a strong relation was found in this study group and this may indicate that women with inherited bleeding disorders bleed heavily throughout the whole menstrual period.

This study also demonstrates that menstruation in patients with inherited bleeding disorders, especially those with objectively confirmed menorrhagia, prolonged duration of menstruation and flooding or passage of clots during menstruation has adverse effects on many aspects of life. These patients have significantly poorer quality of life on all the scales used compared with the control group. A significant proportion had to cut down on the time they spent on their work or other activities, had accomplished less than they would like, were limited in the kind of work or other activities that they can do and had experienced difficulties performing their work during the menstrual period. Half of the patients had moderate, severe or very severe pain associated with menstruation. Over a third reported that menstruation had moderate to severe interference on their normal daily work. Thus in many of these patients menstruation was associated with a poor quality of life. In interpreting these
results, there are two important areas of potential bias which should be considered; firstly, during the study period some of the patients were on treatment for menorrhagia which might have improved their quality of life. Secondly, only 42% of all eligible patients attended for review and only 85% of them completed the quality of life questionnaire. Therefore, the possibility that patients who chose to respond were those with the worst quality of life cannot be ruled out. However, this study reveals that, at least in a substantial proportion of women with bleeding disorders, menstruation can have a negative impact on quality of life and general health.

Women’s experience of menstruation has been assessed in a Swedish survey of 2200 women (Edlung et al 1994). Thirty percent of women included in the survey reported that they had to refrain from social activities because of their period ‘sometimes’, ‘quite often’ or ‘every month’. Some 30% of the women planned social activities with their period in mind. The corresponding figures in the subgroup of women who described their menstruation as excessive was 50% and 57%, respectively. Therefore, menstruation may be a source of inconvenience to women in general, but significantly more so for women with excessive blood loss. Excessive menstrual loss may also have major financial implications. It has been estimated that excessive menstrual loss can lead to more than 300 000 sick days per year in all women of reproductive ages in Sweden (Edlung et al 1994). Women’s estimates of the expense per menstruation is reported to be £6 and £8 in those with normal or excessive menstruation, respectively (Edlung et al 1994).

In women with inherited bleeding disorders, menstruation is usually excessive from menarche and because of the genetic nature of the disease their mothers and sisters
may also have the same bleeding disorder and heavy menstruation. Therefore, these women might consider that it is normal to have heavy menstruation, might not seek medical advice and thus are not aware of treatment options. Hence, it is recommended that women with inherited bleeding disorders should be asked regularly about their periods and an objective assessment of menstrual loss should be performed by PBAC in those who complain of excessive blood loss. Those with normal PBAC scores can be reassured and appropriate investigations, treatment and referral to the gynaecologists should be arranged in those with heavy loss.

This series include a patient with acute abdomen due to haemoperitonium caused by spontaneous ruptured corpus luteum. This has been reported previously in patients with inherited bleeding disorders (Greer et al 1991, Gomez et al 1998). Although, this is a rare complication, it is important that it is considered in these patients, especially those with severe deficiency such as type 3 vWD, before embarking on any surgical intervention with its possible complications. Any surgical interventions, even relatively minor operations, may be associated with significant haemorrhage in patients with inherited bleeding disorders, as demonstrated in this study. Therefore, in all forms of surgery, good liaison between the local haemophilia centre and the surgical/anaesthetic team is essential. Patients’ factor levels should be checked preoperatively and adequate haemostatic cover provided. Monitoring post-operatively is continued depending on the nature of the operation and patient’s factor levels.

In summary, this study demonstrates that menorrhagia is a common and major problem in women with inherited bleeding disorders, especially vWD. Increased awareness among gynaecologists and haematologists of the high prevalence of
menorrhagia among these patients and the available treatment options is necessary for the appropriate management of such patients to improve their quality of life. Appropriate pre-operative assessment and haemostatic cover during any gynaecological procedures, however minor, is essential and patients should be managed in close collaboration with the local Haemophilia Centre to minimise the risk of haemorrhagic complications.
9.1 : INTRODUCTION

DDAVP has an established role in the management of some patients with von Willebrand’s disease and mild to moderate haemophilia A due to its ability to cause an increase in plasma concentrations of FVIII and vWF. It is currently regarded as an important therapeutic alternative to plasma derived coagulation products because of its efficacy in selected cases and because it avoids the risk of infection with blood borne viruses.

Intranasal spray preparation of DDAVP has been shown to provide an effective, accurate and convenient alternative to parenteral administration (Lethagen et al 1987) and is ideal for home use. Administration of 300 μg DDAVP with intranasal pump spray is as effective as 0.3 μg/kg given intravenously with regard to the stimulation of FVIII and vWF and shortening of the bleeding time (Lethagen et al 1995). The spray deposits well-controlled doses into the nostril and allows delivery of the desired volume and concentration of the drug to the site of absorption and has been shown to produce a highly reproducible increase in FVIII, vWF and platelet adhesiveness (Lethagen et al 1987). It is recommended for use as home treatment of menorrhagia in several centres (Aledort 1995). Despite promising results based on women’s subjective assessment (Lethagen & Ragnarson 1993, Seremetis & Aledort 1994, Kobrinsky & Goldsmith 1997) no data have been published on an objective assessment of menstrual blood loss in response to this treatment in a randomised trial. In this study, the effect of DDAVP nasal spray on menstrual blood loss is objectively evaluated using the pictorial blood assessment chart in patients with inherited bleeding disorders complaining of menorrhagia in a placebo controlled double blind trial.
9.2 : PATIENTS AND METHODS

Women aged 18 - 50 years with diagnosed inherited bleeding disorders and objectively confirmed menorrhagia (PBAC score ≥100) were included in the study. Other pathological causes for menorrhagia were excluded by gynaecological examination and pelvic scan. Women with type 2B vWD, history of renal and hepatic impairment, endocrine disorders, thromboembolic disease and nasal pathology interfering with absorption of the spray, including rhinitis, nasal polyp or significantly deviated septum were excluded from the study, as well as those with known hypersensitivity to DDAVP and/or chlorobutanol. Other exclusion criteria included the use of hormonal contraception, intrauterine contraceptive device, medical treatment for menorrhagia and hysteroscopy and/or dilatation and curettage in the last three months. The use of diuretics, carbamazepine, non steroidal anti-inflammatory agents, clofibrate and chlorpropamide were prohibited during and 10 days before the trial. The trial was discussed with the patient, who was given a detailed information leaflet and signed informed consent was obtained.

The design of the study was a randomised, double blind cross-over study. A total of 39 women (type 1 vWD n = 30, FXI deficiency n = 3, vWD/FXI deficiency n = 4, haemophilia A carriers n = 2) were recruited and were randomised to start two months therapy with placebo or Octim spray (DDAVP 1.5 mg/ml, each spray delivering 150 μg). During each of the study periods, the patients were instructed to take one spray in each nostril (i.e. 300 μg of DDAVP in case of Octim spray) twice daily during the second and third day of the period. They were also advised to complete the PBAC for assessment of menstrual loss, to restrict fluid intake to 1.5-2 litres a day during treatment and to weigh themselves daily during the treatment and
at least 3 days afterwards. For the sake of accuracy of menstrual loss assessment, patients were provided with sanitary towels (Kotex Maxi Super towels and/or Tampax Super tampons).

In addition to detailed verbal discussion and demonstration, written instructions about how to take the spray, possible side effects and a 24 hour contact number in case of any queries or adverse effects was provided. For simplicity and accuracy, a dairy which included sections for the study medication and any side effects, fluid intake, body weight, use of concomitant medications as well as the PBAC was provided and the patient was advised to tick or fill in wherever appropriate. After the end of period 1, the patients were given another out-patient appointment to return their completed diary, receive the alternate spray and a dairy for period 2, if they wished to continue with the trial. At the end of period 2, the patients were seen again with their completed dairy and they were asked which period they preferred (i.e. period 1 or 2). The PBACs were scored using the scoring system described by Higham et al 1990. Patients’ quality of life during both periods was assessed using a simple questionnaire which included number of days absent from work or school or avoiding social activities (i.e. patients had to stay at home), seeking medical advice and use of other medications on account of menstrual bleeding. All this information was recorded on their case report form.

For the purposes of analysis, three populations were defined. All women who had formally entered the trial, who had received at least one dose of DDAVP or placebo were included in the ‘safety’ population and were analysed with regard to demographics and adverse events. The primary analyses of efficacy were performed
on an ‘intention-to-treat’ (ITT) population which included those women who were known to have received at least one dose of DDAVP or placebo and who had completed at least one follow-up PBAC score. As a secondary evaluation, the analyses were repeated on a ‘per-protocol’ (PP) population, defined as the subgroup of the ITT population who had completed both periods of testing.

**Statistical methods**

All statistical analysis was performed using SAS (version 6.12 for Windows). The primary endpoint was considered to be the PBAC score over each of the two treatment periods. Secondary endpoints included measures of quality of life (absence from work or school and visits to doctor because of menstrual bleeding), patient preferences for one of the two treatment periods, the development of adverse events, weight changes and fluid intake.

Demographic and clinical factors in the two treatment sequence groups at baseline were compared using Chi-squared tests, Fisher’s exact tests or unpaired t-tests, as appropriate. The primary endpoint was analysed using a parametric mixed effects model which allowed for treatment sequence, treatment received and treatment period as fixed effects, and subject as a random effect. The analysis was also repeated using a fixed effects model, including terms for treatment sequence, treatment received and treatment period, with similar results. All tests performed were two-sided with an overall type I error rate of 5%.
9.3 : RESULTS

A total of 39 women were recruited to the study. Ten of these women (25.6%) did not receive any trial medication (two women were withdrawn for surgery before receiving any medication, two were known not to have received their medication, and the remaining six women did not return and there was no evidence that they had received their medication). Thus, the safety population consisted of 29 women (22 diagnosed as vWD, three vWD/FXI deficiency, two FXI deficiency and two haemophilia carriers). One of these women was known to have taken her first dose of trial medication but did not complete a follow-up PBAC and therefore was not included in the ITT population (n = 28). Four women did not complete the second period of treatment and thus the per-protocol population consisted of 24 women. The number of women withdrawing from the trial was broadly similar between the two treatment sequence groups. The demographics and clinical characteristics of the women, both overall and stratified by treatment sequence, are shown in Table 9.1. The two treatment sequence groups were fairly well matched in terms of these factors.

Overall, there was a significant improvement in PBAC scores with DDAVP (p = 0.0001) or placebo (p = 0.0001) compared to pre-treatment assessment. However, the pre-treatment PBAC scores were not measured under trial conditions and it is likely that the instructions on completion of the charts may have been different in the trial. Thus, these baseline measurements have not been included in further analysis in the study. However, even when the analysis is restricted to those measurements taken under trial conditions, there is a general tendency for the scores to decrease between the first and second treatment period, irrespective of the treatment sequence received.
(Figure 9.1). The PBAC scores at the end of the first and second treatment periods compared to pretreatment scores are shown in Figure 9.2.

Statistical analysis of the PBAC scores (during the trial) revealed that whilst there was large between-individual variability (inter-individual variance=9450), the residual variance was low (1700) suggesting that the scores were reasonably repeatable within a woman. Overall, the analysis confirmed that there was a strong time effect (p=0.01) with the estimated mean PBAC score in the second treatment period being 32 lower than that in the first treatment period (estimated means of 160 and 128 in treatment periods 1 and 2, respectively). This change occurred irrespective of the treatment sequence received. After adjusting for this decrease over time, the mean PBAC score while receiving DDAVP was estimated to be 8 lower than that when receiving placebo (estimated mean scores of 140 and 148, respectively). However, due to the small number of women included in the ITT population, this difference was not significant (p=0.51, 95% confidence interval for difference [placebo - DDAVP] in means, -15.5 to 31.6). The PBAC scores during placebo and DDAVP treatment periods are shown in Figure 9.3.

Generally, few women had been absent from work as a result of bleeding in the month prior to entering the trial (Table 9.1). By the end of the first treatment period, absenteeism had largely remained unchanged (19 unchanged, 4 improved, 5 worsened). By the end of the second treatment period, absenteeism had not worsened in any woman, remained unchanged in 21 and had improved in three. There were no major differences between the women in the two treatment sequences in terms of the
proportions of individuals who had improved, remained unchanged or worsened (p=0.53 for first treatment period, p=0.55 for second treatment period).

Whilst on the trial no woman in either treatment sequence group consulted a doctor on account of menstrual bleeding. When asked which treatment period they had preferred, 21 expressed a preference of whom four (19.0%) preferred the first treatment period and 17 (81.0%) preferred the second treatment period (p=0.001, Sign test for preferences). After unblinding the treatment groups, these results implied that 13 women (61.9%) had preferred the placebo and eight (38.1%) had preferred DDAVP (p=0.37, Sign test for preference). Further analysis, however, revealed that these differences would be expected given the higher proportion of women who preferred the second treatment period. Thus, these results are unlikely to indicate any real preference for the placebo.

Overall, 83% (24/29) of women experienced one or more adverse events during the study. Three women did not start their second treatment, all of whom were receiving placebo followed by DDAVP. Thus these women did not receive DDAVP. Sixty-nine percent (18/26) and 52% (15/29) of women experienced one or more adverse events whilst receiving DDAVP and placebo, respectively (p=0.30, Chi-squared test). The reported adverse events were primarily headache and weight gain. Headache was reported in 23% (6/26) and 24% (7/29) of women receiving DDAVP and placebo, respectively. Analgesics, mainly paracetamol, were administered by six and five women, respectively, to settle the headache. No action was required in the remaining women.
Weight gain was reported in 12% (3/26) of women receiving DDAVP and in none receiving placebo. On the whole, weight fluctuations as recorded in the women’s diaries were larger during the first treatment period than during the second treatment period. However, there was no evidence that treatment with DDAVP spray altered the general pattern of weight changes during menstruation compared to placebo treatment. Generally, fluid intake was higher on the days of treatment and the first day after treatment in the first treatment period (Mean [sd] – treatment day 1: 1625 [1112.8], treatment day 2: 1529 [781.4], post-treatment: 1626 [775.7] ml) than during the second treatment period (treatment day 1: 1392 [389.0], treatment day 2: 1472 [416.0], post-treatment 1427 [420.0]). However, there was no clear evidence of any differences in fluid intake according to the treatment received.

When the analyses were repeated on the per-protocol population, the results were largely unchanged. In addition, when the analyses were performed only in the women with von Willebrand’s disease, the results were again unchanged.
Table 9.1 - Baseline demographic and clinical factors overall, and by treatment sequence group.

<table>
<thead>
<tr>
<th></th>
<th>Treatment sequence</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>overall</td>
<td>DDAVP/placebo</td>
<td>Placebo/DDAVP</td>
<td>p value</td>
<td></td>
<td></td>
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<tr>
<td>Number of women 29 (100%)</td>
<td>16 (100%)</td>
<td>13 (100%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>vWD</td>
<td>22 (75.9%)</td>
<td>14 (87.5%)</td>
<td>8 (61.5%)</td>
<td></td>
<td>0.19</td>
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</tr>
<tr>
<td>Other bleeding disorders</td>
<td>7 (24.1%)</td>
<td>2 (12.5%)</td>
<td>5 (38.5%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Mean (sd)</td>
<td>31.9 (8.7)</td>
<td>32.2 (10.2)</td>
<td>31.6 (6.9)</td>
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<td>0.85</td>
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<td>Weight (kg)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (sd)</td>
<td>66.6 (11.0)</td>
<td>66.2 (10.7)</td>
<td>67.2 (11.8)</td>
<td></td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Low cycle length</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>28 (21-30)</td>
<td>28 (21-30)</td>
<td>28 (21-28)</td>
<td></td>
<td>0.57</td>
<td></td>
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<tr>
<td>High cycle length</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Median (range)</td>
<td>30 (24-42)</td>
<td>30 (24-42)</td>
<td>30 (28-30)</td>
<td></td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>Previous treatment for menorrhagia</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (sd)</td>
<td>21 (72.4%)</td>
<td>12 (75%)</td>
<td>9 (69.2%)</td>
<td></td>
<td>1.00</td>
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</tr>
<tr>
<td>PBAC scores</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (sd)</td>
<td>278.7 (109.0)</td>
<td>292.1 (96.9)</td>
<td>262.1 (124.2)</td>
<td></td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>Absent from work/school*</td>
<td>16 (55.2%)</td>
<td>8 (50%)</td>
<td>8 (61.8%)</td>
<td></td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Consulted a doctor*</td>
<td>8 (27.6%)</td>
<td>4 (25%)</td>
<td>4 (30.8%)</td>
<td></td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

sd, standard deviation; * because of menstrual bleeding
Figure 9.1 - Menstrual scores at the end of the first and second treatment periods. Straight lines - treatment sequence DDAVP/Placebo, Dashed lines - treatment sequence Placebo/DDAVP.
Figure 9.2 - Menstrual scores: pre-treatment, first and second treatment periods. Horizontal lines represent median values.

Figure 9.3 - Menstrual scores: pre-treatment, placebo and DDAVP treated periods. Horizontal lines represent median values.
9.4: DISCUSSION

This study is the first reported randomised trial assessing the use of DDAVP nasal spray for the treatment of menorrhagia in patients with inherited bleeding disorders. Although menstrual blood loss as assessed by PBAC scores were significantly lower with DDAVP treatment compared to pre-treatment, the differences in the scores were not statistically significant when DDAVP nasal spray was used in comparison to placebo nasal spray. However, this study has some limitations which could have led to this result. Firstly the number of patients who completed the trial is small because of the strict exclusion criteria for entry, including patients not taking any antifibrinolytic or hormonal contraceptives three months prior and during the trial period and these are very commonly used by patients with inherited bleeding disorders. Secondly, the comparison between DDAVP or placebo spray is based on assessment of menstrual blood during one period only. However, variation in the amount of menstrual blood loss from one period to the next is considerable and a range of 39 to 271 ml in differences between the lightest and the heaviest period has been reported in patients with menorrhagia (Haynes et al 1977). Thirdly, the dose and duration of treatment may have been inadequate especially for those with very long and heavy menstrual periods. Finally, the level of FVIII and vWF:Ac were not determined after administration of the therapy. Therefore, it is not possible to be certain that therapeutic levels were attained in these women.

In this study, there is a strong placebo effect as demonstrated by the significant improvement of PBAC scores while using placebo nasal spray compared to pre-treatment assessment. The PBAC scores improved throughout the trial and was significantly lower in period 2. The quality of life of the patients was better and the
preference was for the second treatment. This may be related to the improved PBAC score or may be because the second treatment was fresh in mind and therefore selected in preference to the treatment further in the past. Even weight fluctuation during menstrual period was less pronounced and fluid intake was reduced in period 2 compared with period 1. However, there was no treatment effect on PBAC scores, quality of life, adverse effects, changes in weight and fluid effect. The effect of placebo on the treatment of menorrhagia has also been shown by other studies; in a double blind randomised study assessing a prodrug of tranexamic acid (Kabi 2161), 20% of women in the placebo group considered their menstrual bleeding to be less than usual (Edlund et al 1995).

Despite the high withdrawal rate, compliance with the nasal spray was good and the spray was well tolerated by the patients in this study. Side effects of DDAVP are very few, usually mild tachycardia, headache and flushing due to its vasomotor effect. There is also the slight risk of hyponatraemia and potentially water intoxication as DDAVP has an antidiuretic effect. Diminished response following repeated doses, tachyphylaxis, can occur in most patients with intravenous infusions (Mannucci et al 1992). Side effects with intranasal administration are reported to be minimal, the commonest being warmth and flushing (Rose & Aledort 1991, Seremetis & Aledort 1994) and used once daily no tachyphylaxis is seen (Aledort 1995).

In summary, although DDAVP treatment resulted in a decrease in PBAC scores, there was no significant difference when compared to placebo. Performance of further randomised controlled trials with larger number of patients using higher doses and/or longer duration of treatment for selected patients is recommended.
Assessment of efficacy should be based on the measurement of menstrual blood loss of at least three consecutive periods as well as assessing the effect on patients' satisfaction and quality of life. Combination treatment of DDAVP spray and antifibrinolytics should also be considered and assessed.
CHAPTER 10

CONCLUSIONS AND FUTURE STUDIES

10.1 - OVERALL CONCLUSIONS

10.2 - SUGGESTED FUTURE RESEARCH
10.1: OVERALL CONCLUSIONS

This thesis highlights the significant obstetric and gynaecological morbidity associated with inherited bleeding disorders. To minimise this morbidity and to optimise the care provided to women with inherited bleeding disorders, there is the need for the development of national or international guidelines for management and close collaboration between the woman herself and all her care givers, including the social worker, geneticist, perinatologist, obstetrician and gynaecologist and haematologist, i.e. a comprehensive care approach similar to the care provided for males with haemophilia. We have established guidelines relating to pregnancy care and have been published in the British Journal of Obstetrics and Gynaecology (Kadir et al 1997).

The uptake of prenatal diagnosis and termination of an affected pregnancy is low in carriers of haemophilia. Among the study population, the uptake of prenatal diagnosis was 35% and termination of pregnancy was chosen in only 50% of affected male fetuses. The attitudes of carrier women towards these and other reproductive choices is influenced by ethnic and cultural issues and family experience with the disease. The role of counselling is invaluable in helping these women and their partners to make informed choices. Fetal gender determination by ultrasound has important implications in the management of carriers of haemophilia who do not wish to have specific prenatal diagnosis. Knowledge that the fetus is female can be reassuring and the risks of traumatic haemorrhage to a male fetus can be minimised by avoiding invasive monitoring techniques and difficult instrumental deliveries. In addition, lack of prior knowledge of fetal gender can adversely influence the mode of delivery resulting in unnecessary Caesarean sections. Therefore, the importance of
this should be emphasised to the couple and positive diagnosis of the gender should be made and clearly documented for the attending obstetrician in labour.

There is a significant and progressive increase in FVIII in carriers of haemophilia A and FVIII, vWF:Ag, vWF:Ac in women with type 1 vWD during pregnancy. In contrast, carriers of haemophilia B and women with type 3 vWD do not show a significant rise in their plasma clotting factors. In FXI deficiency, the changes in FXI level seemed to be variable as there was an increase in some pregnancies and a decrease in others but the overall changes were not statistically significant. Even in those bleeding disorders with a significant increase in the clotting factors, the rise is variable and unpredictable and a significant proportion of women still have levels below 50 iu/dl at term. Thus monitoring factor levels during pregnancy, especially prior to any antenatal invasive procedure and labour is very important.

Maternal haemorrhagic complications are usually confined to the postabortal and the post-partum periods. The incidence of primary and secondary post-partum haemorrhage was 22% and 11% in carriers of haemophilia, 18.5% and 20% in vWD and 16% and 24% in FXI deficient women, respectively. Most of the significant post-partum haemorrhage occurred in women with low factor levels (< 50 iu/dl) with no prophylactic treatment. In contrast, none of the patients who had prophylactic treatment for labour and puerperium had any significant bleeding complications. The risk of haemorrhage can therefore be reduced by minimising maternal trauma and prophylactic treatment when appropriate. Acquired post-partum haemophilia can be an unusual cause of a severe and unexpected post-partum haemorrhage. Early
recognition and treatment of this rare condition is important to reduce its high morbidity and mortality.

Women with inherited bleeding disorders suffer from heavy and prolonged menstruation. Objectively confirmed menorrhagia using the pictorial blood assessment chart was significantly higher in women with vWD, carriers of haemophilia and FXI deficiency (74%, 57% and 59%, respectively) compared with a control group of women (29%). Menstruation has a negative effect on the quality of life in women with inherited bleeding disorders. Increased awareness among clinicians of the high prevalence of menorrhagia and the available treatment options is necessary for optimal management and to improve quality of life of these patients. Home treatment with DDAVP nasal spray is a safe and attractive option for management of menorrhagia in women with vWD and carriers of haemophilia A. The efficacy of this treatment modality was assessed in this thesis in a prospective randomised placebo controlled trial. Although there was an indication that menstrual bleeding was less heavy when women received DDAVP than when receiving placebo, the difference was not statistically significant.

Undiagnosed inherited bleeding disorders, especially in their mild forms, can be the underlying cause of menorrhagia in a significant proportion of women. vWD was diagnosed in 13% of patients and other hereditary haemorrhagic disorders in another 4% in women with menorrhagia and no obvious pelvic pathology. Although certain factors in the patient’s history predict an increased risk of a bleeding disorder, such as history of menorrhagia since menarche and a history of bleeding after tooth extraction, operations and parturition, menorrhagia may be the only clinical
manifestation of these disorders. Therefore, testing for these disorders, especially vWD, in women with menorrhagia with no obvious pelvic pathology should be considered before embarking on any invasive surgical procedure. Similarly, unexplained post-partum, operative and post-operative haemorrhage that does not respond to general measures should alert obstetricians and gynaecologists to the possibility of bleeding disorders as a causative factor.

There are great inter- and intra-individual variations in coagulation markers in women. These variations can be due to different physiological conditions including age, ethnicity, blood group and hormonal changes during various phases of the menstrual cycle. These factors require consideration in the diagnosis and management of patients with inherited bleeding disorders, especially the most common disorder in women, mild vWD. Timing of blood sampling, when the variability is minimal, is important for accurate diagnosis. In addition, knowledge of the phase when clotting factors are at their peak or nadir could be helpful when contemplating elective surgical procedures in these patients.
10.2 : SUGGESTED FUTURE RESEARCH

Whilst the series of research in this thesis included a relatively large number of women with the most common inherited bleeding disorders (vWD, carriers of haemophilia and FXI deficiency) the results represent the experience and the outcome of only one centre and there is a need for multi-centre studies to confirm these results in a bigger population, to compare different management methods and to gather sufficient information about women with less common bleeding disorders such as prothrombin, fibrinogen, FV, FVII, FX or FXIII deficiency. These multi-centre studies would help in the development of international management guidelines which are of paramount importance in the management of rather uncommon conditions like inherited bleeding disorders.

As yet we do not have sufficient data concerning the rate of miscarriage and recurrent miscarriages in women with various bleeding disorders. In women with type 2 and 3 vWD, the miscarriage rate appeared to be greater than the general population in a multi-centre study by Foster 1995. In women with vWD included in this thesis, there was a higher incidence of vaginal bleeding in the first trimester (i.e. threatened miscarriage), but there was no increase in miscarriage rate. In FXI deficient women, the numbers were too small to draw any conclusions. In carriers of haemophilia, there appeared to be a high number of reported miscarriages (22/82 pregnancies). However due to lack of adequate and prospective information, these figures were not analysed or commented on. A formal large case-control study is needed to assess any possible association between different types and subtypes of bleeding disorders and miscarriages.
DDAVP is an effective and safe drug in the management of women with inherited bleeding disorders and reduces the need for transfusional therapies, thus reducing the risk of transfusion acquired diseases. Some of these conditions are of particular importance in obstetrics due to possible vertical transmission and severe fetal infection, such as Parvovirus B. Therefore, the use of DDAVP ante-partum needs to be assessed and the concerns regarding its safety for the mother and the infant should be re-examined.

There is lack of evidence-based clinical recommendations and guidelines regarding the use of regional analgesia or anaesthesia in labour and for operative deliveries in women with inherited bleeding disorders because of the risk of spinal haematomas. These women are usually denied the benefits of these procedures. Studies to assess the coagulation status and minimum factor levels required for safe administration of these techniques and the role of prophylactic treatment with DDAVP or factor concentrates in association with these procedures are required.

The risk of post-partum haemorrhage is well established in women with inherited bleeding disorders. However, there are insufficient data to answer the following: whether the risk is related to absolute values or changes in values of coagulation factors or bleeding time; whether prophylactic treatment to cover labour and the post-partum reduce the risk of primary and secondary post-partum haemorrhage; should it be administered empirically to all patients or only for those with low levels at term (e.g. < 50 iu/dl) and, in vWD, or those with type 2 and 3; what is the optimal treatment modality, level and duration. Prospective studies are needed to clarify these issues. The use of anti-fibrinolytic agents post-partum is inadvisable according to UK
guidelines for the diagnosis and management of vWD (1997) due to the theoretical risk of post-partum thrombosis. This issue needs to be formally assessed given the efficacy of the agents in the management of intermittent and prolonged secondary post-partum haemorrhage which is a common presentation especially in women with vWD or FXI deficiency.

The high frequency of inherited bleeding disorders in patients with menorrhagia needs to be confirmed by larger studies and whether screening for these disorders should be performed in all women with menorrhagia or only in those with certain clinical criteria needs to be determined. Which test should be used for screening and criteria for referral to the haemophilia centre for confirmation of diagnosis also need further evaluation.

The use of pictorial blood assessment chart as a mean of objective assessment of menstrual loss, its acceptability among women with inherited bleeding disorders and its role in screening women for menorrhagia need to be assessed. The efficacy/failure rate of antifibrinolytics and oral contraceptives for the treatment of menorrhagia requires evaluation. The DDAVP nasal spray is increasingly recommended for treatment of menorrhagia in women with certain types of inherited bleeding disorders. In this thesis, the efficacy of DDAVP was not significantly different to a placebo treatment. However, further assessment in larger studies avoiding the limitations of the study presented in Chapter 9 is required. More precise identification of women responsive to DDAVP is also required prior to treatment. Assessment of efficacy should be based on measurement of menstrual blood loss during at least three consecutive periods as well as assessing the effect on patients
satisfaction and quality of life. Combination treatment of DDAVP spray and antifibrinolytics should also be considered and assessed. Finally the role and effectiveness of Levonorgestrel intra-uterine system, Mirena (LNG IUS) in the treatment of menorrhagia in these women also requires evaluation.

There is little information in the literature concerning the rate of hysterectomy in women with inherited bleeding disorders for refractory menorrhagia. This could be obtained from multi-centre studies and guidelines need to be drawn to reduce unnecessary surgical interventions and pre-operative preparations to minimise their associated bleeding complications. Patient preference, quality of life and cost analyses are also required to compare the long-term use of the commonly used medical treatments in these women (oral contraceptives and antifibrinolytics), nasal DDAVP, Mirena intra-uterine device, versus endometrial ablation and hysterectomy for optimal control of menorrhagia.

The pattern of changes of coagulation factors during the menstrual cycle needs to be confirmed by further studies with larger numbers of women and more frequent measurements. In addition, plasma oestradiol and progesterone levels need to be assessed at the same time as the coagulation factors in order to demonstrate any possible correlation between various coagulation markers and sex hormones. Similarly, the effect or lack of effect of low dose oestrogen (\( \leq 30 \mu g \)) combined oral contraceptive pills also requires assessment.

In addition to the above specific obstetric and gynaecological issues, there are several topics about the diagnosis and management of inherited bleeding disorders that
require future research. This is especially true in type 1 vWD, the commonest bleeding disorder in women. Diagnosis of this disorder, especially its mild forms, is difficult due to the considerable intraindividual phenotypic variance. The same patient may have different results if sampled at different time-points or in different laboratories. The diagnostic accuracy can be considerably improved by improvement of molecular genetic diagnosis of this disorder and elucidation of the genotype in each family. Until genetic diagnosis become the mainstay of diagnosis, better characterisation i.e. guidelines defining criteria for diagnosis of mild forms of vWD are required.
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Appendix 1

Questionnaire used for assessment of attitudes and reproductive choices of women with inherited bleeding disorders

Serial Number:

Date of birth: / / 

Marital Status:

Religion:

1. How many times have you been pregnant?

   If you have never been pregnant but are currently trying to start a family, please go straight to question 6.

   If you have never been pregnant and currently have no plans to start a family please go straight to question 7.

How many children do you have?

   Boys: 

   Girls: 

Do any of the boys have haemophilia?

   Yes 

   No 

If Yes, how many?

2. Did you have any bleeding complications which may have been related to a clotting factor disorder around the time of birth of any of your children?

   Yes 

   No 

Did you require blood transfusion because of excess bleeding at the birth of any of your children?

   Yes 

   No 

3. Did you have antenatal diagnosis for haemophilia in any of your pregnancies?

   Yes 

   No 

If Yes, please tick which pregnancies these were:

First    Second    Third    Fourth    Fifth
4. Were any of your pregnancies terminated?  

Yes [ ]  

No [ ]

IF yes, (i) how many?

(ii) reasons for termination/s:

5. The following are possible factors which may have influenced your decision about whether to have the baby or to terminate the pregnancy. For each pregnancy, please rank the factors from 1 to 7 to indicate how important they were to you in making this decision (1 = most important, 7 = least important)

<table>
<thead>
<tr>
<th>Pregnancy</th>
<th>First</th>
<th>Second</th>
<th>Third</th>
<th>Fourth</th>
<th>Fifth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Religious beliefs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Personal beliefs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partner's beliefs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Own family's beliefs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partner's family's beliefs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Friend's opinions</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other reasons (please specify below)</td>
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</tbody>
</table>

Other reasons:
6. When you decided to start a family, or decided to have more children, what factors influenced you in your decision?

First Child:

Other children:

7. Have you ever made a conscious decision not to have a child, or not to have any more children?

Yes [ ]

No [ ]

If yes, what factors influenced you in this decision?

8. In the past, when you have either made decisions about starting a pregnancy, or have had to make a decision about whether to continue a pregnancy or to have the pregnancy terminated (if applicable), did you find the information provided by any of the sources mentioned below useful?

<table>
<thead>
<tr>
<th>Source</th>
<th>Yes</th>
<th>No</th>
<th>None Provided</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemophilia Centre staff</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>General Practitioner</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Haemophilia Society leaflets</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

9. If you were pregnant now would you prefer to know the sex of the baby

(a) during pregnancy [ ]

OR (b) after the birth of the child [ ]

THANK YOU FOR YOUR HELP

Please return the completed questionnaire in the reply-paid envelope provided.
Appendix 2

Pictorial blood assessment chart and the scoring system used for assessment of menstrual blood loss

<table>
<thead>
<tr>
<th>Date of Start</th>
<th>Date of Birth</th>
<th>Hospital Number</th>
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<tbody>
<tr>
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<table>
<thead>
<tr>
<th>Patient Name</th>
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</table>

<table>
<thead>
<tr>
<th>Towel</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<table>
<thead>
<tr>
<th>Closets/ Flooding</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<table>
<thead>
<tr>
<th>Tampon</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<th>Closets/ Flooding</th>
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</table>

(Closets: size of a coin = 1p/50p etc)

You will see below an example of how to complete the chart, using the detailed scoring system.

Name: Patient
Day Start: 05/11/98

Score: 208

<table>
<thead>
<tr>
<th>Towel</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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<tr>
<th>Closets/ Flooding</th>
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<table>
<thead>
<tr>
<th>Tampon</th>
<th>1</th>
<th>2</th>
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<tr>
<th>Closets/ Flooding</th>
<th>1</th>
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</tbody>
</table>

Assessment of menstrual blood loss using the pictorial blood loss assessment chart (PBAC)
### Scoring System

<table>
<thead>
<tr>
<th><strong>Towels</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 point</td>
<td>for each lightly stained towel</td>
</tr>
<tr>
<td>5 points</td>
<td>for each moderately soiled towel</td>
</tr>
<tr>
<td>20 points</td>
<td>if the towel is completely saturated with blood</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Tampons</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 point</td>
<td>for each lightly stained tampon</td>
</tr>
<tr>
<td>5 points</td>
<td>for each moderately soiled tampon</td>
</tr>
<tr>
<td>10 points</td>
<td>if the tampon is completely saturated with blood</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Clots</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1 point</td>
<td>for small clots (size of 1p coin)</td>
</tr>
<tr>
<td>5 points</td>
<td>for large clots (size of 50p coin)</td>
</tr>
</tbody>
</table>

From Higham et al; British Journal of Obstetrics and Gynaecology; 1990; 97: 734 – 739
Appendix 3

Questionnaire used for assessment of quality of life during menstruation in women with inherited bleeding disorders

Patient No: ___________________ Patient Initials: ______________________

In general, would you say your health is:

- excellent □  very good □  good □
- fair □  poor □

Health and Daily Activities
The following questions are about activities you might do during a typical day. Does your bleeding period/menstruation limit you in these activities? If so, how much?

<table>
<thead>
<tr>
<th>Activity</th>
<th>Yes limited a lot</th>
<th>Yes limited a little</th>
<th>No, not limited at all</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling or playing golf</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Lifting or carrying groceries</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Climbing several flights of stairs</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Climbing one flight of stairs</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Bending, kneeling or stooping</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Walking more than a mile</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Walking half a mile</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Walking 100 yards</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Bathing and dressing yourself</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>

Have you had any of the following problems with your work or other regular daily activities as a result of your bleeding period/menstruation?

<table>
<thead>
<tr>
<th>Problem</th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cut down on the amount of time you spent on work or other activities</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Accomplished less than you would like</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Were limited in the kind of work of other activities</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Had difficulty performing the work or other activities (e.g. it took extra effort)</td>
<td>□</td>
<td>□</td>
</tr>
</tbody>
</table>
How much bodily pain have you had during the last bleeding period/menstruation?

<table>
<thead>
<tr>
<th>None</th>
<th>Very mild</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Very severe</th>
</tr>
</thead>
</table>

During your last bleeding period/menstruation how much did pain interfere with your normal work (including work both outside the home and housework)?

<table>
<thead>
<tr>
<th>Not at all</th>
<th>A little bit</th>
<th>Moderately</th>
<th>Quite a bit</th>
<th>Extremely</th>
</tr>
</thead>
</table>

How much time during your last period

<table>
<thead>
<tr>
<th>All of the time</th>
<th>Most of the time</th>
<th>A good bit of the time</th>
<th>Some of the time</th>
<th>A little of the time</th>
<th>None of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Did you feel full of life?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Have you been a very nervous person?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Have you felt so down in the dumps that nothing could cheer you up?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Have you felt calm and peaceful?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Did you have a lot of energy?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Have you felt down hearted and low?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Did you feel worn out?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Have you been a happy person?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Did you feel tired?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Has your health limited your social activities (like visiting friends or close relatives)?</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Have you even been absent from work / school?</td>
<td>Yes □ No □</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Thank you for helping us by completing this questionnaire and returning it to the nursing staff.