WOMEN WITH INHERITED BLEEDING DISORDERS AND THEIR OFFSPRING – THE UNRESOLVED ISSUES

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I, Joanna Susan Davies, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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ABSTRACT

The past few decades have seen major advances in management of gynaecological conditions and multidisciplinary obstetric care in women with inherited bleeding disorders (IBDs) and their offspring. However, there remain many unresolved issues within the field. A series of observational studies were conducted to address these issues with an overarching aim of improving patient care.

A case-control study determined if there was an association between IBDs and endometriosis. Women with a surgically confirmed diagnosis of endometriosis (n = 84) and controls (n = 30) underwent investigations of haemostasis. Women with endometriosis had significantly more platelet aggregation defects to one and multiple agonists compared to controls (31% vs 4%, p = 0.005 and 15% vs 4%, $p < 0.05$, respectively). Reduced von willebrand factor (VWF) activity correlated with increased laparoscopic stage of endometriosis (r = -0.35, p = 0.01).

A 10-year review and questionnaire study was carried out in carriers of haemophilia to determine their attitudes towards prenatal diagnosis. Sixty-one carriers of haemophilia had obstetric care in 73 pregnancies. Forty-one out of 61 women responded to the questionnaire. The uptake for invasive prenatal diagnosis of haemophilia was reduced compared to previous studies published at the Royal Free Hospital (15% versus 20% in 2008 [1], and 35% in 1997 [2]). Invasive testing to confirm the haemophilia status of the fetus was used to guide management decisions of labour and delivery. The rate of termination of pregnancy (TOP) for haemophilia was lower than in previous case series [1]. Non-invasive determination of fetal gender

using free fetal DNA (ffDNA) was carried out in 58 pregnancies (79%). Fifty-nine deliveries were managed at the Royal Free Hospital over 10-years. The majority of women (66%) in this series underwent elective caesarean section (CS). The primary indication for CS was for haemophilia in 59% of deliveries.

A literature review and meta-analysis assessed the incidence of cranial bleeding at birth in newborns with haemophilia. The incidence of symptomatic intracranial haemorrhage (ICH) was determined by mode of delivery (MOD). Newborns with haemophilia were 44 times (95%CI 34.7-57.1, $p < 0.01$) more likely to experience symptomatic ICH, and 8 times (95%CI 5.38-12.6, $p < 0.01$) more likely to experience extracranial haemorrhage (ECH) at birth, compared to the general population. The OR of experiencing ICH following an assisted vaginal delivery was 4.4 (95%CI 1.46-13.7, $p = 0.008$) compared to vaginal delivery in newborns with haemophilia. The OR of experiencing ICH following CS was 0.34 (95%CI 0.14-0.83, $p = 0.018$) compared to vaginal delivery. CS was associated with the lowest risk of ICH in newborns with haemophilia.

A prospective MRI screening study in term newborns with severe IBDs was undertaken to determine feasibility and incidence of asymptomatic ICH. Cranial MRI within 72 hours of delivery excluded asymptomatic ICH in affected infants. No cases of ICH were reported among eight participants. One newborn experienced cephalohaematoma following an emergency CS. Two of the eight newborns experienced spontaneous ICH in early infancy.

A case-control study analysed the differences in rotational thromboelastometry (ROTEM®) parameters between parturient women with FXI deficiency, and parturient and non-parturient controls. Women with FXI deficiency achieved a hypercoagulable status during the third trimester of pregnancy; however, the changes were not as pronounced as in pregnant controls. Women with prolonged clotting time and clot formation time were considered to have an increased risk of bleeding. A prospective cohort study evaluated the role of ROTEM® analysis in assessment of bleeding risk in women with FXI deficiency. Pregnancy outcomes and haemostatic cover was reviewed in 57 deliveries in women with FXI deficiency. ROTEM® enabled treatment decision and reduced the need for treatment with factor concentrate in women with severe FXI deficiency.

A cohort study assessed the correlation between bleeding score, haemostatic and prothrombotic variables in women with VWD and carriers of haemophilia. The presence of a thrombotic marker (anticoagulant deficiency or prothrombotic gene mutation) altered the bleeding score for a given VWF:RCo level in women with VWD (p = 0.015). Co-inheritance of thrombophilia reduces bleeding severity in women with IBDs, and thrombotic risk must be considered in these women.

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ABBREVIATIONS

APH	Antepartum haemorrhage
APTT	Activated partial thromboplastin time
AVD	Assisted vaginal delivery
BSS	Bernard-Soulier Syndrome
CS	Caesarean section
CT	Computerised tomography
CVS	Chorionic villus sampling
ECH	Extracranial haemorrhage
ffDNA	Free fetal DNA
FVIII	Factor VIII
FVIII:C	Factor VIII activity
FIX	Factor IX
FIX:C	Factor IX activity
FXI	Factor XI
FXI:C	Factor XI activity
GT	Glanzmann's Thrombasthaenia
HMB	Heavy menstrual bleeding
IBD(s)	Inherited bleeding disorder(s)
ICH	Intracranial haemorrhage
IVH	Interventricular haemorrhage
MOH	Massive obstetric haemorrhage
MRI	Magnetic resonance imager

NICE	National institute for Health and Care Excellence
PBAC	Pictorial blood assessment chart
PPH	Postpartum haemorrhage
PFD(s)	Platelet function disorder(s)
PPP	Platelet poor plasma
PRP	Platelet rich plasma
PT	Prothrombin time
rASRM	revised American Society for Reproductive Medicine
rFVIIa	Recombinant activated factor VII
SDH	Subdural haematoma
SGH	Subgalaleal haematoma
SVD	Spontaneous vaginal delivery
TOP	Termination of pregnancy
TXA	Tranexamic acid
UKHCDO	United Kingdom Haemophilia Centre Doctors' Organisation
US	Ultrasound scan
VTE	Venous thromboembolism
VWD	von Willebrand disease
VWF	von Willebrand factor
VWF:Ag	von Willebrand factor antigen
VWF:CB	von Willebrand factor collagen binding capacity
VWF:RCo	von Willebrand factor ristocetin cofactor activity

PUBLICATIONS

List of peer reviewed publications related to thesis

- Davies, J., and Kadir, R.A. Mode of delivery and cranial bleeding in newborns with haemophilia: a systematic review and meta-analysis of the literature. *Haemophilia* 2015; 1-7, DOI: 10.1111/hae.12726. Original article.

- Davies, J., Harper, A., Kadir, R.A. The role of rotational thromboelastometry in assessment of haemostasis during pregnancy in women with factor XI deficiency. *Haemophilia* 2015. DOI: 10.1111/hae.12807. Original article.

List of other publications and invited reviews related to thesis

- Davies, J and Kadir, R.A. The management of factor XI deficiency in pregnancy. *Semin Thromb Haemost* 2016. DOI: 10.1055/s-003601587685. Review article

- Kadir, R.A., Davies, J. Hemophilia Carriers: Diagnosis, Bleeding Risk and Treatment. May 2014 for the *International monitor on Hemophilia*, Issue 22. Review article

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- Davies, J and Kadir, R.A. Endometrial haemostasis and menstruation. *Rev Endocr Metab Disord* 2012. 13(4): p. 289-99. DOI: 10.1007/s11154-012-9226-4. Review article

- Davies, J and Kadir, R.A. Reply to von Willebrand's disease and postpartum haemorrhage by Chee et al. *Haemophilia* 2012. 18(6): p. e399-400. DOI: 10.1111/hae.12029. Letter to editor.

List of studies under review for publication

- Davies, J., Hussein, B., Riddell, A., Rahimy, O, and Kadir, R.A. The prevalence of bleeding disorders in women with endometriosis: a case-control study. Submitted to *AJOG* Jan 2016 (under review).

- Davies, J., Chi, C., Shareif, L., Tuddenham, E., Kadir, R.A. The effect of thrombotic markers on severity of bleeding symptoms in women with inherited bleeding symptoms.

CHAPTER 1

INTRODUCTION

1.1 General introduction

1.2 Aims and layout of thesis

1.1 General Introduction

Inherited bleeding disorders (IBDs) result from a deficiency or abnormal functioning of coagulation factors or proteins. Obstetric and gynaecological (O&G) bleeding specific to IBDs have long been underestimated in women. However, an increasing number of publications have reported O&G morbidity in women with IBD in recent years [3-5] (Fig 1.1). Bleeding is expected during menstruation and childbirth therefore excessive bleeding in women may be overlooked [6].

Von Willebrand disease (VWD), haemophilia A and haemophilia B are the most common IBDs. They account for 75% of all patients registered on the United Kingdom Haemophilia Centre Doctors' Organisation (UKHCDO) registry in 2012. At the Royal Free Hospital, these disorders account for 62% of all patients registered at the Katharine Dormandy Haemophilia Centre and Thrombosis Unit. As the centre is located in North London, an area with a large Jewish population, factor XI (FXI) deficiency constitutes most (22%) of the remaining patients. The centre registry also includes patients with platelet function disorders (PFDs) and other rare bleeding disorders.

A multidisciplinary, joint O&G and haemophilia clinic was set up in 2002 for women with IBDs. The aim was to provide joint specialist expertise in treating gynaecological bleeding and provide obstetric input during pregnancy in women with IBD. Research conducted at the Royal Free initially reported the high prevalence of IBDs in women with heavy menstrual bleeding (HMB) and normal pelvic examination [3]. A high frequency of menstrual problems were subsequently reported

in women with IBD [7]. Efficacy of various treatments for HMB in women with IBDs has been explored [8].

The Royal Free was the first centre to report pregnancy outcomes in carriers of haemophilia [1, 2], and in women with VWD, and FXI deficiency [3, 9]. From the late 1980s, carriers of haemophilia were offered invasive prenatal diagnosis to determine the haemophilia status of the fetus. The Royal Free Hospital is collaborating with the Li Ka Shing Institute of Health Sciences in Hong Kong, to develop a non-invasive method of diagnosing haemophilia in the fetus using free fetal DNA (ffDNA) [10]. Women with rare bleeding disorders (including coagulation factor (F) defects; FVII, FX, combined FV and FVIII, and FXIII) are also managed in the Joint clinic, as well as women with PFDs, including rare platelet defects such as Glanzmann's thrombasthenia and Bernard-Soulier Syndrome. The Joint clinic is a tertiary referral clinic and women are referred from across the UK. This has amounted to an increasing experience with managing such rare bleeding disorders, and reporting their outcome. In the last 20 years, five MD(Res) theses and over 100 publications have been produced from the centre in relation to O&G problems and management in women with IBDs. Consequently, the subject of this thesis is focused on O&G issues for women with IBDs and their offspring that have not been tackled in previous research theses or publications.

1.2 Aims and layout of thesis

The main objectives of this thesis are to provide a structured background review of O&G morbidity in women with IBDs and their offspring. From the background review, the unresolved issues that have not been addressed will be highlighted. The series of studies included in this thesis are designed to address these issues and provide better evidence to optimise patient care. Due to the diversity of study populations, each study presented by chapter will have a distinct hypothesis and a description of participants and methods used in that study. Laboratory methodologies used throughout the thesis are presented in Chapter 3.

A National Research Ethics Service (NRES) committee approved each study included in this thesis, with written informed consent obtained from each participant.

This thesis is comprised of a series of studies that address the unresolved issues in women with IBDs and their offspring. These include:

1. Case-control, laboratory-based study to assess the frequency of haemostatic abnormalities in women with endometriosis compared to controls (Chapter 4).

2. 10-year cohort study reviewing pregnancy outcomes following prenatal diagnosis in carriers of haemophilia. A survey to explore women's attitudes towards prenantal diagnosis and non-invasive methods, and how they have affected reproductive choice and MOD was conducted and reported (Chapter 5).

3. Systematic review of the literature to assess the incidence of symptomatic and asymptomatic intracranial and extracranial haemorrhage in neonates with haemophilia compared to the general population. Meta-analysis to assess the effect of MOD on rates of ICH (Chapter 6).

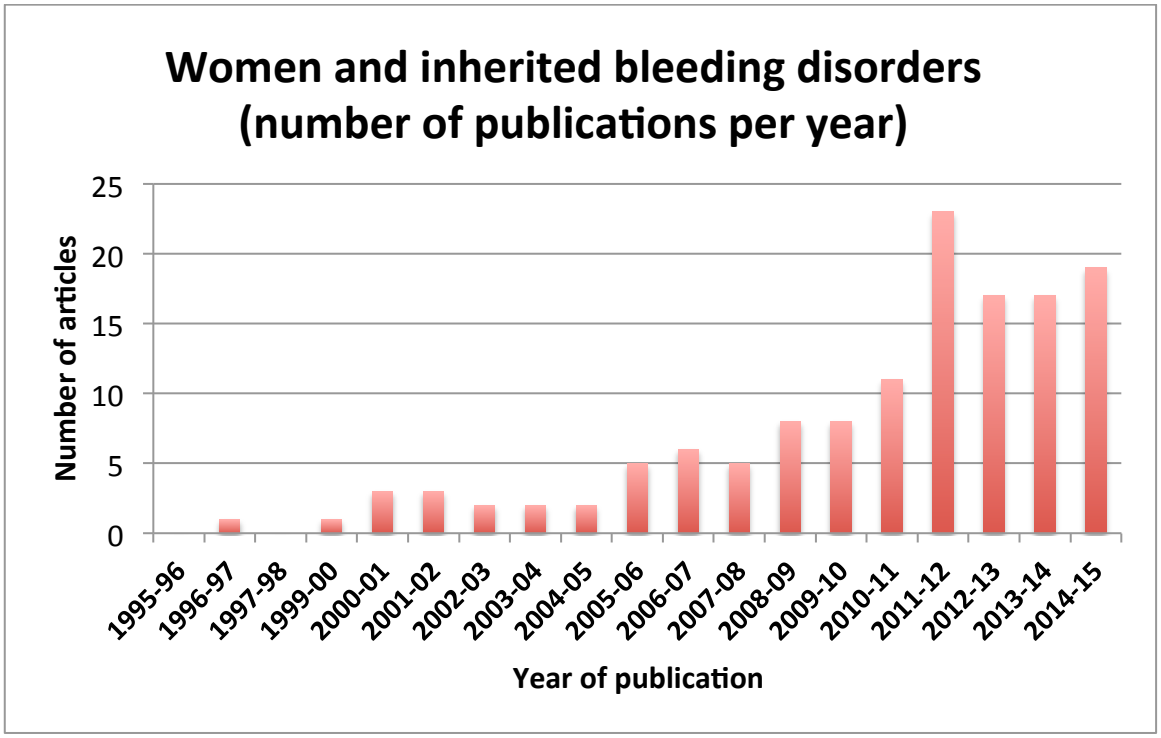
4. Prospective cohort study to determine the incidence of asymptomatic cranial bleeding in newborns with haemophilia, using cranial MRI imaging in the first few days of life (Chapter 6)

5. Case-control study to compare rotational thromboelastometry (ROTEM®) changes in parturient women with FXI deficiency compared to parturient, and non-parturient controls (Chapter 7).

6. Cohort study to assess the role of ROTEM® in the third trimester of pregnancy in assessment of bleeding risk and provision of haemostatic cover, in women with FXI deficiency (Chapter 8).

7. Cohort study to assess the correlation between bleeding phenotype, and level of coagulation factors, and prothrombotic factors in women with IBDs (Chapter 9).

Figure 1.1 Women and inherited bleeding disorders – number of published articles in the last 20 years.



CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

2.4 The neonate and inherited bleeding disorders

2.4.1 Neonatal haemostasis

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2.1 Inherited bleeding disorders

2.1.1 Von Willebrand Disease

Von Willebrand Disease (VWD) is the most prevalent IBD in women. Initially described by Erik von Willebrand in 1926, his index case was a young girl who bled to death at the age of 13 during her fourth menstrual period [11]. Epidemiological studies indicate that up to 1% of the general population has the condition [12]. It is characterised by a deficiency or dysfunction of von Willebrand factor (VWF).

VWF is a large adhesive glycoprotein synthesised by vascular endothelial cells, bone marrow megakaryocytes and subendothelial connective tissue. Multimers of VWF can be extremely large > 20,000 kDa, and consist of over 80 subunits. Only the large multimers are functional. VWF plays an important role in primary haemostasis by promoting the tethering and adhesion of circulating platelets as well as platelet aggregation. It also serves as a carrier protein for the procoagulant factor VIII (FVIII) in plasma, thus indirectly contributing to the generation of fibrin [13]. Normal variations in VWF levels result from ABO blood group, ethnicity and phases of the menstrual cycle [14]. VWF levels increase during pregnancy, with the use of combined oral contraceptives and are elevated in the neonatal period [15, 16]. The disorder is classified into three main categories (Table 2.1).

Classification

Type 1 VWD is the most common form of VWD, accounting for around 75% of all cases. It results from a quantitative deficiency in VWF due to mutations that suppress secretion, or enhance clearance [17]. In type 1 VWD the plasma VWF levels are

reduced with a proportionate reduction in VWF antigen (VWF:Ag), VWF ristocetin cofactor activity (VWF:RCo) and VWF collagen binding capacity (VWF:CB) at levels between 10-40 IU/dL (normal range 45-175 IU/dL). The factor VIII activity (FVIII:C) is normal or mildly decreased (normal range 50-150 IU/dL).

Type 2 VWD is the result of a qualitative functional deficiency in VWF and is further sub-classified into four variants depending on the functional defect present (Table 2.2). In type 2A VWD there is deficiency of high molecular weight (HMW) VWF due to abnormal intracellular processing and/or secretion [18], or due to an increase in proteolytic breakdown of large multimers [19]. This results in a reduction in VWF-dependent platelet adhesion with normal or near normal levels of VWF:Ag and FVIII:C but significant reduction in VWF:RCo and VWF:CB.

Type 2B VWD is characterised by the presence of variants of VWF with an increased affinity for glycoprotein Ib (GPIb) resulting in spontaneous binding to platelets and subsequent rapid clearance of platelet-bound HMW VWF multimers. The condition may be associated with thrombocytopaenia, which can be exacerbated by pregnancy, surgery or 1-desamino-8-D-arginine vasopressin (DDAVP) [20-22].

Type 2M VWD is characterised by a decreased ability of VWF to bind GP1b receptor on platelets [23, 24]. Although laboratory findings are similar to type 2A, it is distinguishable by multimeric analysis with the findings of normal levels of ultra large HMW VWF multimers.

In type 2N VWD there is deficiency in binding to coagulation FVIII. The FVIII:C level is disproportionately reduced (5-30 IU/dL) relative to the VWF:Ag. Type 2N VWD can be misdiagnosed as mild haemophilia A. An important distinction is the pattern of inheritance; autosomal recessive as opposed to X-linked. It can be confirmed by laboratory analysis using FVIII binding assay that measures the affinity of VWF to FVIII [25].

Type 3 VWD is characterised by virtual absence of VWF. The coagulation screen shows an isolated prolonged APTT and laboratory assays show markedly reduced (<5 IU/dL) levels of VWF:RCo, VWF:CB, and VWF:Ag and FVIII:C less than 10 IU/dL. Multimeric analysis shows no multimers due to a marked reduction in VWF.

Table 2.1 Primary classification of VWD

Category	Defect	VWF protein function	Proportion of cases
Type 1	Quantitative partial deficiency	Normal	70-80%
Type 2	Qualitative functional deficiency	Abnormal	15-20%
Type 3	Quantitative complete deficiency	Undetectable	1%

Table 2.2 Secondary classification of type 2 VWD

Subtype	Platelet-associated function	Factor VIII binding capacity	HMW VWF multimers
2A	Decreased	Normal	Absent
2B	Increased affinity for GP1b	Normal	Usually reduced/absent
2M	Decreased	Normal	Normal and occasional ultra-large forms
2N	Normal	Markedly reduced	Normal

GP1b; glycoprotein 1b. HMW VWF; high molecular weight von Willebrand factor.

Genetics and inheritance

VWD is caused by a mutation(s) in the *VWF gene,* located on the short arm of chromosome 12 [15]. Mutations can affect the complex biosynthesis of VWF multimers to impair assembly, intracellular targeting and secretion. Since chromosome 12 is an autosome (i.e. not on a sex chromosome), VWD occurs equally in men and women. Most VWD type 1, most type 2A, type 2B and type 2M are inherited in an autosomal dominant manner [26]. Most affected individuals have an affected parent. The proportion of cases caused by a *de novo* mutation is not known. The offspring of an autosomal dominant affected parent has a 50% chance of inheriting the condition.

VWD type 2N, type 3 and some VWD type 1 and type 2A are inherited in an autosomal recessive manner [26]. Siblings of individuals with autosomal recessive VWD have a 25% chance of being affected, a 50% chance of being an asymptomatic carrier, and a 25% chance of being unaffected and not a carrier of the condition.

Prenatal diagnosis, carried out mostly for type 3 VWD, is only possible if the genetic mutation in the family is known, or linkage analysis using intragenic markers is informative (see section 2.1.2).

Clinical manifestations

Typical bleeding symptoms in women with VWD are from mucosal surfaces such as easy bruising, epistaxis, oral cavity bleeding, PPH and HMB [3, 14, 27, 28]. The bleeding phenotype is highly variable and the severity of bleeding symptoms among family members with the same genetic mutation varies, reflecting the diverse

phenotype and variable penetrance of this disorder [29]. The response to a haemostatic challenge such as surgery, childbirth, trauma and invasive procedures such as dental extraction is variable. The bleeding risk can be challenging to predict and haemostatic prophylaxis requires careful consideration prior to any challenge. Type 3 VWD results in a severe bleeding phenotype classically likened to haemophilia. Spontaneous gastrointestinal, soft-tissue, joint and cerebral bleedings are rare, and only encountered in severe or type 3 VWD [30].

2.1.2 Carriers of haemophilia

Haemophilia A and B are caused by a deficiency in coagulation factors VIII (FVIII) and factor IX (FIX) respectively. The factor deficiency is a result of a genetic mutation in the corresponding genes, both of which are situated on the long arm of the X chromosome. Subsequently, they are X-linked recessive conditions with males inheriting the condition, and women are affected as carriers. Although rare, they are the most common severe IBD. Haemophilia A occurs with an incidence of 1 in 5,000 per live male births and haemophilia B affects one in 25,000 live male births. Approximately one third of cases of haemophilia A, and one fifth of cases of haemophilia B arise from a new mutation, without previous family history of the disorder.

Genetics

Haemophilia A is highly heterogenous at the mutational level with over 1000 different mutations described on the international Haemophilia A Mutation Database (http://hadb.org.uk, formerly the Haemophilia A Mutation, Structure, Test and Resource Site [HAMSTeRS] database) [31]. Roughly half of all cases of severe haemophilia A are the result of an inversion mutation involving intron 22, and roughly 5% involve intron 1. The remaining genetic defects are a result of stop codons, deletions, other inversions, or individual missense mutations. For the purpose of genetic counselling the large online database is of particular value because it allows inference as to the severity of the condition that is associated with a particular mutation.

Haemophilia B is also highly heterogenous at the mutational level with over 1000 unique mutations described in the international *f*9 gene database (www.factorix.org

[32]. Although smaller than the *f*8 gene, the mutational variation explains the range in clinical severity encountered in affected males. Sequencing the essential *f*9 gene regions or linkage analysis will identify the mutation in around 95% of cases of haemophilia B, which can then be used to confirm carrier status [33].

Inheritance and confirmation of carrier status

Haemophilia A and B are X-linked recessive disorders, meaning women who are carriers have a 50% chance of passing the defective gene mutation to a male offspring who will be affected. In addition, there is a 50% change of passing the defective gene mutation on to a female offspring, who will also be a carrier. The daughters of men with haemophilia are obligate carriers. The sons of men with haemophilia will not be affected. The first step in the diagnosis of carrier status, which is still carried out in clinical practice today, is the construction of an accurate family tree.

During the 1980s, DNA analysis evolved from haplotyping to mutation analysis, which offered certainty of carrier status. Initial genetic diagnosis was carried out using linkage analysis that uses a non-pathogenic normal variation in the gene sequence to track the mutated gene through a family. Linkage analysis is possible without knowledge of the gene mutation of the index family member. When the technology for automatic sequencing became available, mutation-specific diagnosis became possible. With this technique carrier determination by direct mutation detection without an affected family member is possible in 95% of cases [33].

Clinical manifestations

Carriers of haemophilia have one normal and one abnormal *f8* or *f9* gene. Therefore they are expected to have FVIII and FIX levels around 50% of the normal range (50-150 IU/dL). However, a wide range of factor levels (5-219 IU/dL) has been observed among carriers of haemophilia [34]. The reason for the lower end of this range in haemophilia carriers is attributed to a phenomenon known as extreme lyonisation, which can occur in early embryonic life. There is random inactivation of one of each pair of X chromosomes [35]. This can result in factor levels that are in the range of a male with mild (FVIII/FIX >5 IU/dL to <50 IU/dL) or moderate haemophilia (FVIII/FIX >1 IU/dL to <5 IU/dL) and thus carriers can experience bleeding manifestations in keeping with this condition. Rarely, the female offspring of a carrier mother and a father with the same form of haemophilia may inherit a homozygous form of the condition (factor activity levels <1%). In addition full or partial monosomy X, as in Turner syndrome, may result in a female with haemophilia [36].

The classification of haemophilia carriers according to the male definition of disease severity is controversial because females with factor levels in the low to normal range can experience significant bleeding symptoms [34]. This is due to women having the additional haemostatic challenges of menstruation and childbirth. Thus, women with a self-reported increased bleeding tendency and low FVIII and FIX levels are described as ‘symptomatic’ carriers of haemophilia.

Mauser-Bunschoten *et al* first investigated the bleeding tendency in haemophilia carriers in a case-control study in 1988 [37]. This study described a relationship between FVIII/FIX activity level and a tendency to bleed. A more detailed assessment was subsequently carried out by Plug *et al* who found an increased prevalence of

prolonged bleeding after tooth extraction, tonsillectomy and surgical procedures in carriers with factor levels < 40 IU/dL [34]. In addition, haemophilia carriers with low to normal factor levels (41-60 IU/dL) had an increased bleeding risk compared to non-carrier women (Table 2.3). They were twice as likely to bleed after surgery (RR 2.2, 95% CI 1.2-4.1) and twice as likely to develop anaemia (RR 2.0, 95% CI 1.0-4.3) and have restrictions in daily life (RR 2.0, 95% CI 0.8-4.9) due to HMB [34].

In a single institutional study, the phenotype of 46 haemophilia A carriers was compared to the underlying genetic mutation and FVIII levels. Carriers were categorised into three groups according to their underlying bleeding tendency. Thirty-two (70%) out of the 46 carriers reported spontaneous bleeding symptoms such as recurrent easy bruising (31 carriers, 67%), HMB (23 carriers, 50%), recurrent nose bleeding (seven carriers, 15%) or recurrent gum bleeding (two carriers, 4%). Whilst the FVIII levels were concordant between the groups, significant variations in genetic mutations were reported. In Group 1 (no bleeding tendency), 10 missense mutations and one small deletion were found. In Group 2 (weak bleeding tendency), 11 missense mutations and two intron 22 inversion were found, and in Group 3 (strong bleeding tendency) 9 missense mutations, seven intron 22 inversion, one deletion and two nonsense mutations were found (Fig 2.1) [38]. The study was limited by a small study population. In addition, there was no standardised questionnaire or bleeding score to objectively assess the severity of bleeding symptoms among participants. The correlation with genotype and phenotype in carriers of haemophilia requires further consideration. A larger study incorporating the quantification of bleeding symptoms using a bleeding score would be more informative.

Table 2.3 Bleeding tendency of both carriers and non-carriers according to decreasing clotting factor level (reproduced from Plug *et al*, 2006)

Figure removed due to copyright restrictions

Women who ever received treatment with clotting factor concentrate, tranexamic acid or DDAVP before tooth extraction, tonsillectomy, or operations were excluded from the analysis.

Figure 2.1 Bleeding tendency and *f*8 gene mutation (reproduced from Miesbach *et al*, 2011

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2.1.3 Factor XI deficiency

Severe factor XI (FXI) deficiency is a rare IBD with a prevalence of 1:1,000,000 in most populations [39]. Its incidence is much higher in the Ashkenazi Jewish population due to the founder effect for three mutations, such that the heterozygous state is present in up to 8% of this group. [40] It is also common in the Basque population of Southern France [41] with an estimated prevalence of 9% in heterozygotes.

Genetics

FXI deficiency arises from genetic defects in the FXI gene, located on the long arm of chromosome 4. Over 190 causative mutations in the FXI gene have been reported and are described on the interactive database (www.factorxi.org) [42]. The three most common FXI gene mutations were first described in six Ashkenazi Jewish patients with severe deficiency [40]: type I – mutation at a splice site of the last intron, type II – a Glu117stop mutation, and type III – a Phe283Leu substitution. Types II and III mutations predominate in Ashkenazi Jewish populations. An increasing number of mutations are also reported in the non-Jewish population, most of which are missense point mutations that occur throughout the FXI gene [42].

The exact role of FXI in coagulation is still not fully understood. In the consolidation phase of the coagulation pathway FXI is responsible for activating FIX to augment thrombin generation on activated platelets [43]. Although originally described as part of the contact phase of the intrinsic pathway, the physiological counterpart of this pathway remains an enigma. Factor XII (FXII) is auto-activated by negatively charged surfaces in the presence of prekallikrein (PK) and high molecular weight

kininogen (HK), which in turn activates FXI. However, this pathway was challenged by the observation that deficiencies in FXII or HK do not result in a bleeding tendency and an alternative activation pathway for FXI was proposed [44]. Thrombin activation of FXI in the absence of FXII was confirmed experimentally in a plasma environment [45]. In this pathway, thrombin activation of FXI is positive feedback that augments further thrombin generation through the activation of tenase (aFXI/aFVIII) and prothrombinase (FXa/FVa) complexes. In addition FXI activation results in reduced fibrinolysis due to activation of the thrombin activation pathway inhibitor (TAFI) [46]. Therefore high concentrations of thrombin are required for inhibition of clot lysis and activation of TAFI in contrast to the small amounts of thrombin required for platelet activation and fibrin formation [47].

Inheritance

The inheritance of FXI deficiency is autosomal recessive with severe deficiency occurring in homozygotes or compound heterozygotes (FXI activity levels of 15-20 IU/dL and below) and partial deficiency in heterozygotes (FXI activity levels 20-70 IU/dL) [48]. The normal range of FXI activity is 70-150 IU/dL.

Clinical manifestations

FXI deficiency results in trauma-related bleeding and bleeding from tissue with high fibrinolytic activity, such as the endometrium, oral cavity and urogenital tract. Spontaneous bleeding is rarely associated with this condition. Woman with FXI deficiency are at risk of PPH [49] and HMB [50]. FXI deficiency is notable for its wide range of symptom severity that has poor correlation with FXI activity levels. This was evident from early case reports [51-53] and has been confirmed in a larger

study that assessed bleeding tendency in FXI patients with partial deficiency [54]. Individuals with severe FXI deficiency may be asymptomatic whilst individuals with mild deficiency may experience severe bleeding symptoms. This variability in bleeding tendency and lack of correlation with FXI levels can make the clinical management of FXI deficiency particularly challenging.

2.1.4 Platelet function disorders

Inherited platelet function disorders (PFDs) fall into the categories of either disorders of platelet function or structure. The prevalence of PFDs in the general population has not been properly evaluated. Severe, clearly defined PFDs are rare and usually diagnosed in childhood due to the severity of bleeding symptoms. However, mild PFDs are less frequently encountered in clinical practice and are likely to be under-diagnosed due to milder bleeding manifestations and the complexity of investigations.

Classification

PFDs that arise with inherited thrombocytopathies include defects of platelet adhesion, receptors, secretion, enzymes and signalling pathways [55, 56]. The most commonly encountered are deficiencies of glycoprotein (GP) mediators of adhesion (Bernard-Soulier syndrome [BSS]) and aggregation (Glanzmann thrombasthaenia [GT]). Partially reduced or absent expression of the GPIb/IX/V complex due to mutations in *GPIBA*, *GPIBB*, *GP9* genes can give rise to BSS [57]. Partially reduced or absent expression of the integrin GPIIb/IIIa (α-IIb/β-3) or qualitative defects of the receptor due to mutations in either *ITGA2B* or *ITGB3* genes result in the disorder GT [55].

Disorders of platelet secretion and signal transduction are heterogenous disorders that result in the inability of platelets to release intracellular granule contents on activation of platelet-rich plasma with agonist. Signal transduction defects result from defects in the agonist receptors for thromboxane A_2, adenosine diphosphate (ADP) and collagen [58]. Platelet granule disorders include storage pool disorders with deficiency in the number and/or content of dense granules, alpha granules, or both granules [59, 60].

Quebec platelet disorder is associated with abnormal proteolytic degradation of alpha granule proteins. Defects in the membrane G-proteins and in the prostaglandin pathway enzymes cyclo-oxygenase and thromboxane A_2 synthase can result in disorders that interfere with the platelet conformational changes associated with activation. Disorders of platelet procoagulant activity have also been reported [61]. A classification of inherited PFDs is shown in Table 2.4.

Genetics and inheritance

BSS is usually transmitted in an autosomal recessive manner, patients being homozygous, or compound heterozygous for *GPIBA*, *GPIBB*, and *GP9* gene mutations. The majority of carriers are asymptomatic [62]. However, there are case reports of *GPIBA* mutation being transmitted in an autosomal dominant manner in different Mediterranean families [63, 64]. GT is also inherited in an autosomal recessive manner, with over 100 defects found in the genes that code for the α-IIb/β-3 integrin in megakaryocytes [65]. Platelet activation and secretion disorders, and platelet storage pool disorders, with a milder phenotype, are usually inherited in an autosomal dominant trait.

Clinical manifestations

PFDs give rise to spontaneous bleeding that is usually mucocutaneous in nature and of varying clinical severity. Mucocutaneous bleeding includes easy bruising, epistaxis, and HMB. Rapid onset of bleeding during surgery or trauma can be severe and life threatening although severity of bleeding symptoms varies considerably and bleeding may not occur with every haemostatic challenge. Spontaneous joint

bleeding, muscle bleeds, urinary, gastrointestinal or ICH are not associated with inherited PFDs [57].

Table 2.4 Classification of inherited platelet function disorders (reproduced from Rao & Gabbeta 2000)

Figure removed due to copyright restrictions

vWF; von Willebrand factor. GPIb; glycoprotein Ib. GP IIb-IIIa; glycoprotein IIb-IIIa. Tx; thromboxane. PLC; phospholipase

2.2 Gynaecological conditions and inherited bleeding disorders

2.2.1 Heavy menstrual bleeding

Menstruation is a complex, highly regulated physiological process that is under strict hormonal control. The cessation of menstrual bleeding relies on both local hormonal, endocrine factors and intact systemic haemostasis. HMB is defined as regular excessively heavy menstruations with blood loss of greater than 80 mL per cycle. As a consequence of modern life women menstruate approximately 10 times more frequently from menarche to menopause than in primitive human cultures. Thus HMB represents a regular haemostatic challenge and can become a major health problem for women with IBDs.

Assessment of menstrual blood loss

Mean menstrual blood loss is around 30 mL per cycle and menstrual losses of 60-80 mL per cycle give women a greater tendency towards iron deficiency anaemia [66]. The average menstrual course is 5 days duration and is considered abnormal when it lasts for longer than 7 days. Due to the inheritable nature of bleeding disorders the quantity and/or duration of menses may be overlooked or rationalised as normal when comparisons are made with other female family members.

Attempting to ascertain the true prevalence of HMB in women with IBDs is not easy. The subjective estimation of a women's menstrual blood loss can be inaccurate and there is limited correlation between self-assessment, quantity of sanitary protections required, and duration of menses with actual blood loss [30]. Menstrual blood loss can be recorded with a high degree of accuracy using the alkaline haematin method. This

compares the venous blood haemoglobin with the measured alkaline haematin content of all the soiled sanitary products collected by patients during menstruation [66]. However, this method is inconvenient, laborious and costly for women in clinical practice. In addition, there has been doubt as to the validity and the clinical usefulness of the 80 mL criterion. In a later study, the proportions of women with haemoglobin <12g/dl and low ferritin levels were significantly increased only in the group of women that had menstrual blood loss >120 mL [67].

Pictorial blood assessment chart (PBAC) is a semi objective method that takes into account the number of towels and tampons used, the degree to which individual items are soiled, and the passage of clots and flooding (Appendix 1). From this information a score is ascertained, and a score >100 has a sensitivity and specificity of >80% for HMB (menstrual blood loss of 80 mL or more with the alkaline haematin method). Subsequently PBAC is a useful tool that has been adopted more readily for use in research as well as in clinical practice [68].

The menstrual cup (Mooncup®) is an alternative to using sanitary towels and tampons. A high satisfaction and continuation rate has been reported among users [69]. The menstrual cup rated significantly higher than towels and tampons for comfort, quality, menstrual blood collection, appearance and preference in a randomised crossover trial in 124 women [70]. This may provide a simple and more accurate method of measuring menstrual blood flow, although studies have not yet been published to evaluate this method in women with HMB.

HMB in women with inherited bleeding disorders

HMB is the commonest bleeding symptom in women with IBDs with reported prevalence varying depending on the underlying disorder and population studied (Table 2.5).

Von Willebrand disease

HMB is the most common presenting symptom in women with VWD, occurring with a frequency of 78-97%, in females with VWD [7, 71]. In a case-control study of 66 women with VWD assessment of menstrual blood loss was made using a PBAC score. Women with VWD had increased menstrual blood loss (PBAC score >100) (p = 0.001), increased menstrual flooding (p = 0.001), and longer duration of menses (p = 0.001), compared to 69 women without VWD [7]. In addition, women with VWD reported significantly increased towel/tampon use during menses (p = 0.002), history of anaemia (p = 0.001) [27] and reduced quality of life during menstruation [50].

An international survey of 44 women with severe VWD, mainly types 2 and 3 reported severe HMB requiring treatment with blood product therapy in 80% [72]. A survey undertaken in the Netherlands in 423 women with moderate and severe VWD demonstrated that 20% had undergone hysterectomy for HMB [73].

Carriers of haemophilia

HMB is a common gynaecological issue for carriers of haemophilia resulting in significant morbidity. PBAC chart was used to assess menstrual blood loss in 30 carriers of haemophilia and 69 age-matched controls. The median PBAC score was significantly higher in carriers (113 vs 73) than in the control group (p < 0.001). The

incidence of HMB, as defined by a PBAC score greater than 100, was 57% in haemophilia carriers compared to 29% in the control group [7].

FXI deficiency

FXI deficiency is associated with bleeding during times of high-fibrinolytic activity, which occurs during menstruation. In a case-control study, 20 women with FXI deficiency were interviewed and invited to fill in a PBAC chart. The median PBAC score was 122 (range 38-482) in cases versus 72 (range 9-310) in controls (p = 0.0001) [7]. HMB (PBAC >100) was reported in 59% of women with FXI deficiency. Nineteen of 46 (41%) of premenopausal women had symptoms indicative of HMB (flooding, using double towels, staying at home in bed) in the kindred study reported by Bolton-Maggs *et al*. Three women (18%) had undergone early hysterectomy [54].

Platelet function disorders

HMB has been reported with a high frequency (50-98%) in women with inherited PFDs. This is usually dependent on the underlying disorder (Bernard-Soulier syndrome and Glanzmann's thrombasthaenia typically present with severe HMB, often in adolescence).

HMB has also been reported in women with rare bleeding disorders such as deficiency in prothrombin, fibrinogen, factors V, VII, X, and XIII. In a systematic review of the literature HMB was reported in 35-98% of women with these disorders [74].

Table 2.5 Prevalence of heavy menstrual bleeding in women with inherited bleeding disorders

Study	Sample size and population	Type of study	Prevalence
Von Willebrand Disease			
Silwer *et al*. 1973 **[75]**	136 women > 15 years of age with VWD and 500 controls	Case-control	60% among women with VWD; 25% among controls
Kadir *et al.* 1999 [7]	66 women with VWD	Prospective cohort	74% with PBAC score >100
Ragni *et al.* 1999 ***[71]***	29 females with type 1 VWD	Case series	93%
Kouides *et al.* 2000 **[27]**	99 females with type 1 VWD	Survey	79%
Lak *et al*. 2000 **[76]**	130 females with type 3 VWD	Case series	69%
Woods *et al*. 2001 **[77]**	921 females > 13 years with VWD	Case series	47%
Perry and Alving. 1990 **[78]**	36 women with VWD	Case series	42%
Greer *et al*. 1991 **[79]**	8 women with VWD (type 1 and 2)	Case series	100%
Kirtava *et al.* 2003 **[80]**	102 women with VWD and 88 controls	Case-control study	95% among women with VWD; 61% among controls
De Wee *et al*. 2011 **[73]**	423 women > 16 years with moderate and severe VWD	Nation-wide cross-sectional study	81% with >2 menorrhagia symptoms
Foster *et al.* 1995 **[72]**	30 women with VWD unresponsive to DDAVP in an international registry	Case series	80% with at least one episode requiring blood product
Federici, 2004 **[29]**	Women with VWD in Italian registry	Case series	32% with type 1; 32% with type 2; 56% with type 3
Haemophilia Carriers			
Mauser Bunschoten *et al*. 1988 **[37]**	102 carriers of haemophilia A and 19 carriers of haemophilia B	Case series	31% in HA; 10% in HB
Greer *et al*. 1991 **[79]**	18 carriers of haemophilia A and 5 carriers of haemophilia B	Case series	22% in HA; 40% in HB
Kadir *et al.* 1999 [14]	30 carriers of haemophilia	Prospective cohort	57% with PBAC score > 100
Plug *et al*. 2006 **[34]**	274 carriers of haemophilia and 245 controls	Postal survey	23% consulted with GP for HMB; 20% in controls
Meisbach *et al*. 2011 **[38]**	46 carriers of haemophilia A	Cohort	50%
Factor FXI deficiency			
Bolton-Maggs et al. 1995 **[54]**	46 women with FXI deficiency	Questionnaire study	41% with symptoms indicating menorrhagia
Brenner et al .1997 **[81]**	82 women with FXI deficiency	Case series	12% with prolonged menstrual bleeding (> 7 days)
Kadir et al. 1999 [14]	20 women with FXI deficiency	Prospective cohort	59% with PBAC score >100
Platelet Function Disorders			
Lopez et al. 1998 **[82]**	35 women with Bernard-Soulier syndrome	Summary of case reports	51%
George et al. 1990 **[83]**	55 women with Glanzmann's thrombasthaenia	Summary of case reports	98%
McKay et al. 2004 **[84]**	3 out of 6 in affected women with Quebec Platelet Disorder	Case series	50%

Prevalence of bleeding disorders in women with HMB

Over the last 10 years there has been increasing realisation that IBDs as a cause of HMB are under-estimated in women in terms of their frequency and severity. Up to one third of cases of HMB could be due to an undiagnosed bleeding disorder. Thus HMB represents an important bleeding symptom that can be used as a predictor for identification and diagnosis of women with IBDs. In a systematic review, an overall prevalence of 13% (confidence intervals 11%, 15.6%) of VWD was reported among 988 women in 11 studies [4]. Furthermore, the prevalence of VWD in women with HMB varies with ethnicity. A higher prevalence of VWD was reported in the European studies of mainly white Caucasian women. A lower prevalence was reported in the North American studies that included a higher proportion of black African women. It has been demonstrated that black women have higher levels of VWF:Ag, VWF:Ac and FVIII:C than white women [7, 85] and that ethnic variations in VWF can influence the diagnosis of VWD [86]. The difference in prevalence of VWD between white and black women persists even when using race-specific laboratory reference ranges [87].

Disorders of platelet function, especially mild defects are also underestimated in women with HMB. Laboratory diagnosis of these disorders requires specialised experience leading to under diagnosis in general. In women with HMB, PFDs have been observed to be more common than VWD [5, 88]. A multicenter study in the US included 232 women with unexplained HMB and a PBAC score >100. A laboratory defect in haemostasis was detected in 73.3% of the women. The frequency of platelet aggregation defects among women with HMB was 51.5%, which was significantly higher (17.3%) than the control group [89]. As with VWD, the prevalence of PFDs

varies depending on ethnicity [5]. In a study that evaluated platelet function defects in 74 women with unexplained menorrhagia, abnormalities of platelet aggregation were seen in 69% of black women compared to 39% of white ($p = 0.06$).

IBDs are prevalent (10-20%) in adolescents presenting with HMB at menarche [88, 90-93]. HMB since menarche [92], positive family history of bleeding symptoms, and clinical anaemia [91] are predictive of bleeding disorder as an underlying cause for HMB in young women.

2.2.2 Other gynaecological conditions

Bleeding can occur around the time of ovulation as the ovum ruptures from the surface of a mature ovarian follicle. Ovulation is a physiological occurrence and intraperitoneal bleeding may be experienced as 'Mittelschmerz' or mid-cycle pain. A high frequency of Mittlesmerz has been reported in women with VWD [27]. Carriers of haemophilia also experience significantly more mid-cycle pain which impacts on quality of life [50]. The underlying pathology behind these symptoms has not been fully explored in women with IBDs.

In an ultrasound study the presence of pelvic fluid was seen more often in cycles where women experienced Mittelschmerz [94]. Assuming that the visualised fluid was blood, this suggests that Mittelschmerz is associated with bleeding at the time of ovulation. Therefore women with IBDs are particularly vulnerable to either bleeding into the peritoneal cavity or bleeding into the residual follicle (the corpus luteum) resulting in haemorrhagic cyst [74] [95, 96].

Women with VWD can present with an acute abdomen from haemoperitoneum due to rupture and subsequent bleeding of the corpus luteum [96-98]. Severe intraperitoneal bleeding secondary to ruptured corpus luteum has also been reported in carriers of haemophilia [14], in women with FXI deficiency [99] and congenital afibrinogenaemia [100]. It is particularly associated with FXIII deficiency, occurring in 8% of 121 women identified in a systematic review [101].

Multiple case reports have described women with IBDs presenting, often repeatedly, with haemorrhagic ovarian cysts [7, 72, 75, 79]. Jarvis and Olsen reported a case of type 1 VWD that presented with recurrent haemorrhagic ovarian cysts [97]. Silwer *et al* reported that nine out of 136 women with VWD experienced this problem [75]. Bleeding from haemorrhagic ovarian cysts results in severe pain, and/or severe, life-threatening haemoperitoneum in women with IBDs.

Endometrial hyperplasia is a condition of increased proliferation of the endometrium due to increased exposure to oestrogen. Endometrial polyps arise from proliferation of endometrial glandular tissue. Both endometrial hyperplasia and uterine polyps can present with HMB and/or irregular intermenstrual bleeding. A case-control study of 102 women with VWD reported a higher prevalence of endometrial hyperplasia (10% in women with VWD versus 1% in controls). In the same group of women there was also an increased prevalence of endometrial polyps (8% versus 1%) and fibroids (32% versus 17%) [80]. The increased prevalence of these pathologies in women with IBDs may be due to the bleeding disorder exacerbating symptoms, leading to increased detection and diagnosis. Further research is required to determine if there is a genuine increased prevalence of these conditions among women with IBDs.

2.2.3. Endometriosis

Background

Endometriosis is an oestrogen-dependent, benign inflammatory disorder caused by deposition of ectopic endometrial glands and stroma. These are usually located within the pelvis, but may be found in other locations such as the bowel, diaphragm, umbilicus and pleural cavity. The exact prevalence is difficult to determine, partly due to the variability in clinical presentation, and partly due to the fact that surgical visualisation of endometriotic implants is regarded as the gold standard for a definitive diagnosis [102]. Population-based studies report a prevalence of around 1.5%, compared with 6-15% in hospital-based studies [103]. However, the prevalence of endometriosis appears to be around 5% of reproductive aged women, with a peak between 25 and 35 years of age [104].

Pathogenesis

The exact pathogenesis of endometriosis is not known. The most commonly accepted hypothesis is the retrograde menstruation theory; viable endometrial fragments pass back through the fallopian tubes, possibly due to a pressure gradient originating from dysynergic uterine contractions [105]. Once in the peritoneal cavity they can implant, grow and invade pelvic structures. The likelihood of this occurrence may be influenced by menstrual and reproductive factors that increase pelvic contamination to regurgitated endometrium, such as duration and heaviness of menstrual blood flow [102]. The risk of developing endometriosis is increased in women with a duration of menses > 6 days (OR; 2.5, 95%CI; 1.1-5.9) [106].

Aberrant immunological mechanisms are also implicated in the pathogenesis of endometriosis with increased activation of circulating monocytes and macrophages and release of inflammatory cytokines into the peritoneal fluid, which stimulate ectopic endometrial cell proliferation [107]. Another theory is that endometriosis is derived from a metaplastic process occurring in the peritoneal mesothelium [108]. This theory is still supported for ovarian endometriosis. Over the past few decades there has been a concerted research effort to understand the pathogenesis of endometriosis. The genetics and molecular make of the ectopic and eutopic cells have been well described. The results of genome-wide association studies are consistent with a heritable component in endometriosis [109].

Clinical manifestations

Endometriosis results in chronic pelvic pain, dysmenorrhoea, deep dyspareunia (pain on deep penetration), dyschezia (pelvic pain with defecation) and dysuria (pain with micturition). The pain is usually cyclical and can occur with lower back pain. Endometriosis rarely results in abnormal bleeding symptoms such as rectal bleeding or haemoptysis [110, 111]. A case-control study of 5540 women demonstrated that 25% of women with endometriosis reported dysmenorrhoea to their general practitioner in the three years prior to diagnosis, 24% reported urinary tract symptoms, 11% reported symptoms related to painful sexual intercourse, and 2% reported rectal bleeding or dychezia [103]. The proportion of women with endometriosis who are asymptomatic is difficult to determine. A well-described paradox of endometriosis is that severity of disease seen at laparoscopy staging (Fig 2.2) does not correlate well with severity of symptoms, and the reason for this is unclear [112].

An estimated 25-50% of women with infertility have endometriosis, and around 30-50% of women with endometriosis have infertility [113]. The mechanism for how endometriosis affects fertility is not well understood. Causation is thought to be due to adhesions that alter tubal patency, reduced ovarian reserve with endometrioma formation, altered embryo transport and impaired implantation due to pro-inflammatory milieu.

The chronicity of the condition has a significant impact on women's lives including work, social functioning and sexual relationships [114]. Endometriosis is a chronic condition that affects the young, working-age population. Social, indirect and intangible costs contribute to the overall economic burden. These include time lost from education and work, the reduced ability to carry out normal everyday activities, loss in earned income, social withdrawal and psychological disorders such as depression [115]. A prospective, multi-centre European study estimated the average annual total cost per patient with endometriosis was €10,000 including cost of healthcare, and loss of productivity [116].

Management

Laparoscopy may be indicated to investigate pain symptoms or subfertility. Diagnosis is made by direct visualisation of endometriotic deposits, which are classically dark "powder-burn" lesions, although may also resemble more subtle clear vesicles. At laparoscopy the stage of the disease can be recorded according to the revised American Society for Reproductive Medicine (rASRM) classification (Fig 2.2).

Surgical excision or ablation of endometriosis results in effective improvement in endometriosis symptom severity and quality of life scores [117, 118].

The combined oral contraceptive (COC) pill [119], progesterone therapy (i.e. oral medroxyprogesterone acetone [120], norethindrone acetate [121], the levonorgestrel-releasing intrauterine system (LNG-IUS) [122], subcutaneous depot medroxyprogesterone acetate (DMPA) injection [123], and etonogestrel subdermal implants [124]) and gonadotropin-releasing hormone (GnRH) analogues [125] are established hormonal therapies with proven efficacy in reducing pain severity and progression of endometriosis. COCs can be used long-term for patients with endometriosis until pregnancy is desired. They are contraindicated in women with hypertension, history of venous thromboembolism, breast cancer and migraine with aura [126]. High dose progesterone therapies are associated with weight gain, mood disturbance, depression and irritability [127]. Continuous use COC and progesterone therapy are associated with breakthrough bleeding. GnRH analogues are first-line therapy for symptomatic patient with moderate and severe endometriosis. Their use is restricted to short-term administration (6-12 months) due to side effects of hypoestrogenism (hot flushes, bone mineral density loss, and vaginal dryness).

Figure 2.2 Revised American Society for Reproductive Medicine (rASRM) classification system for endometriosis

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Endometriosis is classified into four stages: stage I (minimal), stage II (mild), stage III (moderate), and stage IV (severe) depending on the extent, and depth of endometriosis implants; presence and severity of adhesions; and the presence and size of ovarian endometriomas

Endometriosis and inherited bleeding disorders

The only existing evidence to support an association between the two conditions is studies that assess the prevalence of endometriosis in women with IBDs, usually

against controls. In a study that assessed the reproductive experience of women with VWD, endometriosis was reported in 30% of women with VWD compared to 13% of controls (p = 0.005) [80]. In a postal survey that interviewed 168 women, dysmenorrhoea and its interference with daily life were reported significantly more often (p = 0.001) in women with IBDs compared to controls. Of the 99 women with IBDs included in the survey, 50% reported moderate, severe or very severe pain during their menses [50]. Surveillance data from the US Centers for Disease Control and Prevention (CDC) reported that over 50% of the 217 women with IBDs experienced dysmenorrhoea, and the prevalence of a confirmed diagnosis of endometriosis in these women was 13% [89].

Menstruation is a complex regulated process that occurs following progesterone withdrawal, vasoconstriction of spiral arterioles, and shedding of the endometrium. Local factors within endometrial tissue promote a pro-haemorrhagic environment. Downregulation of tissue factor (TF) and plasminogen-activator inhibitor 1 (PAI-1) occurs following progesterone withdrawal [128]. Inducible nitrous oxide (iNO) results in inhibition of platelet aggregation, relaxation of smooth muscle and vasodilatation with increased menstrual blood flow [129]. VWF levels fluctuate during the menstrual cycle and are lowest (VWF:Ag and VWF:RCo) during the first four days of menses [130]. Hormonal fluctuations around the time of menstruation may impair both systemic and local primary haemostasis (Fig 2.3). Impaired haemostasis leads to increased menstrual blood flow and prolonged duration of menses, both of which may exacerbate endometriosis, according to the retrograde menstruation/implantation theory [131]. The association between endometriosis and IBDs is explored in Chapter 4 of this thesis.

Figure 2.3 Pathogenesis of abnormal haemostasis leading to heavy menstrual bleeding (reproduced from Kouides and Kadir [132])

Figure removed due to copyright restrictions

2.3 Pregnancy and inherited bleeding disorders

2.3.1 Prenantal diagnosis

Prenatal diagnosis for haemophilia has been available since the mid 1950s when amniocentesis was first performed to determine fetal gender. Fuchs and Riis correctly reported fetal sex based on the presence or absence of the Barr body found in fetal cells of amniotic fluid [133]. In 1979 fetal blood samples obtained by fetoscopy at 18-20 weeks gestation was undertaken to determine the fetal clotting factor level [134]. Due to advances in ultrasound technology, fetal blood sampling under fetoscopic-guidance was later replaced by cordocentesis under ultrasound-guidance, which was associated with a lower rate of procedure-related fetal loss [135]. The technique of chorionic villus sampling (CVS) to obtain a specific prenatal genetic diagnosis for haemophilia in the first trimester was first reported in 1985 by Gitschier *et al* [136].

Currently, definitive prenatal diagnosis of haemophilia can only be achieved by invasive testing. CVS at 11-14 weeks gestation is the preferred and most commonly used method. It involves the biopsy of placental tissue via trans-abdominal ultrasound-guided needle aspiration. Fetal DNA is extracted from the chorionic villus tissue and used for PCR-based fetal gender determination. Thereafter further testing using direct mutation detection or polymorphism linkage analysis is carried out to assess the haemophilia status if the fetus is male. Amniocentesis is also used for prenatal diagnosis in some centres after 15 weeks gestation. It involves an ultrasound-guided collection of amniotic fluid containing fetal cells (amniocytes). The incidence of procedure-related pregnancy loss following both CVS and second-trimester

amniocentesis is less than 1% [137, 138]. Cordocentesis at 18-20 weeks remains an option when genetic testing is non-informative, or when genetic services are not available in developing countries [9].

Preimplantation genetic diagnosis (PGD) is another method of very early prenatal diagnosis, pioneered during the late 1980s [139]. It combines assisted reproduction techniques with molecular genetics and cytogenetics to identify abnormal mutations in embyros prior to implantation. The advantage of this technique is that the couple are reassured from the outset that the pregnancy is unaffected and can avoid invasive prenatal testing and the difficult decision regarding whether to have a termination of pregnancy (TOP). Attitudes towards PGD vary considerably worldwide owing to scientific, cultural and religious differences. The PGD-related legal and ethical issues have been debated both nationally in the UK, and internationally, in particular in relation to its potential eugenic dimension [140]. Nevertheless, PGD has been widely practiced worldwide for various indications and can substantially reduce the risk of passing an undesirable genetic condition to offspring [141-144]. The first successful live birth of a baby unaffected by haemophilia following PGD was reported in 2002 [145]. This case involved diagnosis of haemophilia status through testing of the polar body extruded during meiosis, thus avoiding the requirement to biopsy the embryo. However, due to concerns regarding 'crossing over' of genetic material during meiosis this technique was not considered ideal. In many countries, PGD for families with haemophilia provides diagnosis only by sex determination of the embryos. This results in the unnecessary disposal of healthy male embryos and reduces success rates by diminishing the pool of embyros available for transfer. In 2006, the UK reported the first live birth following PGD with mutation specific diagnosis of embryos [146].

A recent Spanish cohort reported 12 live births amongst 34 couples affected by haemophilia, using a combination of embryo sexing and direct mutation analysis PGD, with a live birth rate of 10-24% per cycle [147].

Third trimester amniocentesis is offered to couples who wish to avoid the risk of miscarriage associated with early prenatal diagnosis but wish to know the haemophilia status of the fetus in advance of labour. This information can help to determine management of labour and delivery including the decision regarding mode of delivery (MOD). The procedure-related complications are approximately 1% and include preterm labour, rupture of membranes, and infection [148]. Another disadvantage of third trimester amniocentesis is the approximate 1% risk of failure to obtain a sample and a higher culture failure rate (10% versus 1%) compared to samples obtained in the second trimester [149]. This could result in the onset of labour prior to obtaining a diagnosis in the potentially affected fetus. Current data on the safety and efficacy of third trimester amniocentesis are limited. A recent case series reported the outcome in 9 pregnant carriers of haemophilia that underwent amniocentesis for prenatal diagnosis at or after 34 weeks gestation [150]. There were no reported complications associated with the procedure and all women delivered after 37 weeks gestation. One out of the 9 cases had an inconclusive result. Five out of 9 were confirmed to be unaffected which enabled routine obstetric management [150].

The discovery of cell-free fetal DNA (ffDNA) in maternal plasma proved to be an important step in the advance of non-invasive prenatal diagnosis technology. Fetal gender determination using amplified Y chromosome sequences (Y-PCR) found in

ffDNA is now a well-established aspect in prenatal diagnosis of sex-linked conditions such as haemophilia [151]. ffDNA in maternal plasma has been detected as early as five weeks gestation, however sensitivity and specificity increases with advancing gestation. A meta-analysis and systematic review of the literature found a sensitivity and specificity of 94.5% and 98.9% respectively in tests performed from 7 to 12 weeks gestational age [152].

Y-PCR testing with ffDNA has the advantage of negating the need for invasive testing and its associated risk of fetal loss for female fetuses [151]. However, invasive testing is still required for pregnancies with male fetuses that have a 50% chance of being affected with haemophilia. Despite recent advances in ffDNA technology, its use for prenatal diagnosis of haemophilia in pregnancies with a male fetus remains challenging because the mothers are carriers of the mutation and the maternally inherited fetal allele is indistinguishable from the maternal DNA. In a recent study, using quantitative PCR technology and relative mutation dosage approach, accurate identification of the mutant or wild type alleles was achieved in pregnant carriers of haemophilia with male fetuses [10]. The fetal genotype was identified from as early as 11 weeks gestation demonstrating the potential of a non-invasive method for specific prenatal diagnosis of haemophilia in the first trimester. However, this specialised technology currently has important limitations. A specific real time PCR assay is necessary for each mutation of haemophilia A and B [33]. About 50% of cases of severe haemophilia are caused by intron 22 inversion mutation. Therefore a specific assay targeting this mutation would be highly beneficial in providing a universal non-invasive test that can be used globally for carriers of severe haemophilia A, who are more likely to opt for and benefit from prenatal diagnosis.

Attitudes of haemophilia carriers towards prenatal diagnosis

The uptake for prenatal diagnosis varies worldwide but remains generally low in high resource countries that can provide effective lifelong care [153]. The determinants affecting the choice for prenatal diagnosis were evaluated in a nationwide survey in the Netherlands. Forty-eight out of 207 carriers underwent early prenatal diagnosis in the first trimester (Y-PCR testing or CVS), 26 pregnancies were positive for haemophilia and 18 underwent termination of pregnancy. The decision to have early prenatal diagnosis was associated with having severe haemophilia in the family (relative risk (RR) 20.2; 95% confidence interval (CI) 2.7-153.6), a liberal view towards termination of pregnancy (TOP) (RR 12.5; CI 3.1-51.2), older age (RR 2.0; CI 1.0-3.9) and absence of a religion (RR 1.9; CI 1.1-3.1) [154]. Many carriers of haemophilia did not consider the condition to be severe enough to justify TOP [154]. In addition, haemophilia carriers state fear of fetal loss associated with invasive tests, as reasons against opting for prenatal diagnosis [154]. The attitudes of haemophilia carriers towards non-invasive options, including the potential for ffDNA prenatal diagnosis of haemophilia, have not been addressed. In addition the impact of knowledge of haemophilia status towards decision regarding MOD and risk of cranial bleeding has not been evaluated. This is addressed through a questionnaire study presented in Chapter 5 of this thesis.

2.3.2 Haemostatic changes in normal pregnancy

Normal pregnancy is associated with a progressive increase in procoagulant activity due to elevated levels of coagulation factors VII (FVII), FVIII, FX, FXII, fibrinogen and von WIllebrand factor (VWF) [155-157]. The rise in coagulation factors, especially FVIII and VWF, are most pronounced in the third trimester [155]. FII, FV and FIX levels are slightly increased or unchanged during normal pregnancy [157, 158] (Table 2.6). Reports are inconsistent regarding FXI levels in normal pregnancy, with some studies reporting a slight increase [159], no change [158], or a decrease [155]. The change in coagulation factor levels is accompanied by a reduction in physiological anticoagulants, most significantly a reduction in protein S activity levels and an acquired protein C resistance [160]. Placental derived plasminogen activator inhibitor 2 (PAI-2) impairs fibrinolytic activity. Local endometrial haemostasis is enhanced at the placental trophoblast level by increased tissue factor expression and reduced expression of the inhibitor thrombin activatable fibrinolysis inhibitor (TAFI) [160]. Pregnancy is associated with a reduction in platelet count due to the increase in plasma volume and resultant physiological haemodilution. However, there is limited data on changes in platelet function during normal pregnancy and these changes are poorly understood. Some studies indicate there is change in platelet activation during pregnancy with increased platelet aggregation and reactivity at term [161-163]. Other studies have found no change in platelet function [164] or else platelet hypoactivity [165]. In a small prospective longitudinal study that assessed platelet reactivity throughout pregnancy, a significant reduction was reported in the first trimester, compared to non-pregnant controls. In addition a significant increase was seen in third compared to first trimester [166].

Table 2.6 Haemostatic changes in normal pregnancy

Clotting factor	Increase (↑) No significant change (↔)
Fibrinogen	↑↑
FVII	↑
FVIII	↑↑
FX	↑
FXII	↑
VWF	↑↑
FII	↔
FV	↔
FIX	↔
FXI	↔

F, factor; VWF, von willebrand factor

This table gives an overview of haemostatic changes in normal pregnancy. The changes in haemostatic variables and inhibitors result in a global hypercoagulable status considered a physiological protective mechanism to prevent excessive bleeding at the time of delivery.

2.3.3 Postpartum haemostatic changes

Following expulsion of the placenta, the pro-haemostatic changes associated with pregnancy are reversed. Not all haemostatic indices have been studied in the postpartum period; for example, there is a lack of data on postpartum FXI levels.

James *et al* performed a case-control study measuring postpartum changes in von Willebrand factor activity levels (VWF:RCo, VWF:Ag and FVIII:C) in 40 parturient women without VWD [167]. VWF:RCo and VWF:Ag levels rose in the immediate postpartum period (within 24 hours), fell rapidly after delivery, approached the nadir within 1 week and appeared to plateau for 3 weeks following delivery [167]. In contrast, FVIII:C levels dropped to 20% of their value in the immediate postpartum period, and then gradually declined to baseline by 3 weeks postpartum (Fig 2.4). The initial decline in FVIII:C was more gradual than VWF, which was also observed in a longitudinal study assessing factor levels on days 1-3 postpartum [8].

Saha *et al* performed a prospective observational study measuring platelet count and function, fibrinogen levels and fibrinolysis, and performed ROTEM® analysis, in 46 healthy parturient women from pre-delivery to 6 weeks postpartum [168]. Platelet count decreased further and reached a nadir by day 2 postpartum. Platelet count rose rapidly due to the release of fresh platelets [169]. There was a significant increase, compared to pre-delivery platelet count, with maximum levels reached by day 11-15 (Fig 2.5). Platelet function also increased although the change was gradual, and not as significant (Fig 2.6) [168]. Increased platelet aggregation to agonist ADP and epinephrine have been noted in postpartum women [170].

Fibrinogen levels initially peaked on day 3, and then reduced to baseline and appeared to stabilise by day 25 postpartum (Fig 2.5) [168]. Fibrinolysis is reduced during pregnancy due to decreased t-PA activity. A brisk fall in PAI-1 and rise in t-PA activity immediately following delivery results in a rapid reversal with an increase in fibrinolytic activity, and a return to non-pregnant levels within 3-5 days [157, 171]. However, high levels of PAI-2 antigen are detected in maternal serum for a further few days. Free protein S appears to return to pre-pregnancy levels by the end of the first week postpartum [172]. Saha *et al* found a sharp rise in all three anticoagulants (protein S, protein C and antithrombin) by day 3-5 (Fig 2.6) [168]. Table 2.7 summarises postnatal haemostatic changes.

Figure 2.4 Postpartum VWF:RCo, VWF:Ag and FVIII levels

Figure removed due to copyright restrictions

Trends in mean and 95% confidence intervals of (a) von Willebrand factor ristocetin activity level (VWF:RCo), (b) von Willebrand factor antigen (VWF:Ag) and (c) factor VIII (FVIII) activity level from predelivery to day 42 postpartum. Reproduced from James *et al* [167].

Table 2.7 Postpartum haemostatic changes

Clotting factor	Change	Normalisation to baseline
Factor VIII	↑ and then ↓	3 weeks
VWF	↑ and then ↓↓	1 week
Fibrinogen	↑ and then ↓↓	3-5 weeks
Fibrinolysis	↑↑	3-5 days
Platelet count	↓ and then ↑	2 weeks
Antithrombin	↑↑	3-5 days
Protein C	↑↑↑	3 days
Free Protein S	↑↑	3-5 days

VWF, von willebrand factor

Figure 2.5 Postnatal changes in platelet count, fibrinogen, PT and APTT

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Trends in the mean and 95% confidence intervals of (A) platelets (PLT), (B) fibrinogen (FBG), (C) prothrombin time (PT), and (D) activated partial thromboplastin time (APTT) at different intervals from predelivery to day 40/42 postnatal. Reproduced from the Haemostatic changes In the Pueriperium (HIP) study [168].

Figure 2.6 Postnatal changes in protein C, protein S, antithrombin and platelet function

Figure removed due to copyright restrictions

Trends in the mean and 95% confidence intervals of (A) protein C (PC), (B) free protein S (PS), (C) antithrombin (AT), and (D) platelet function assessment (PFA - 100) at different intervals from predelivery to day 40/42 postnatal. Reproduced from the Haemostatic changes In the Pueriperium (HIP) study [168].

2.3.4 Changes in factor levels during pregnancy in women with IBDs

Carriers of haemophilia

Due to the physiological changes mentioned above, carriers of haemophilia A may experience partial or complete correction in the haemostatic defect during pregnancy. Carriers of haemophilia A with low baseline FVIII:C may have factor levels in the normal range (>50 IU/dL) by term. However, as FIX levels do not rise significantly during pregnancy, carriers of haemophilia B with low baseline levels will not have an increase and thus remain at risk of bleeding at delivery [1]. The study performed by Chi *et al* assessed 90 pregnancies in 53 carriers of haemophilia to clearly demonstrate this trend (Fig 2.7).

Figure 2.7 Pregnancy changes in factor VIII and factor IX levels in carriers of haemophilia (reproduced from Chi *et al*, 2008)

Figure removed due to copyright restrictions

The median pre-pregnancy and third trimester FVIII:C in haemophilia A carriers was 42 IU dL (range 20-85) and 120 IU dL (range 38-205) respectively. The median pre-pregnancy and third trimester FIX levels in haemophilia B carriers was 27 IU dL (range 21-48) and 45 (range 36-68) [1]. The risk of bleeding at delivery is inversely related to FVIII and FIX levels, although carriers with mildly reduced factor activity levels (41-60 IU/dL) also have an increased risk of bleeding [2, 37].

Von Willebrand disease

Due to the progressive increase in FVIII:C and VWF:Ag associated with pregnancy most women with type 1 VWD have normal haemostasis (factor levels > 50 IU/dL) by term [79, 173] (Fig 2.8). However, pregnancy in women with specific mutations (C1130F and R1205H) that result in severe type 1 VWD (increased clearance of VWF) are associated with a lack of correction of the haemostatic defect with only a slight increase in FVIII and VWF levels [174] (Fig 2.8). Similarly, women with type 3 VWD have little or no increase in factor levels during pregnancy [175]. Women with type 2 VWD may have an increase in FVIII and VWF levels but there is an ongoing functional defect with abnormal multimers. Variations between the different subtypes of VWD type 2 have been observed. In type 2A usually VWF:RCo remains markedly low compared to the increase of VWF:Ag and, by far more significant, of FVIII (Fig 2.8) [176, 177]. Women with subtype 2M have no significant change in

both VWF and FVIII levels during pregnancy, and the abnormal VWF:Ag/VWF:RCo remains unchanged [178]. In homozygous subtype 2N, FVIII is normalised by the end of pregnancy, but its level remains significantly reduced compared to largely increased VWF, which maintains its reduced ability to bind FVIII (Fig 2.8). Women with subtype 2B may have a worsening of thrombocytopaenia in pregnancy due to the increased production of abnormal VWF multimers, with an increased affinity for GP1b, causing spontaneous platelet aggregation [21, 179]. When this subtype presents for the first time in pregnancy it is frequently misdiagnosed as idiopathic thrombocytopaenic purpura, resulting in inappropriate management [180].

Figure 2.8 Changes in FVIII and von Willebrand factor levels during normal pregnancy and in women with the more frequent types of von willebrand disease. Reproduced from Castaman 2013 [181].

Figure removed due to copyright restrictions

Factor XI deficiency

FXI levels are unaffected by pregnancy [157, 158] and the persistence of low factor levels in the third trimester is common in women with this condition [49, 175, 182]. Chi *et al* demonstrated no significant change in FXI levels during pregnancy in 30 deficient women in 61 pregnancies [49]. Myers *et al* found no consistent change in FXI levels during pregnancy in 33 women with FXI deficiency [182].

Platelet function disorders

There is currently a lack of data in the literature on the effect of pregnancy on platelet function in women with PFDs [183].

2.3.5 Antenatal bleeding complications

An increased risk of miscarriage is only confirmed in rare bleeding disorders; congenital factor XIII deficiency and fibrinogen disorders. In a systematic review of 192 pregnancies in women with factor XIII deficiency, 127 (66%) resulted in miscarriage [101]. A high miscarriage rate is reported in women with untreated congenital afibrinogenaemia [184, 185]. At present there is limited evidence to support an increased rate of early pregnancy loss in women with VWD, carriers of haemophilia, or women with FXI deficiency. Most studies report a rate that is comparable to the 10-12% rate of clinically recognised first trimester miscarriage documented in cohort studies in the general population [186].

Women with IBDs are exposed to several haemostatic challenges throughout pregnancy. In the first trimester bleeding can occur following spontaneous miscarriage, invasive prenatal diagnostic procedures, and TOP. The pregnancy associated increase in FVIII and VWF levels (see Chapter 2.3.1) is typically not pronounced in the first trimester, in haemophilia A carriers and women with VWD. In haemophilia B carriers and women with FXI deficiency there is unlikely to be any change from baseline levels. Therefore these women may be at increased risk of prolonged or excessive bleeding during such events [1, 2, 9, 175, 182].

Bleeding that occurs after the 24th completed week of pregnancy and prior to delivery is less common and termed antepartum haemorrhage (APH). APH occurs in 3-5% of all pregnancies and is a leading cause of perinatal and maternal morbidity worldwide [187]. APH occurs from bleeding at the placental site, lesions of the cervix or vagina

and occasionally is of fetal origin. Among the most important causes that result in major haemorrhage include placenta praevia (low implantation of the placenta), and placental abruption (premature detachment of placenta from the uterus), which account for 31% and 22% of APH, respectively. Vasa praevia is the presence of unsupported fetal blood vessels below the fetal presenting part, usually resulting from a valementous cord insertion. It complicates approximately 1 in 6000 deliveries, but if undiagnosed, carries a 60% mortality rate in the infant following rupture of membranes [188].

The risk of antepartum bleeding complications was significantly increased compared to controls in a large case-control study conducted in the United States including 4067 pregnancies in women with VWD (odds ratio [OR] 10.2, 95% confidence interval [CI]: 7.1-14.6) [189]. In two published reviews of 172 pregnancies among 65 carriers of haemophilia A, and 20 carriers of haemophilia B, there were no reported episodes of APH [1, 2]. Severe placental abruption necessitating urgent caesarean delivery occurred in one out of 28 (3.6%) pregnancies in woman with FXI deficiency reported by Kadir *et al* [175]. Mild and self-limited APH was reported in three out of 61 (4.9%) pregnancies reported in the case series by Chi *et al* [49]. Numerous case reports and case series have reported an increased risk of bleeding complications in pregnancy in women with BSS and GT. In a systematic review of the literature, APH complicated four out of 30 pregnancies in women with BSS [190]. GT is associated with adverse perinatal outcome, although APH rate does not appear to be increased, according to a literature review in 20 pregnancies [83].

APH occurs more frequently in congenital factor XIII deficiency and fibrinogen disorders. APH affected 5 out of the 65 (8%) pregnancies that reached viability in the systematic review of congenital FXIII deficiency [101]. Placental abruption, reported in numerous case reports and case series, is particularly associated with congenital afibrinogenaemia [184, 191, 192].

Regional analgesia/anaesthesia

Regional block provides the most effective pain relief in labour. It is required for instrumental vaginal delivery and caesarean section. It is regarded as the safest option as general anaesthesia in term pregnant women is associated with a higher morbidity and mortality, from complications such as failed intubation and aspiration pneumonia [193]. Women with IBDs have previously been denied this option as coagulopathy is regarded as a contraindication. Blood vessel puncture after placement of epidural catheter and bleeding into the epidural space can result in spinal haematoma leading to permanent neurological damage. However, the incidence of this potentially devastating complication is extremely low (5 per million in the general obstetric population) [194]. In a retrospective review of 80 pregnancies amongst 63 women with IBDs (including 19 women with FXI deficiency, 16 carriers of haemophilia, 15 with VWD, 7 with PFDs) regional block was administered safely following individualised assessment involving a multidisciplinary team [195]. However, due to the small number of participants, and the low incidence of spinal epidural haematoma, an increased incidence of this complication in women with IBDs could not be excluded. FXI deficiency in particular poses a unique challenge, as the bleeding risk does not correlate well with factor levels. Chapter 8 of this thesis addresses

haemostatic assessment and provision of haemostatic prophylaxis for regional anaesthesia in women with FXI deficiency.

2.3.6 Postpartum haemorrhage

Postpartum haemorrhage (PPH) is an important global cause of maternal morbidity and mortality. Obstetric haemorrhage was the third most common direct cause of maternal death from the 2015 MBRRACE-UK report, with a rate of 0.59 per 100,000 maternities [196]. Primary PPH is defined as an estimated blood loss greater than 500 mL following vaginal delivery, and 1000 mL following caesarean delivery. The prevalence of primary PPH varies according to definition, but is estimated to be around 3-6% according to hospital discharge summaries in the United States, Canada and Australia [197-199]. There is no standard definition of massive obstetric haemorrhage (MOH). It is variably defined as estimated blood loss > 1500 mL within 24 hours of delivery, a decrease in haemoglobin of > 4 g dL^{-1}, or transfusion requirement of > 4 units packed red cells [200, 201].

The most common cause of primary PPH is uterine atony, or failure of the uterus to adequately contract, which accounts for up to 80%. [202]. Genital tract or uterine 'trauma' is responsible for around 20%, and comprises perineal, vaginal, and cervical lacerations, in addition to spontaneous or iatrogenic uterine rupture during operative delivery. Other aetiologies include retained placenta, and abnormal placental implantation, which account for 10%, and often coexist with uterine atony [201]. Coagulopathy is a recognised cause of PPH, which can be either congenital or acquired. Acquired coagulopathy develops during a PPH due to consumption of

coagulation factors and fibrinogen, and haemodilution due to massive transfusion [203]. This is also accompanied by hyperfibrinolysis with rapid clot turnover. Certain obstetric events can result in an acquired coagulopathy, including pre-eclampsia/eclampsia, placental abruption, amniotic fluid embolism or intrauterine stillbirth causing disseminated intravascular coagulopathy [204]. Congenital coagulopathy, as in IBDs, are another important aetiology of PPH, which may be the presenting symptom.

Secondary PPH is excessive lochia loss from 24 hours until six weeks postnatal [205]. The prevalence of secondary PPH in the general population is harder to measure as it is often managed in the community and therefore under-reported. Current estimates are that secondary PPH complicates around 1% of deliveries in the UK [206]. In a study that assessed quantity and duration of lochia loss using a PBAC chart, the median duration of lochia loss was significantly higher (39 days; range 21-58, versus 31 days; 10-62 days, $p = 0.03$) in women with IBDs compared to controls [207].

Von Willebrand disease

Women with all types of VWD are at an increased risk of bleeding complications at the time of delivery. Kouides *et al* reported a significant increase in childbirth related bleeding in 99 women with type 1 VWD compared to 150 controls (31% versus 10%, $p = 0.001$) and furthermore 17% had PPH that required a blood transfusion compared to 3% of controls ($p = 0.002$) [27]. A case-control study in women with known IBD demonstrated that primary PPH occurred with a significantly higher frequency in women with VWD (32% versus 7.5%) compared to controls [208].

A large epidemiological study including 4067 deliveries among women with VWD in the United States showed that 6% of women with VWD experienced a PPH compared with only 4% of women without VWD. These numbers were small and the odds did not reach statistical significance. However, there was a statistically significant risk of other bleeding complications such as perineal haematoma (OR, 3.3; 95% CI: 0.8, 13.4), a five fold increased risk of receiving blood transfusion (OR, 4.7; 95% CI: 3.2, 7.0) and the maternal mortality rate was 10 times higher in women with VWD than in women without (123 vs 12.7 per 100 000 deliveries)[189]. In a large population-based study that assessed risk factors for MOH in Norway, VWD was second only to emergency caesarean delivery in having the highest adjusted OR (3.31, 95% CI; 1.01-10.85) [209][101].

Carriers of haemophilia

Carriers of haemophilia are at a significantly increased risk of bleeding during labour and delivery. Whilst some haemophilia A carriers have normal FVIII levels at term, a significant proportion may still have low factor level (< 50 IU/dL), especially those with severe deficiency [1, 2, 79]. Factor IX levels are not affected by pregnancy, therefore haemophilia B carriers will be at an increased risk of bleeding if they have low baseline factor levels.

The incidence of primary PPH among haemophilia carriers is significantly higher (12-22%) [1, 2, 79] than in the general population (5-8%) [210]. The incidence of secondary PPH is also increased in carriers of haemophilia (2-11%) [1, 2] compared to the incidence reported in the general population (0.8%) [206]. FVIII and FIX activity correlates with the risk of bleeding among carriers of haemophilia with the

most significant PPH occurring in those with baseline factor levels below 50 IU/dL [2, 37].

FXI deficiency

The incidence of both primary and secondary PPH is increased in women with FXI deficiency, as demonstrated in numerous case series and cohort studies [49, 175, 182, 211]. Chi *et al* evaluated 49 deliveries in women with both partial and severe FXI deficiency and found an incidence of both primary and secondary PPH to be 11% of all deliveries [49]. Similarly, the incidence of PPH in 33 women with FXI deficiency was 13% in the study performed by Myers *et al* [182]. In a study performed in Israeli women with severe FXI deficiency (FXI:C <17 IU/dL), 19 out of 62 women (30%) experienced PPH in one or more pregnancies [211].

Platelet function disorders

There is a lack of data in the literature in relation to mild PFDs and pregnancy complications [212]. In women with severe disorders such as BSS and GT, the published literature is limited to case reports and small case series only, which indicate a high risk of severe bleeding complications in both mother and neonate [212]. In a systematic review of the literature, 30 pregnancies among 18 women with BSS were identified [190]. Primary and secondary PPH occurred in 34% and 40% of deliveries, respectively, with 50% requiring blood transfusion. Two women underwent obstetric hysterectomy at the time of delivery. In a systematic review of the literature, 40 pregnancies in 35 women with GT were identified. Similarly, the rate of primary and secondary PPH was high (34% and 20%, respectively), and PPH was frequently severe.

There are multiple case reports and small case series that document incidence of PPH in women with rare IBDs. PPH was the most common obstetric complication occurring in 14 out of 31 deliveries (45%) among 10 women affected with hypofibrinogenaemia. Blood transfusion was required in all cases and two women underwent hysterectomy [213]. Women with FV deficiency are at increased risk. PPH was reported in 13 out of 17 deliveries (76%) among nine women [214]. PPH was reported at a rate of 30% in 13 and 14 pregnancies in women with FVII and FX deficiency, respectively [215, 216]. PPH occurred in 16 (25%) out of 65 pregnancies in women with FXIII deficiency. There is limited data on pregnancy in women with combined FV and FVIII deficiency. Due to the rarity of these IBDs, there is limited data available of haemostatic assessment and prophylactic treatment at delivery.

2.3.7 Haemostatic assessment and haemostatic agents used for bleeding prophylaxis and treatment in pregnancy

Von Willebrand disease

Haemostatic assessment in women with VWD includes regular monitoring of VWF:Ag, VWF:Ac and FVIII:C, particularly during the third trimester to determine the risk of bleeding at delivery. The platelet count should be monitored in women with type 2B VWD as pregnancy may exacerbate thrombocytopaenia [21]. A personal and/or family history of bleeding complications is an important factor to consider in the management of these women.

Prophylaxis and treatment of pregnant women with VWD depends upon the anticipated MOD and use of regional anaesthesia, factor levels, VWD subtype, previous bleeding history and response to 1-deamino-8-D-arginine (DDAVP) treatment, the presence of an inhibitor and the potential risks of treatment [217]. Haemostatic cover is not usually required for women with type 1 VWD, unless third trimester VWF:Ag or FVIII:C are less than 50 IU/dL [183]. The bleeding risk can be challenging to predict in women with type 2 VWD. The defect is functional therefore changes in factor levels in pregnancy do not necessarily affect bleeding risk. The treatment will depend on the subtype, and is individualised for each woman. Treatment with factor concentrate is almost always necessary for women with type 3 VWD. The main therapeutic options available include pharmacological agents such as tranexamic acid and DDAVP, or VWF/FVIII-containing concentrates.

Antifibrinolytic agents

The three main antifibrinolytic agents are Epsilon-Amino Caproic Acid (EACA), tranexamic acid and Aprotinin (APR). APR is the most potent antifibrinolytic agent but due to increased mortality rates reported with several clinical trials it has been withdrawn from the market and its use is currently not recommended [218]. Tranexamic acid is more potent than EACA and it is the main antifibrinolytic agent used for the treatment of VWD. It inhibits the binding of plasminogen to fibrin by reversible blockage of lysine-binding sites and thus inhibition of fibrinolysis. It has a maximum clinical efficacy if administered prior to delivery when there is a sufficient circulating volume in plasma [217]. It can be used in combination with DDAVP or factor concentrates as prophylaxis to prevent bleeding during surgery or delivery.

The use of tranexamic acid during pregnancy, delivery and postpartum was evaluated in a systematic review [219]. Tranexamic acid demonstrated a small reduction (33 mL, 95%CI, -4.1-69.1; $p = 0.08$) in blood loss during elective caesarean section and vaginal delivery in prospective studies. Another systematic review found a significant reduction in blood loss (92 mL; 95%CI, 76-109 mL) following administration of 0.5-1 gram of intravenous tranexamic acid for PPH in three trials [220]. This review stressed that the evidence of tranexamic acid use for reducing blood loss in PPH was weak, as the studies included were not randomised or blinded to bias. A French randomised controlled trial administering high dose tranexamic acid to women with PPH > 800 mL demonstrated a significant reduction in blood loss over six hours (173 mL versus 221 mL, $p = 0.04$), shorter duration of bleeding and reduction in progression to severe bleeding in the treatment group compared to controls [221]. The World Maternal Antifibrinolytic (WOMAN) trial is a large multinational trial that is currently ongoing to evaluate the efficacy and safety of tranexamic acid use in PPH

[222]. An extended course of tranexamic acid is recommended in women with VWD with low baseline factor levels, as these are likely to decrease rapidly after delivery, resulting in secondary PPH [167]

Tranexamic acid can be given orally (15-25 mg kg^{-1} tds), intravenously (10 mg kg^{-1} tds), or specifically for dental and oral cavity bleeding as a mouthwash (10 mL of a 5% v/w solution qds). The adverse effects of tranexamic acid include nausea, vomiting and gastrointestinal upset, especially during prolonged durations of treatment. Rapid intravenous infusion can result in dizziness and hypotension. Tranexamic acid should be used with caution in patients with a previous history of thromboembolism, and is currently contraindicated in the UK in patients with active thromboembolic disease [223].

1-deamino-8-D-arginine (DDAVP)

DDAVP is a synthetic vasopressin analogue that enhances endogenous levels of VWF and FVIII [224]. Despite the extensive clinical use of DDAVP as a haemostatic agent the cellular mechanism of action is not fully understood. DDAVP induces a release in VWF stores in endothelial Weibel-Palade bodies and tissue plasminogen activator (t-PA), which subsequently stabilises plasma FVIII levels [225]. However, in patients with type 2N VWD, where there is a decreased affinity of VWF for FVIII, DDAVP also induces a rise in FVIII concentration. This indicates that DDAVP induces a direct release from FVIII storage pools [226]. Recent experimental models have proven the source of FVIII to be predominantly derived from endothelial cells in the liver and elsewhere throughout the circulation [227, 228].

DDAVP can be administered by slow intravenous or subcutaneous infusion (0.3 μg kg^{-1} over 20 mins), or via intranasal spray (300 μg). Typically the effect of one metered-dose intranasal spray, which can be easily administered at home, is comparable to that of a 0.2 μg kg^{-1} intravenous injection and sufficient to induce the required haemostatic effect [229]. The side effects of DDAVP are attributed to the vasodilatory effect and include mild tachycardia, headache and flushing. Hyponatraemia and volume overload due to the antidiuretic effect of DDAVP are rarely encountered. The risk is greater in patients receiving multiple doses, and can be minimised by fluid restriction at the time of treatment [230].

DDAVP is usually very effective at inducing an increase in VWF and FVIII levels in patients with type 1 VWD. It has no therapeutic benefit in type 3 VWD. In types 2A and 2M VWD, DDAVP enhances the levels of abnormal VWF and has a variable clinical effect. A trial of DDAVP administration to measure the response is recommended in these patients. DDAVP can be used in patients with type 2N VWD, but the short half-life of the FVIII response makes this of limited therapeutic benefit. The use of DDAVP in patients with type 2B VWD is controversial as the abnormal VWF with an increased affinity for GPIb may induce platelet aggregation and thrombocytopaenia [20, 231].

DDAVP can be used with caution during pregnancy but prolonged administration is not recommended due to the risk of fluid retention and should be avoided in pregnancies complicated by pre-eclampsia. DDAVP has a greater affinity for V2 receptors and therefore has little if any oxytocic effect. It should be used with caution in women receiving a synthetic oxytocin infusion (Synotocinon®) to induce or

augment labour. A systematic review reported on the safe and efficacious use of DDAVP for prophylaxis during invasive procedures carried out during the first and second trimester in 50 pregnancies [232]. In addition, there were no postpartum bleeding complications in 167 out of 172 pregnancies where DDAVP was administered. One case of water intoxication was reported and one case of premature delivery in all 216 pregnancies in 30 studies of DDAVP use in pregnancy. VWD was the most common indication for DDAVP use.

Blood products

A plasma-derived concentrate containing VWF is the optimum treatment when DDAVP is not effective or contraindicated. There are various concentrates available that have different ratio of VWF/FVIII. The dose and duration of treatment is dependent on the haemostatic challenge. The most commonly used blood products that are licenced in the UK are Haemate-P®, and Alphanate SD/HT®. Haemate-P is a lyophilised, intermediate-purity concentrate that contains a similar quantity of VWF HMW multimers compared to normal plasma. The concentrate contains approximately 2.5 IU of VWF:Ag for every FVIII:C [233]. Its efficacy has been rated as good to excellent at achieving haemostasis in VWD patients with acute bleeding or for surgical prophylaxis [234]. Alphanate SD/HT® is a high-purity, lyophilised concentrate of VWF and FVIII and other plasma proteins. The concentrate contains approximately 0.6 IU VWF:Ag for each unit of FVIII:C [235]. A multicentre prospective study in 81 patients with VWD demonstrated adequate haemostasis for surgical interventions with Alphanate SD/HT® usage. The concentrate effectively stopped active bleeding in most cases following administration of one or two doses, even when the bleeding time was not adequately corrected [235]. Although

VWF:RCo and FVIII levels required for haemostasis in VWD patients have not been established in clinical trials. The general consensus however, is that VWF:RCo and FVIII should be raised above 50 IU/dl for delivery and for at least 3-5 days postpartum, or until haemostasis and wound healing is achieved [236]. Sustained high levels of FVIII increases the risk of venous thromboembolism (VTE) therefore when repeated infusions of VWF:FVIII concentrates are necessary, the FVIII:C levels should be monitored daily to avoid excess of 150 IU/dL [237-239]. In addition to the pregnancy induced hypercoagulable state there may be other VTE risk factors such as surgery or immobility [237]. Wilfactin® is a highly purified VWF concentrate containing very little FVIII. Combined data from various clinical trials assessing the efficacy of Wilfactin® have shown excellent or good haemostasis control in 89% of patients with spontaneous bleeding [29]. Wilfactin® is therefore considered safer in pregnant women with hormonally induced enhanced FVIII levels who require VWF replacement. Other adverse symptoms of plasma product usage include allergic reactions and anaphylaxis, chest tightness, urticaria, prutitis and oedema.

Carriers of haemophilia

The risk of bleeding at delivery in carriers of haemophilia correlates well with FVIII and FIX levels, which should be assessed during the third trimester of pregnancy. This is especially important in women with low pre-conceptual (baseline) factor levels. Carriers with factor levels < 50 IU/dL at term require appropriate haemostatic cover.

The treatment of symptomatic haemophilia carriers is dependent on baseline factor levels, the presenting bleeding complaint and/or haemostatic challenge anticipated. In

the majority of circumstances tranexamic acid are sufficient to prevent bleeding in carriers of haemophilia with mildly reduced factor levels (50-60 IU/dL). DDAVP is used in carriers of haemophilia A with moderately reduced (40-50 IU/dL) factor levels. Usually only one or two intravenous administrations provide sufficient cover during labour, and tranexamic acid can be continued thereafter. DDVAP has no effect on FIX levels therefore cannot be used in the treatment of haemophilia B.

Factor concentrates are reserved for the treatment of haemophilia carriers who do not respond to DDAVP and have factor levels < 50 IU/dL at term. Recombinant factor products (rFVIII and rFIX) are the treatment of choice if available. FVIII and FIX levels should be maintained at > 50 IU/dL for three days after delivery. An extended course of tranexamic acid is recommended in carriers with low baseline factor levels, as these are likely to decrease rapidly after delivery, resulting in secondary PPH [207].

Factor XI deficiency

Factor XI (FXI) deficiency poses a particular problem for the obstetrician as the bleeding phenotype is highly variable and does not correlate well with the underlying FXI level. An early cohort study of bleeding tendency yielded an odds ratio (OR) of 13 (95% CI, 3.8-45) in homozygotes or compound heterozygotes, and of 2.6 (95% CI, 0.8-9.0) in heterozygotes [81]. However, excessive bleeding has been well documented in heterozygotes with borderline FXI level between 50-70 IU/dL [39, 54].

Criteria have been suggested, based on personal bleeding history, to categorise patients with FXI deficiency into 'bleeders' and non-bleeders' (Table 2.8). Two haematologists with expertise in haemostasis independently obtained a bleeding history in 172 patients with FXI deficiency, and any patient with 2 ≥ positive criteria were described as 'bleeders'. In addition, they evaluated FXI deficient patients for co-inheritance of von Willebrand disease (VWD). Patients with blood group O had a lower von Willebrand factor (VWF) level, and an increase in bleeding symptoms [54].

Bleeding tendency in relation to genotype has also been assessed. In a cohort study including 52 patients with severe FXI deficiency, the mean number of injury or surgical related bleeding was significantly higher in patients with the II/II genotype (1.6 ± 2.4) versus patients with a II/III (1.4 ± 1.5) or a III/III (1.0 ± 1.1) genotype ($p <$ 0.05 for both comparisons) [40]. In contrast, another study found no difference in bleeding tendency in relation to genotype. Twenty patients who were compound heterozygotes for the type II mutation were compared with 41 partially deficient patients with an unknown mutation [54]. Approximately half the patients in each group were classified as 'bleeders'; 45% in compound heterozygotes compared with 49% in patients with an unknown mutation. In a study by Salomon *et al* the type II/III mutation was reported to be associated with the highest frequency (12%) of PPH among women with severe FXI deficiency [211]. This genotype was associated with a mean FXI level of 20 IU/dL. The frequency of PPH was lowest (2%) in women with type II/II mutation, where the mean FXI level was 60 IU/dL. However, the difference in frequency of PPH between the groups did not reach statistical significance ($p =$

0.24), and there was no statistical correlation between PPH and FXI level (p = 0.46) [211].

Lately there has been a growing interest in using thrombin generation studies to distinguish bleeding risk in FXI deficient patients. Low velocity and delayed thrombin generation suggests a higher bleeding tendency, independent of FXI level [240]. Thrombin generation induced by recalcification is able to distinguish between bleeding and non-bleeding phenotype in patients with severe FXI deficiency [241]. In a study including 24 patients with both mild and severe FXI deficiency, those exhibiting severe bleeding tendency had markedly impaired thrombin generation. The opposite was also demonstrated in that patients with severe deficiency (FXI level = 1 IU/dL) and normal thrombin generation had a non-bleeding phenotype [240]. Clot structure and stability has also been analysed in patients with severe FXI deficiency to distinguish bleeding tendency. Symptomatic 'bleeders' were found to exhibit a lower fibrin network density and clot stability compared with asymptomatic 'non-bleeders' [242].

Personal bleeding history, factor level, the presence of any co-inherited VWD, blood group and the nature of the haemostatic challenge should be taken into account when determining the need for therapy. Relying on FXI levels alone may result in inappropriate assessment of the bleeding risk and overuse of plasma products, which are associated with a risk of thromboembolic complications, and the potential for viral transmission. In addition, regional anaesthesia may be withheld in a woman with FXI deficiency due to concerns over bleeding complications resulting in spinal injury. Part

of this thesis aims to assess the role of rotational thromboelastometry (ROTEM®) in assessment of bleeding tendency in pregnant patients with FXI deficiency.

Table 2.8 Criteria to assess bleeding tendency in patients with FXI deficiency [54]

1.	Bruising tendency
2.	Mucous membrane bleeding; epistaxis; GI; haematuria
3.	Menstrual bleeding: Requiring treatment – OCP; TXA; surgical procedure
4.	Bleeding post surgery
5.	Bleeding post dental extractions(s)
6.	Bleeding in relation to parturition

GI; gastrointestinal, OCP; oral contraceptive pill, TXA; tranexamic acid
Criteria to classify as 'bleeder'; requires presence of 2 out of 6 bleeding symptoms on assessment by haematologist with expertise in haemostasis

Current treatment options for FXI deficiency at delivery include FXI concentrates, fresh frozen plasma (FFP), recombinant factor VIIa (rFVIIa) or antifibrinolytic agents such as tranexamic acid. In the majority of patients with mild heterozygous FXI deficiency, tranexamic acid is sufficient to prevent bleeding. The combined use of tranexamic acid with FXI concentrates or rFVIIa is not recommended due to the increased risk of thromboembolism. A course of oral tranexamic acid can be administered in the postnatal period, 14-48 hours following blood product administration.

Only a plasma-derived FXI concentrate is currently licenced for use in the UK (Hemoleven®, LPB, Lille, France). Even though the risk of viral transmission is negligible with modern inactivation processes, viruses can still be present in commercial blood derivatives and the transmission rates of prion disease are unknown [243]. FXI concentrates have a considerable thrombotic potential and should be administered with caution in pregnancy [244, 245]. Due to the thrombotic risk associated with FXI concentrates, treatment should be initiated with caution, and avoided if possible in older patients with a history of cerebrovascular disease. The target plasma FXI level should ideally be in the lower end of normal range (60-70 IU/dL) with avoidance of peak FXI levels >70 IU/dL.

The disadvantages with FFP administration are due to the larger volumes required which increase risk of volume overload and take longer to infuse. In addition there is potential for serious hypersensitivity reactions (i.e. transfusion related acute lung injury [TRALI]) [246].

Low dose rFVIIa, previously used as a bypassing agent for patients with FXI deficiency with inhibitors, is now an established treatment for patients without inhibitors who wish to avoid plasma-derived products. However, high levels of FVII activity have been reported following rFVIIa administration resulting in thrombotic complications [247, 248].

Platelet function disorders

Predicting bleeding risk can be challenging in patients with platelet function disorders. Bleeding occurs following surgery therefore MOD is an important consideration. However, bleeding does not occur with every haemostatic challenge. Women with severe PFDs should be managed in a tertiary unit affiliated with a haemophilia centre where the relevant expertise and access to platelets and blood products are available. The options for haemostatic prophylaxis are DDAVP, tranexamic acid, rFVIIa and platelet transfusion. Platelet transfusion should be avoided where possible due to the associated risk of alloantibody formation [57, 249].

There is limited data on patients with mild PFD, such as storage pool disease, signalling defects or ADP receptor abnormality. DDAVP and tranexamic acid only are usually sufficient to prevent bleeding at delivery in these women [212].

2.3.8 Mode of delivery and cranial bleeding

Special consideration should be given to reduce the bleeding risk in a potentially affected fetus. It is recommended that women with a severe IBD have a multidisciplinary management plan drawn up in advance of labour, and delivery should be at a centre affiliated with a haemophilia laboratory with readily available factor concentrates [250]. If the woman is carrier of a severe condition with a potentially affected fetus this is also recommended to minimise the risk of bleeding complications in the offspring. During labour, invasive procedures such as fetal blood sampling or fetal scalp electrode monitoring should be avoided where possible.

The most serious bleeding complication that can arise in the affected offspring is cranial bleeding during labour and delivery. Intracranial haemorrhage (ICH), defined as an accumulation of blood within the cranial vault, can result in serious long-term neurological sequelae or death. Extracranial haemorrhage (ECH), defined as haemorrhage or haematoma that occurs outside the cranial vault, can be rapid and result in life threatening hypovolaemia, due to persistent bleeding into the subgaleal space. In the general population the risk of cranial bleeding is increased with assisted deliveries and prolonged labour. Planned caesarean section (CS) is associated with the lowest risk [251]. The safest MOD for fetuses at risk of IBDs is still debated. There is limited data available from prospective studies on the risk of cranial bleeding according to MOD in newborns with IBDs.

Prenatal diagnosis and multidisciplinary management have potential to reduce the risk of cranial bleeding in deliveries where an affected offspring is anticipated. Chapter

6.2 summaries the available literature on the risk of cranial bleeding in newborns with haemophilia compared to the general newborn population. A meta-analysis is presented to determine the effect of MOD on rates of ICH in newborns with haemophilia.

2.4 The neonate and inherited bleeding disorders

2.5.1 Neonatal haemostasis

The haemostatic system develops with age and is an evolving process in the fetus and neonate. The capacity of newborns to generate thrombin is reduced at birth due to a physiological reduction in vitamin K-dependent coagulation factors (FII, FVII, FIX and FX) and contact factors (FXI, FXII, prekallikrein and high molecular weight kinnogen) that are about 50% of the normal adult range [252]. The reduction in procoagulant factors in neonates may be the result of decreased production, accelerated renal clearance, or consumption due to angiogenesis and inflammation. Therefore the deficiencies of prematurity are further exacerbated by sepsis and asphyxia [253].

The risk of bleeding secondary to reduced coagulation factors is counterbalanced by a physiological deficiency in natural anticoagulants (protein C and antithrombin) and coagulation inhibitors (tissue plasminogen activator inhibitor) as well as a reduction in fibrinolysis [254]. Consequently, in the healthy term infant the haemostatic system is sufficiently balanced for appropriate clot formation and haemostasis.

Platelet number and volume are relatively similar to adult ranges in the neonate. There is a reduction of platelet surface glycoproteins, resulting in a reduced response to agonists and granule secretion. However reduced platelet function is not observed in the neonate, which may be explained by a compensatory increase in plasma

concentrations of VWF levels and a greater percentage of large VWF multimers [254, 255].

2.5.2 Bleeding manifestations in the neonate with IBD

The pattern of bleeding in the neonate is decidedly different from that of an older child or adult with an IBD. Bleeding in the neonatal period was thought to be a rare event, however more recent studies suggest that 15-33% of newborns with IBDs present with bleeding manifestations during the first few weeks of life [256]. Birth trauma can result in cranial bleeding that remains a major cause of mortality and morbidity in this group. Cranial bleeding in relation to labour and deliver is the most serious bleeding manifestation in the neonate with an IBD, because it has potential to be life threatening or to cause life-long neurological morbidity. This is covered in detail in Chapter 6.

A significant proportion of bleeding manifestations in the neonate are iatrogenic, caused by intramuscular injections for vitamin K administration, heel prick, venepuncture, and procedures such as circumcision. In a study conducted in the US that assessed the sites of initial bleeding episodes in the first 30 days of life in 207 infants with haemophilia, 135 (65%) were due to iatrogenic causes [256]. The majority of these bleeding events occurred in infants with no previous family history of haemophilia. Prolonged umbilical stump bleeding is characteristic of an inherited defect in fibrinogen production or function and FXIII deficiency.

CHAPTER 3

LABORATORY INVESTIGATIONS OF HAEMOSTASIS

3.1 Routine coagulation tests and factor assays

3.2 Platelet light transmission aggregometry

3.3 Rotational thromboelastometry

3.1 Routine coagulation tests and factor assays

The principle tests of haemostasis are described below with a brief methodology for each test. These investigations have been employed throughout this research project and each study will refer back to this section when a description is required.

Activated partial thromboplastin time

The activated partial thromboplastin time (APTT) is the so-called measure of the intrinsic and common pathway of coagulation in recalcified citrated plasma. It depends upon the mixing of citrated plasma with 'partial thromboplastin', a reagent that lacks the apoprotein component of complete thromboplastin. Thus this excludes the involvement of FVII and tissue factor (TF) of the extrinsic pathway. Theoretically APTT should detect deficiencies in coagulation factors II, V, VIII, IX, X, XI, XII and fibrinogen, although the sensitivity to fibrinogen deficiency is low. Due to the variability in the composition of phospholipids available for reagents there is marked variation in the sensitivity of different reagents to coagulation factor levels and inhibitors. In addition the performance of APTT can be affected by incubation time, type of coagulometer, and type and concentration of activator used to provide a surface area for the initiation of coagulation (e.g kaolin or celite). This can produce inconsistencies in APTT results in different laboratories [257]. Isolated prolonged APTT can occur in the presence of a lupus anticoagulant, which targets the phospholipid used to determine APTT. An additional limitation of the APTT is that it detects deficiencies in factors XII, prekallikrein and high-molecular weight kininogen that are of no clinical significance and prevalent in up to 2% of the population [258].

The principle of APTT measurement uses an automated system to determine the optical density that determines the threshold for clot formation. Platelet poor plasma (PPP) is incubated at 37°C, the phospholipid (cephalin) and contact activator (kaolin) are added followed by calcium (all pre-warmed to 37°C). Addition of calcium initiated coagulation and timing begins. The APTT is the time taken from addition of calcium to clot formation [259].

Prothrombin time

The prothrombin time (PT) assess the extrinsic and common pathways. It measures the time for clot formation when TF, thromboplastin and calcium are mixed with citrated plasma. The PT should detect deficiencies in FII (prothrombin), factors V, VII and X, in addition to very low fibrinogen levels. However, like APTT the PT sensitivity is also dependent on reagents used [257]. The international normalised ratio (INR) was developed to overcome the reagent-dependent variability for the purposes of monitoring patients on oral anticoagulants. Prolongation to PT is seen most often in clinical practice as a result of hepatic impairment or nutritional deficiency of vitamin K from malabsorption.

The principle of PT measurement uses an automated system to determine the optical density that determines the threshold for clot formation. PPP is mixed with TF (containing phospholipid) incubated at 37°C. An excess of calcium chloride (25 mM) is added to initiate coagulation [259].

Coagulation factor assays

The principles of coagulation factor assays rely upon measuring the degree of correction of the APTT when plasma is added to a sample specifically deficient in the factor to be measured. Coagulation factor assays can be performed by a standardised one-stage or two-stage technique or by chromogenic assay. The one-stage assays are simple and easy to perform but are limited by susceptibility to interference from preactivation of FVIII or anti-phospholipid antibodies and misleading results when assaying rFVIII. Two-stage assays require an initial step to produce activated factor X (FXa) in a quantity proportional to the amount of factor present that is being measured. The chromogenic assay measures the amount of FXa present through its action on a highly specific chromogenic substance. FXa is formed by the conversion of factor X (FX) by activated FIX and FVIII to cleave a chromogenic substrate. The sample is combined into a mixture with activated FXI in the presence of factor IIa (thrombin), incubated, combined with FX and FVIII, and incubated [260]. The colour intensity produced is directly proportional to the amount of FXa, which in turn is directly proportional to the amount of factor present. The factor levels can be calculated from absorbance of the sample at the specific wavelength [259]. In this thesis FVIII, FIX, and FXI were assayed on thawed PPP by one-stage clotting methods as described above. FXIII antigen levels were measured using a FXIII ELISA assay [Affinity Biologicals, Quadratech Diagnostics Ltd, UK].

Von Willebrand factor assays

A summary of the VWF assays used to help distinguish the different type and subtypes of VWD are given in Table 3.1

Table 3.1 Summary of von Willebrand factor assays

Designation	Abbreviation	Summary
von Willebrand Factor Ristocetin Cofactor Activity	VWF:RCo	A functional assay of plasma VWF based upon the degree of platelet agglutination induced after the addition of ristocetin
von Willebrand Factor Collagen Binding Activity	VWF:CB	A functional assay of plasma VWF that quantifies the ability of VWF to bind collagen coated ELISA plates
von Willebrand Activity	VWF:Ac	A functional assay of plasma VWF that uses monocloncal antibody that targets the part of VWF molecule that binds to GP1b receptor. Usually reduced in all types of VWD. Most sensitive assay used for screening purposes
von Willebrand Factor Antigen	VWF:Ag	An immunological assay that quantifies the amount rather than the function of VWF in plasma. Usually reduced in type 1 VWD, and absent in type 3 VWD
Factor VIII	FVIII	A clotting factor that acts as a cofactor in the formation of *Xase* 'Tenase' complex
Factor VIII activity	FVIII:C	A functional assay of FVIII coagulant activity
Ristocetin-Induced Platelet Aggregation	RIPA	A test that measures the ability of VWF to bind to platelets

ELISA, enzyme-linked immunosorbent assay; GP1b, glycoprotein 1B

The ratios of VWF assays can help to distinguish the types, and subtypes of VWD. For example, a VWF Ristocetin Cofactor activity (VWF:RCo) to VWF antigen (VWF:Ag) ration <0.7 suggests a dysfunctional VWF which differentiates type 2A, 2B and 2M VWD from type 1 VWD. The ratio of VWF Collagen Binding activity (VWF:CB) to VWF:Ag helps to distinguish the different subtypes of type 2 VWD. In VWD type 2A the ratio is usually low. In VWD type 2B the ratio is usually low but can be normal. In VWD type 2M the ratio is usually <0.7.

Specialised laboratory tests for VWD include FVIII binding studies that are of value in diagnosis of VWD type 2N, and Ristocetin Induced Platelet Agglutination (RIPA) which is of value in distinguishing VWD type 2B. In addition a VWF multimeric

analysis is a qualitative assay that depicts the variable concentrations of different sized multimers [259].

3.2 Platelet light transmission aggregometry

Principles of LTA

The gold standard for *ex vivo* measurement of platelet reactivity is light transmission aggregometry (LTA) on platelet rich plasma (PRP). There is an increase in light transmission as platelets aggregate in response to an agonist, which is recorded over a predetermined time.

Prior to aggregation testing

Platelets are separated from white and red cells by centrifugation at 150 *x g* for 12 minutes at room temperature to make a platelet suspension in plasma. The supernatant PRP is transferred into a 10 mL capped tube (D10 plastic tube) avoiding red cells or the buffy coat layer disturbance. The PRP samples are held at room temperature undisturbed for at least 30 minutes prior to testing. The prepared PRP samples are checked visually for haemolysis and/or red cell contamination, in which case these samples are discarded. The aggregation test was completed within four hours of venous blood collection. After removing the PRP, the original tubes are centrifuged again at 450 *x g* for 15 minutes and then the supernatant poor platelet plasma (PPP) is transferred into a 5 mL capped tube, avoiding contamination with buffy coat residues [261]. Prior to the LTA, platelet count is measured using an aliquot of 0.5 mL PRP on a SYSMEX analyser (Sysmex, UK) to ensure platelet count is within appropriate range of 100,000µL- 600,000µL.

LTA methodology

Platelet aggregation was carried out using a dual channel Payton aggregometer 600B (Payton Associates, Canada). Using 10 millivolts deflection, the PRP settings were 0.5 and 5.5 millivolts for channel one and two respectively which represent 0% aggregation and the corresponding blank value setting using PPP was 4.5 and 9.5 millivolts for each channel which represent 100% aggregation. The aggregometer was turned on and set at 37°C to warm up and stirring speed was set at 9000 rpm.

Prior to LTA a stir bar was deposited into two clean cuvettes containing 360 µL PRP and 400 µL PPP. The cuvettes were placed into the incubation wells of the aggregometer and heated to 37°C for 3 minutes. The PPP cuvette was placed into channel one of the aggregometer and after few seconds the PPP button was set. When the PPP light was activated this indicates that the aggregometer calibration for the PPP sample was set. The PPP cuvettes were replaced with PRP cuvettes and the PRP button on the aggregometer is pushed. After a few seconds, the PRP button lights up indicating that the machine was calibrated for the PRP sample. These steps are repeated for channel two. The aggregometer pen for channel one was at 45% of full scale of the chart sheet and for channel two was at 95% of the full scale. The chart speed was 1cm/min and the chart drive was switched on. The pen was lowered to the baseline trace for 2 minutes.

To obtain an aggregation curve, 40 µL of working reagent was added to the PRP sample. The aggregation trace was monitored by a chart recorder. PRP optical density and percentage of aggregation was recorded at 3 and 5 minutes on a work sheet for

both ADP and epinephrine and at 3 minutes for collagen, ristocetin, arachidonic acid and U46619. The optical density (light transmission) was measured using work sheet small squares. The maximum number of small squares reached by platelet aggregation curve over a fixed period of time was converted to percentage using a platelet aggregation conversion table. The parameters recorded while monitoring the aggregation curve for each agonist included lag phase, shape change, maximal aggregation amplitude, primary and secondary aggregation phase, disaggregation and delay response [262]. Usually with weak aggregation agonists (ADP and epinephrine) there would be biphasic aggregation curve (primary and secondary waves) whilst with strong agonists like collagen, ristocetin, arachidonic acid and U46619 only a single aggregation phase was seen. The minimum final aggregation cut off was considered to be 65% for all used reagents apart from ristocetin 0.5 mg/mL, which was less than 10%.

3.3 Rotational thromboelastometry

Thromboelastometry measures the viscoelasticity during blood clot formation. The resistance measured is quantified by computer software that produces the thromboelastogram. The method was first developed by Hartert over 50 years ago [263]. Two commercially available devices for performing thromboelastometry are TEG (Thromboelastograph; Haemoscope/Haemotinics, Nils, Ill) and ROTEM® (Rotational thromboelastometry; TEM International, Munich, Germany). The TEG system has been in use particularly in the United States for many years.

Point-of-care devices such as ROTEM®/TEG are predominantly used to guide transfusion during cardiac and hepatic surgery or major haemorrhage. Thromboelastometry is used to estimate clot formation, thrombin generation, fibrinogen levels, platelet function, and clot dissolution by fibrinolysis [264]. It provides information regarding the interactions of coagulation factors during the different phases of coagulation and lysis over time.

Principles of thromboelastometry

A sample of citrated whole blood is placed into a cuvette into which a cylindrical pin is immersed. A gap of 1mm exists between the pin and cuvette, which is bridged by blood. In the TEG system, a disposable cup moves back and forth through an arc of 4.75 degrees around a fixed plastic pin. In case of ROTEM®, the plastic pin rotates back and forth through an angle of 4.75 degrees in the centre of the plastic cup. During clot formation, fibrin strands are formed, increasing the torque between the pin and the cup. During clot retraction, dissociation of fibrin strands and degradation

of fibrin by fibrinolysis reduces the torque [265]. The change in torque is detected by a strain gauge, which converts the output into a signal, which is delivered to a computer where it is processed by proprietary software, into a trace (Fig 3.1).

Figure 3.1 Rotational thromboelastogram (reproduced from http://www.rotem.de)

ROTEM® is used with specific assays to measure different aspects of coagulation. EXTEM uses tissue factor as an activator of the extrinsic pathway; INTEM uses ellagic acid/phospholipids for contact activation. The HEPTEM is a modified version of INTEM that includes a heparin-degrading enzyme. FIBTEM assesses the extent of fibrin polymerisation by inhibiting platelet function with cytochalasin-D after tissue factor activations [266]. NATEM is without an added activator or inhibitor and although it takes longer to perform it is highly sensitive to the equilibrium of coagulation.

ROTEM® assays can detect changes in coagulation including hypo and hypercoagulation and hyperfibrinolysis [267]. Clotting Time (CT) is representative of clot initiation and clot formation time (CFT) and alpha angle are representative of clot consolidation. Maximum Clot Firmness (MCF) is an indication of clot strength and the Lysis Index in 60 minutes (LI 60) and Maximal Lysis (ML) are representative of clot dissolution (Table 3.2).

Table 3.2 ROTEM® parameters (reproduced from http://www.rotem.de)

Curve parameter	Definition
Clotting time (CT) in seconds	The period of time from the start of the measurement until a 2 mm amplitude occurs
Clot formation time (CFT) in seconds	The period of time in seconds starting from an amplitude of 2 mm (CT) until an amplitude 20 mm is reached
Alpha angle in degrees	The angle between the centre line and a tangent to the curve through 2 mm amplitude point
Maximum clot firmness (MCF) in millimetres	The maximal amplitude of clot firmness during 60 minutes
Lysis index at 60 minutes (LI 60) in percentage	The percentage of maximum clot strength present at 60 minutes
Maximum lysis (ML)	The percentage of lost clot stability (relative to MCF) at 60 minutes

CHAPTER 4

THE PREVALENCE OF BLEEDING DISORDERS IN WOMEN WITH ENDOMETRIOSIS

4.1 Introduction

Endometriosis is a complex gynaecological condition that affects around 5% of women of reproductive age. It is characterised by the presence and growth of ectopic endometrial tissue outside of the uterus resulting in dysmenorrhoea, dyspareunia, and subfertility. The chronicity of the condition has a significant impact on women's lives including work, social functioning and sexual relationships.

The formation of endometriotic lesions depends on attachment and invasion at ectopic sites. This may be due to abnormalities of the eutopic endometrium itself, predisposing the cells to survive and implant ectopically [268]. Endometriosis deposits continue to be under hormonal regulation and as such still undergo monthly proliferation, differentiation and shedding [268]. The pain resulting from endometriosis is cyclical suggesting there is localised internal bleeding within or around the endometrial deposits. Endometriomas or 'chocolate cysts of the ovary', arise due to repeated haemorrhage into ovarian tissue. The cyst contents contain a dark, gelatinous material that is high in iron content and indicative of chronic bleeding [269].

The aim of this study was to assess whether laboratory abnormalities of haemostasis are increased in women with endometriosis, which may in turn be implicated as part of the pathogenesis of endometriosis. In addition the correlation between symptom severity, stage of disease and a bleeding tendency is investigated.

4.2 Materials and methods

The study was conducted from July 2013 until July 2014 at the Royal Free Hospital, north London.

4.2.1 Study population

Case participants were identified through a local hospital database that provided diagnostic information regarding laparoscopic procedures. Women aged 18-55 years with a surgically confirmed diagnosis of endometriosis were invited to attend the Royal Free Hospital for an interview and laboratory investigations of haemostasis. Participants were excluded if they had a known IBD such as VWD, PFD, carriers of haemophilia or other rare factor deficiency. Age-matched female control subjects were staff members recruited from the Royal Free Hospital without a diagnosis of endometriosis, or symptoms suggestive of the condition. Control subjects were matched, as far as possible, in ethnicity, blood group, and smoking status. Participants were only included if they were willing to abstain from taking medication that interfered with platelet function for seven days prior to laboratory testing. In addition they were asked to avoid herbal preparations, caffeine, and excessive exercise on the day of testing. Participants found to have abnormal results were invited back for repeat testing and consultation with a specialist in haemostasis.

Participants meeting the inclusion criteria were asked to complete the Pain Impact Questionnaire (PIQ-6) (Appendix 2), a six question health survey designed to subjectively measure severity and impact of pain on an individual's functional health

and wellbeing [270]. Each participant completed a pictorial blood assessment chart (PBAC) to semi-objectively quantify menstrual blood loss [68]. The stage of endometriosis was recorded according to laparoscopic findings where documented. The revised American Society for Reproductive Medicine (rASRM) classification system was used to define disease severity (Fig 2.2) [271].

4.2.2 Laboratory methods

A sample of 30 mL venous blood was collected from each participant using a 19-gauge butterfly with minimal occlusion of the antecubital fossa vein into blood collection tubes containing 106 mol/L sodium citrate [Sarstedt monovettes, Sarstedt, Leicester, UK]. Samples were analysed within 30 minutes of collection. Please refer to Chapter 3.1 Coagulation tests and factor assays, and Chapter 3.2 Platelet light transmission aggregometry for laboratory methods used in this study.

4.2.3 Statistical analysis

Student's *t* test was used to assess difference in age, and mean haemostatic variables between cases and controls. Chi squared or Fisher's exact test was used to compare nominal demographic data, frequency of factor deficiencies, and abnormal platelet aggregation responses between cases and controls. Multiple logistic regression analysis was used to determine if there were any correlation between PIQ-6 score, PBAC score and haemostatic variables. Ordinal data (laparoscopic stages), PIQ-6 score and haemostatic variables were analysed by Spearman's Rho test. All statistical tests were carried out using SPPS version 22.0.

3.3 Results

A total of 84 women with a surgical diagnosis of endometriosis were recruited in the case group. Three women were excluded from the final analysis as they had a previously confirmed diagnosis of IBD. Thirty asymptomatic women without a diagnosis of endometriosis were recruited as controls. There was no significant difference in age, ethnicity, blood group O, or smoking status between cases and controls (Table 4.1).

The primary indication for undergoing laparoscopy in women with endometriosis was: 51/81 (63%) for dysmenorrhoea/pelvic pain, 10/81 (12%) for subfertility, 8/51 (10%) for ovarian cysts, and 6/81 (7%) for abnormal menstrual bleeding. Seven women (8.6%) had undergone hysterectomy for treatment of endometriosis. rASRM laparoscopic staging was available for 65 case participants and were distributed as follows: 18 (28%) women with stage I, 15 (23%) women with stage II, 14 (22%) women with stage III, and 18 (28%) with stage IV endometriosis.

Table 4.1 Baseline characteristics of cases and controls

Characteristic	Cases (n = 81) N (%)	Controls (n = 30) N (%)	P value
Median age (range)	39 (22-55)	35 (23-53)	0.174
Ethnicity			
White	57 (70)	22 (73)	0.742
Black	4 (5)	1 (7)	1.00*
Asian	20 (25)	7 (23)	0.716
Smoking status			
Current smoker	4 (5)	1 (3.3)	1.00*
Non smoker	77 (85)	29 (97)	1.00*
Blood group 'O'**	19/42 (45)	6/12 (50)	0.972

p value determined by Chi squared test for parametetric data (frequency > 5) or Fisher's exact test (*) for non-parametric data (frequency < 5). Missing data (**)

There were significantly more defects of platelet aggregation with one agonist among the women with endometriosis compared to the control group (31% versus 4%, p = 0.005). A significantly higher frequency of abnormal platelet aggregation response to epinephrine was detected in women with endometriosis compared to controls (25% versus 4%, p = 0.02) (Table 4.2). In addition, there were significantly more abnormal aggregation responses to multiple agonists (≥2) in women with endometriosis (15% versus 4%, $p < 0.05$). Three women (4%) in the endometriosis group were diagnosed with a PFD following retesting. Two women had reduced aggregation response to weak agonists ADP and epinephrine. Another woman had an inappropriate response to ristocetin at low concentration. One woman in the control group had abnormal aggregation to multiple agonists, which was normal on repeat testing.

Among the women with endometriosis, two had abnormalities of VWF consistent with mild VWD. One woman had both VWF:Ag and VWF:RCo level below 45 IU dL^{-1} and another had a VWF:RCo level below 45 IU dL^{-1}. The woman with low VWF:RCo was diagnosed with type 2 VWD, whilst the other woman had normal VWF on repeat testing. Among the control group, there was one woman with low VWF:Ag and VWF:RCo level, which was normal on repeat testing. Thus the frequency of VWF below the laboratory reference range did not differ significantly between cases and controls (p = 0.57) (Table 4.3).

Table 4.2 Frequency of platelet aggregation abnormalities in cases and controls

	Cases (*n* = 81) *n* (%)		Controls (*n* = 30) *n* (%)		*p* value
Platelet count Mean (± SD)	384 (102)		344 (81)		0.060
Agonist					
Adenosine diphosphate	12	(14.8)	1	(3.6)	0.179
Epinephrine	20	(24.7)	1	(3.6)	0.023
Collagen	3	(3.7)	1	(3.3)	1.000
Arachidonic acid	4	(4.9)	0	(0)	0.578
Ristocetin	3	(3.7)	0	(0)	0.497
U46619	0	(0)	0	(0)	
One agonist	25	(30.9)	1	(3.6)	0.005
Multiple agonists (2 or more)	12	(14.8)	1	(3.6)	0.047
Platelet function disorder	3	(3.7)	0	(0)	0.385

p value computed by Fisher's exact test. Areas highlighted in grey are statistically significant ($p < 0.05$)

Table 4.3 Abnormal haemostatic variables

	Reference range	Cases (n = 81)		Controls (n = 30)		*p* value
		***n* (%)**	**Level**	***n* (%)**	**Level**	
Factor XI	70-150 IU dL^{-1}	4 (4.9)	48, 61, 65, 67,	1 (3.3)	64	1.000
Factor XIII	70-175 IU dL^{-1}	5 (6.2)	59, 62, 63, 64, 66	3 (10)	64, 65, 67	0.680
VWF:Ag	45-145 IU dL^{-1}	1 (1.2)	44	1 (3.3)	41	0.566
VWF:RCo	45-145 IU dL^{-1}	2 (2.4)	38, 27	1 (3.3)	39	1.000

p value computed by Fisher's exact test

VWF:Ag, von willebrand factor antigen; VWF:RCo, von willebrand factor ristocetin cofactor activity level

Deficiencies in coagulation FXI or FXIII (below the laboratory reference range) were detected in both cases and controls (Table 4.3). Four women (5%) with endometriosis and one woman (3%) in the control group had FXI level below 70 dL^{-1}. These women were tested for the common FXI gene mutations and no abnormalities were detected. Similarly, five women (6%) with endometriosis and three women (10%) in the control group had FXIII below 70 IU dL^{-1}. The isolated deficiencies in either FXI or FXIII levels were between 48-67 IU dL^{-1} and were thought to represent >2 standard deviations from the mean in the general population. No significant differences in the frequency of abnormalities were detected between the groups (p = 1.00 and 0.68 for FXI and FXIII, respectively).

Overall no significant difference was detected in the mean haemostatic variables between women with endometriosis and controls (Table 4.4). The only haemostatic variable that approached clinical significance (p = 0.06) was difference in mean platelet count (384 x 10^9/L in cases versus 344 x 10^9/L in controls).

VWF:RCo level demonstrated a significant downward trend with increasing rASRM stage (r = -0.35, p = 0.01) (Fig 4.1). Five women out of 18 with severe endometriosis had a VWF:RCo level < 50 IU dL^{-1} (two women had VWF:RCo level below the laboratory reference range of 45 IU dL^{-1}). Patients with severe (stage IV) endometriosis (n = 18) had a significantly reduced mean VWF:RCo level compared to controls (60 IU dL^{-1} in severe cases versus 77 IU dL^{-1} in controls, p = 0.02). Patients with severe endometriosis had an increased platelet count compared to controls (429 x 10^9/L in severe cases versus 344 x 10^9/L in controls, p = 0.01) (Table 4.5).

Figure 4.1 Scatter plot demonstrating the distribution of von Willebrand Factor Ristocetin Cofactor activity level (VWF:RCo) according to revised American Society For Reproductive Medicine (rASRM) stages of endometriosis

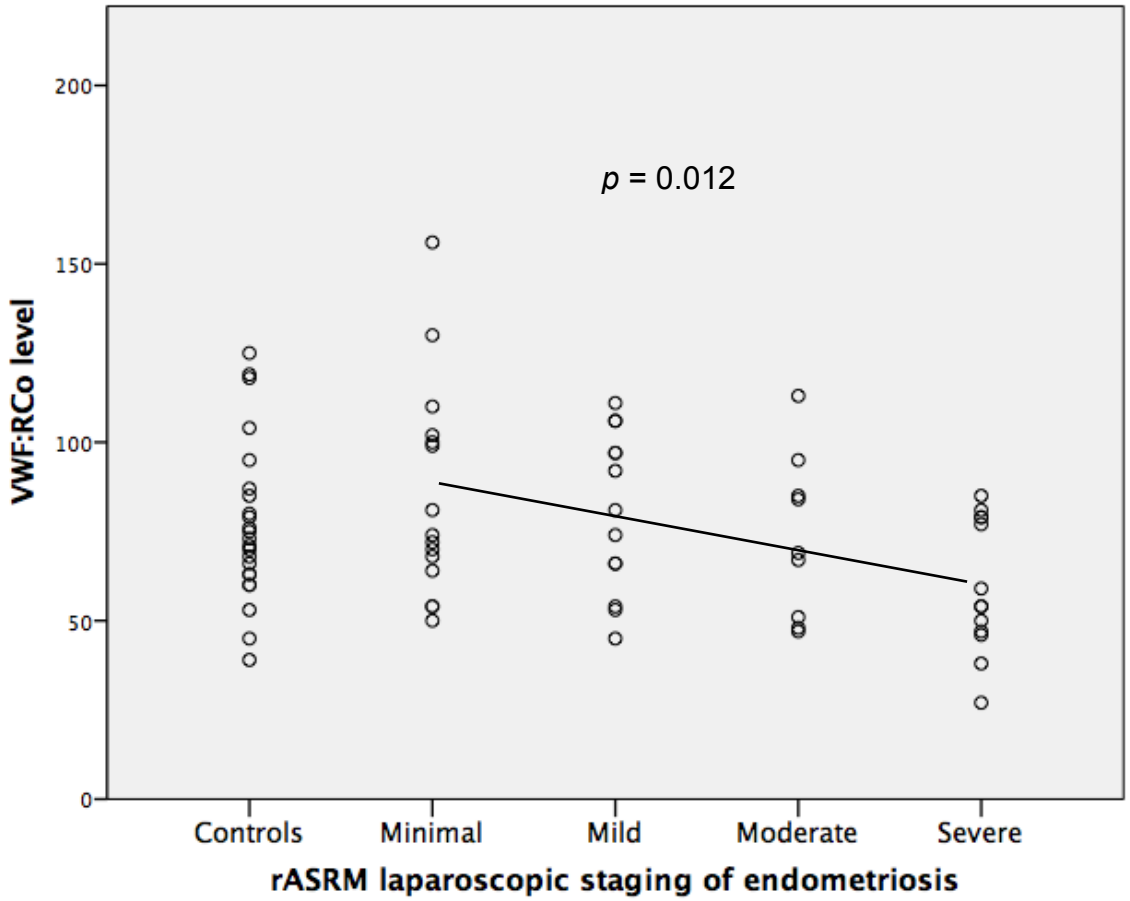


Table 4.4 Difference in haemostatic variables between cases and controls

	Reference range	Cases Mean (± SD) (n = 81)	Controls Mean (± SD) (n = 30)	*p* value
PT	9-13.5 secs	11.3 (0.9)	11.3 (0.8)	0.810
INR	0.9-1.2	0.9 (0.1)	1.0 (0.1)	0.362
APTT	28-36 secs	30.9 (3.0)	30.8 (3.0)	0.820
Fibrinogen	1.5-4.0 g L^{-1}	2.6 (0.6)	2.5 (0.5)	0.852
Platelet count	150-450x10^{9}/L	384 (102)	344 (81)	0.060
Factor VIII	50-150 IU dL^{-1}	98 (28.0)	97 (30.0)	0.887
Factor IX	50-150 IU dL^{-1}	104 (20.7)	104 (16.6)	0.924
Factor XI	70-150 IU dL^{-1}	91 (14.8)	88 (14.2)	0.563
Factor XIII	70-175 IU dL^{-1}	103 (30.1)	104 (24.3)	0.974
VWF:Ag	45-145 IU dL^{-1}	92 (34.3)	91 (32.6)	0.904
VWF:RCo	45-145 IU dL^{-1}	72 (25.5)	77 (23.1)	0.385

p value determined by student's t test

PT; prothrombin time, INR; internal normalised ratio, APTT; activated partial thromboplastin time, VWF:Ag, von willebrand factor antigen; VWF:RCo, von willebrand factor ristocetin cofactor activity level

Table 4.5 Difference in haemostatic variables in cases with severe (stage IV) endometriosis compared to controls

	Reference range	Severe cases Mean (± SD) (n = 18)	Controls Mean (± SD) (n = 30)	*p* value
PT	9-13.5 secs	11.1 (0.6)	11.3 (0.8)	0.285
INR	0.9-1.2	0.9 (0.1)	1.0 (0.1)	0.317
APTT	28-36 secs	31.2 (2.5)	30.8 (3.0)	0.987
Fibrinogen	1.5-4.0 g L^{-1}	2.6 (0.7)	2.5 (0.5)	0.797
Platelet count	150-450x10^9/L	429 (116)	344 (81)	0.005
Factor VIII	50-150 IU dL^{-1}	93 (25.0)	98 (29.7)	0.569
Factor IX	50-150 IU dL^{-1}	108 (21.8)	103 (16.4)	0.387
Factor XI	70-150 IU dL^{-1}	92 (15.5)	91 (16.2)	0.820
Factor XIII	70-175 IU dL^{-1}	112 (47.7)	104 (24.3)	0.535
VWF:Ag	45-145 IU dL^{-1}	90 (37.9)	88 (30.6)	0.773
VWF:RCo	45-145 IU dL^{-1}	60 (18.6)	77 (22.17)	0.024

p value determined by student's t test

PT; prothrombin time, INR; internal normalised ratio, APTT; activated partial thromboplastin time, VWF:Ag, von willebrand factor antigen; VWF:RCo, von willebrand factor ristocetin cofactor activity level

Women with endometriosis had significantly increased mean PBAC score compared to controls (319, SD ±366 in case group vs 147, SD ±166 in controls, $p = 0.024$). No statistically significant difference was detected between any haemostatic variable and the PBAC score. However, the mean PBAC score was significantly increased in women with platelet aggregation defects to one agonist (408, SD ±418, $p = 0.021$), and multiple agonists (489, SD ±589, $p = 0.015$) compared to the mean PBAC score of women without platelet aggregation defects (266, SD ±297).

Platelet count was the only haemostatic variable to demonstrate a weak positive correlation with PIQ-6 score in the logistic regression analysis ($r^2 = 0.031$, $p = 0.03$) (Table 4.6). No statistically significant difference was detected between any haemostatic variable and the PBAC score. Figure 4.2 shows distribution of PIQ-6 scores across the rASRM laparoscopic stages. No significant trend in PIQ-6 score was detected across the different stages ($p = 0.241$).

Figure 4.2 Scatter plot demonstrating the distribution of Pain Impact Questionnaire (PIQ-6) score according to revised American Society For Reproductive Medicine (rASRM) stages of endometriosis

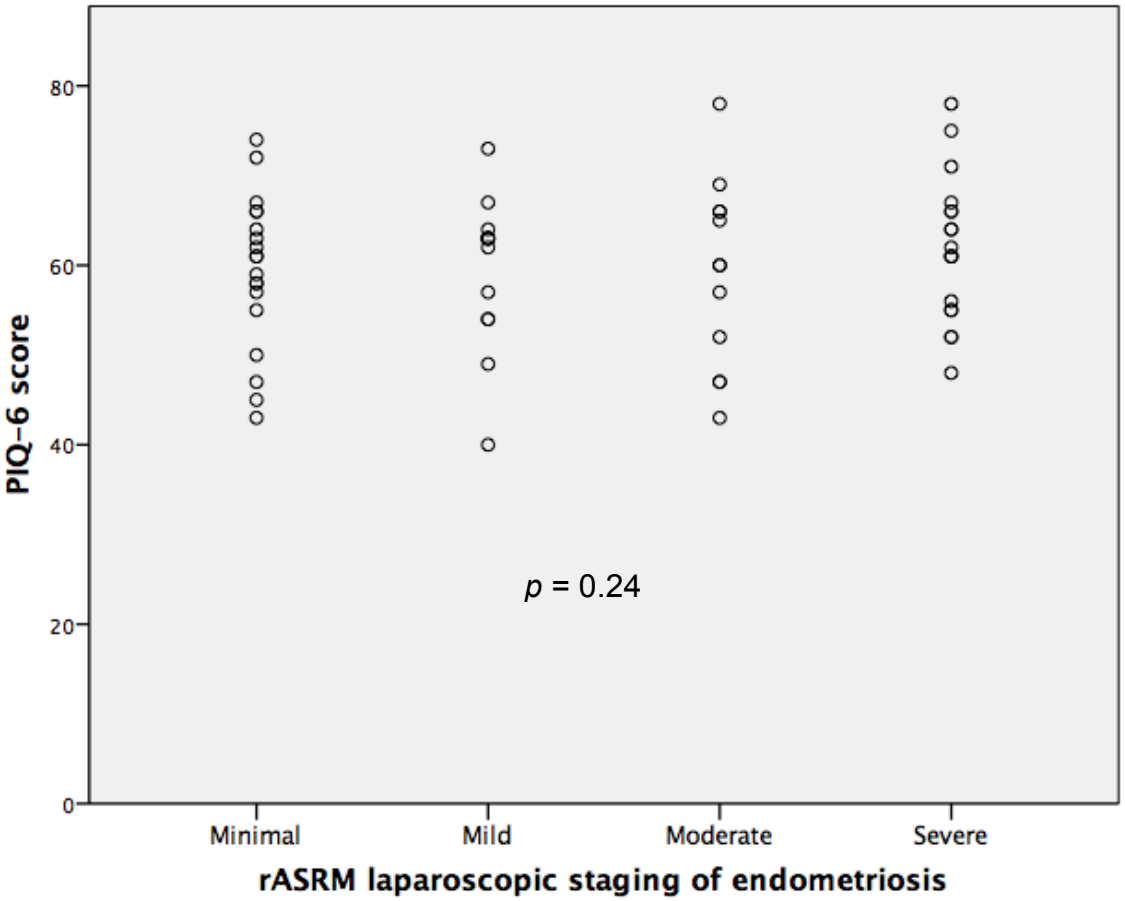


Table 4.6 Correlation between Pain Impact Questionnaire (PIQ-6) Score and haemostatic variables

	Correlation coefficient	***p* value**
Fibrinogen	0.069	0.696
Factor VIII	0.051	0.795
Factor IX	-0.219	0.315
Factor XI	-0.049	0.781
Factor XIII	0.086	0.583
VWF:Ag	0.083	0.611
VWF:RCo	-.162	0.423
Platelet count	0.351	0.033

p value determined by multiple regression analysis

VWF:Ag, von willebrand factor antigen; VWF:RCo, von willebrand factor ristocetin cofactor activity level

4.4 Discussion

In this study a significantly higher proportion of women with endometriosis had abnormal platelet aggregation response to one and multiple agonists compared to controls. Furthermore the women with platelet aggregation defects were more symptomatic with HMB and increased PBAC score ($p = 0.024$). A high frequency of HMB has been reported in women with PFDs and VWD (Table 2.5) [27, 50, 71, 80, 82, 83]. The increased frequency of abnormal platelet aggregation and low VWF:RCo seen in women with stage IV endometriosis indicate that primary haemostasis defects are associated with the condition.

These findings could have implications in the pathogenesis of endometriosis; firstly with increased retrograde menstruation, which may in turn increase the rate of endometriosis formation. Secondly, impaired local haemostasis within endometriotic implants may result in recurrent cyclical internal bleeding, exacerbating the spread of the condition throughout the pelvic cavity. Endometrial tissue that implants ectopically continues to be under hormonal regulation and undergoes monthly proliferation and shedding [268]. The pain results from localised internal bleeding within or around the endometrial deposits.

The downward trend of VWF activity level (VWF:RCo) with increased disease severity detected in this study indicates that endometriosis may be associated with a functional defect in primary haemostasis. Inflammation and chronic bleeding associated with severe endometriosis may result in a consumptive microvascular process that could explain reduction in VWF:RCo level. In addition, severe

endometriosis may be different from minimal/mild stages, and more likely to be associated with impaired local haemostasis. Alternatively, impaired systemic platelet aggregation may result in progression to more advanced disease. Future research should include VWF multimeric analysis in women found to have low VWF:RCo levels, which would help to determine the nature of the functional impairment.

Other studies have suggested that there might be an association between VWD and endometriosis. In a study that assessed the reproductive experience of women with VWD, endometriosis was reported in 30% of cases compared to 13% of controls (p = 0.01) [80]. However, the increased detection of endometriosis may be higher in women with VWD, who suffer from HMB, and therefore are more likely to consult with a gynaecologist.

An increased platelet count was seen in women with severe endometriosis and a positive correlation with platelet count and PIQ-6 score. An increased platelet count in women with severe endometriosis has been confirmed previously [272]. Thrombocytosis is a marker of chronic inflammation, and inflammation is strongly implicated in the pathogenesis of endometriosis. In addition, platelet count increases with chronic active bleeding and iron deficiency states [273]. There was no correlation with bleeding tendency and symptom severity. This may indicate that a bleeding tendency does not exacerbate endometriosis symptoms. However, the women were asked to objectively assess symptom severity over the past four weeks, and thus the PIQ-6 score may not have reflected the women's' symptoms when they were at their peak of severity (i.e. prior to laparoscopic ablation).

Traditional testing of platelet function such as bleeding time, light transmission aggregation (LTA), impedance aggregometry, and investigation of platelet activation by flow cytometry require a high degree of expertise to perform and interpret, and are limited to specialised haemostasis laboratories. In addition, LTA is limited by lack of reproducibility. The platelet aggregation defects reported in this study were found at initial testing. Only three women with endometriosis had abnormal platelet function when repeat testing was performed. Thus, future research should address this and any abnormalities found at initial testing should be reproducible.

More recently point-of-care testing dedicated to platelet function has become available using the platelet function analyser (PFA-100®, VerifyNow System, Multiplate Electrode Aggregometry). This devise employs simpler methodologies using whole blood without the necessity of sample processing, which is rapid, easier to use and more readily available in general laboratories [274]. The PFA-100® is also sensitive at detecting type 1 VWD [275]. In addition thromboelastometry platelet mapping (TEGPM), primarily used to detect platelet inhibition to antiplatelet medication, is a novel method of assessing platelet function that is far more convenient for large scale studies [276].

Routine testing for a disorder of primary haemostasis in women with endometriosis would be laborious and time consuming using traditional laboratory methods such as those utilised in this study. On the other hand, a diagnosis of a PFD would aid treatment decisions in such cases; women with a positive diagnosis should be advised to avoid non-steroidal anti-inflammatory medication, which further impairs platelet function and is commonly used to treat the pain of endometriosis. In addition, it

would be an important diagnosis to establish before undergoing surgical treatment. In a large prospective study that attempted to identify haemostatic disorders in 5649 patients prior to surgical intervention, the addition of screening with a standardised bleeding questionnaire and PFA-100® testing ensured the identification of impaired haemostasis in almost every case [277]. Those patients identified as having a bleeding disorder were treated with DDAVP to minimise the bleeding risk during surgery [277]. Thus, a more cost-effective approach to identify a haemostatic disorder in women with endometriosis could include screening with a concerted bleeding history or bleeding score prior to laboratory testing.

One of the limitations of this study is that the control population comprised of asymptomatic women who had never sought medical attention for symptoms of endometriosis (including subfertility). The prevalence of endometriosis in asymptomatic women ranges from 1-22%, depending on the diagnostic criteria and the population studied [278-281]. Thus, it is not feasible to fully exclude whether women in the control population had the condition. In addition, abnormalities in platelet aggregation detected with LTA do not always signify a bleeding disorder. An individual with suboptimal response to epinephrine only, and no bleeding history should not be considered as having a functional platelet abnormality with current clinical testing [282]. All abnormalities in platelet aggregation found on initial testing should be repeated with the addition of flow cytometry, nucleotide studies, and genetic testing if appropriate to establish or exclude a diagnosis of PFD. Further research is required to determine whether the finding of a high frequency of abnormal platelet aggregation in women with endometriosis is detected in a larger cohort,

ideally with control subjects including women who attend for laparoscopic sterilisation.

The impact of haemostatic treatment during menstruation should be investigated in women with endometriosis who are found to have a disorder of haemostasis. The effect of administering haemostatic therapy, in addition to hormonal treatment, to women with a primary haemostatic disorder and endometriosis should be assessed in a clinical trial. Antifibrinolytic agents (i.e. oral tranexamic acid) or DDAVP (i.e. intranasal desmopressin) can be administered prior to or during menses to assess the effect on endometriosis symptom severity and/or rate of endometriosis stage progression.

4.5 Conclusion

A significantly higher frequency of abnormal platelet aggregation response to one agonist ($p = 0.005$), multiple agonists ($p < 0.05$) and epinephrine ($p = 0.02$) was found in women with endometriosis compared to controls. Three women with endometriosis were diagnosed with a PFD. A reduced level of VWF:RCo was seen in women with severe (stage IV) endometriosis compared to controls, with a downward trend of VWF:RCo seen with increasing rARSM stage ($p = 0.02$). A higher platelet count was noted in women with endometriosis compared to controls, and was associated with increased symptom severity. A dysfunction in primary haemostasis may account for disease progression and thus contribute to the underlying pathogenesis of endometriosis. Selective screening of symptomatic women with a positive bleeding history could have important implications for the treatment of endometriosis. Women found to have a co-existing platelet aggregation abnormality should be managed accordingly, and advised to avoid antiplatelet medication.

CHAPTER 5

PRENATAL DIAGNOSIS AND MODE OF DELIVERY IN CARRIERS OF HAEMOPHILIA: A TEN-YEAR EXPERIENCE

5.1 Introduction

Haemophilia carriers face difficult choices in relation to prenatal diagnosis and management of delivery. Non-invasive cell free fetal DNA (ffDNA) in maternal plasma is now used to determine fetal gender in early pregnancy. This has the advantage over ultrasound scan (US), as gender can be determined more accurately in early pregnancy prior to invasive testing [151]. Using ffDNA to determine fetal gender in early pregnancy means that invasive testing in female fetuses can be avoided. A woman can then opt for chorionic villus sampling (CVS) or amniocentesis to determine if the male fetus is affected. This is usually offered to women who are carriers of severe or moderate haemophilia. It may also be indicated for other reasons (i.e. to rule out chromosomal abnormalities) and the haemophilia status of the fetus is determined opportunistically. Both CVS and amniocentesis are invasive procedures that are associated with an approximate 1% risk of pregnancy loss [137, 138].

Invasive prenatal diagnosis is offered to women with IBDs if they wish to opt for termination of an affected pregnancy. Prenatal diagnosis is also undertaken to determine whether the fetus is affected, to guide intrapartum management, and decision making regarding place and mode of delivery (MOD) to minimise the risk of fetal and neonatal haemorrhagic complications. If the result from invasive testing is negative, the woman can be referred back to her local hospital for obstetric management. On the other hand, if the fetus is affected with haemophilia, the mother is advised to deliver at the Royal Free Hospital; being a tertiary centre with access to expertise in the field of haemophilia. MOD is planned in advance of delivery, ensuring optimal circumstances to minimise cranial bleeding. In addition, delivery at

a tertiary centre ensures access to haemostatic laboratory facilities and factor concentrate if required, for the mother and the newborn.

Research is currently ongoing in the field of non-invasive prenatal diagnosis of haemophilia to determine the status of the fetus through analysis of ffDNA in maternal plasma. A previous pilot study at the centre has proven the feasibility of this approach [10]. The technique for this is undergoing further development with the aim of providing a universal diagnostic test for prenatal diagnosis of inversion 22 mutation, the most common mutation resulting in severe haemophilia A.

Preimplantation genetic diagnosis (PGD) is an early method of prenatal diagnosis that involves *in-vitro* fertilisation and selection of non-affected embryos to implant back into the uterus. This can be offered to couples with haemophilia who wish to avoid invasive testing and termination of affected pregnancy.

A previous study reported aspects of pregnancy care, including prenatal diagnosis, in carriers of haemophilia at the Royal Free Hospital over a 10-year period [1]. The aim of this study was to review prenatal diagnosis in carriers of haemophilia over the subsequent 10-years, and to determine if the introduction of non-invasive prenatal diagnosis had impacted on reproductive choice and management of delivery. In addition, the attitudes of carriers towards non-invasive prenatal diagnosis and mode of delivery were explored.

5.2 Methods

Carriers of haemophilia who attended the Royal Free Hospital for obstetric management from January 2006 until January 2016 were invited to participate in the study. A standardised questionnaire (Appendix 3) was sent by post to all eligible carriers over the age of 18. Carriers who attended the joint Obstetric Haemophilia clinic were consented to be contacted by telephone to complete the questionnaire. Written informed consent was obtained to review medical records, to obtain clinical data, including; age at the time of pregnancy, year of pregnancy, severity of haemophilia, baseline factor levels, invasive and non-invasive prenatal diagnosis, mode of delivery (MOD), estimated blood loss, and outcome of each pregnancy.

The questionnaire consisted of three parts. Part 1 included general information regarding the type and severity of haemophilia, relationship of carrier to index case, and family history of haemophilia-related complications. Part 2 included details of each pregnancy, including details of prenatal diagnostic testing, the reasons for and against opting for different types of prenatal diagnosis, and the outcome of that pregnancy. In part 3 the women were asked about their views on future developments in non-invasive prenatal diagnosis of haemophilia, and of obstetric management. This included their views regarding risk of intracranial haemorrhage and mode of delivery in affected pregnancies.

5.2.3 Statistical analysis

Descriptive statistics was used to demonstrate pregnancy outcomes and responses to the questionnaire. Chi-squared and Fisher's exact was used to detect whether any

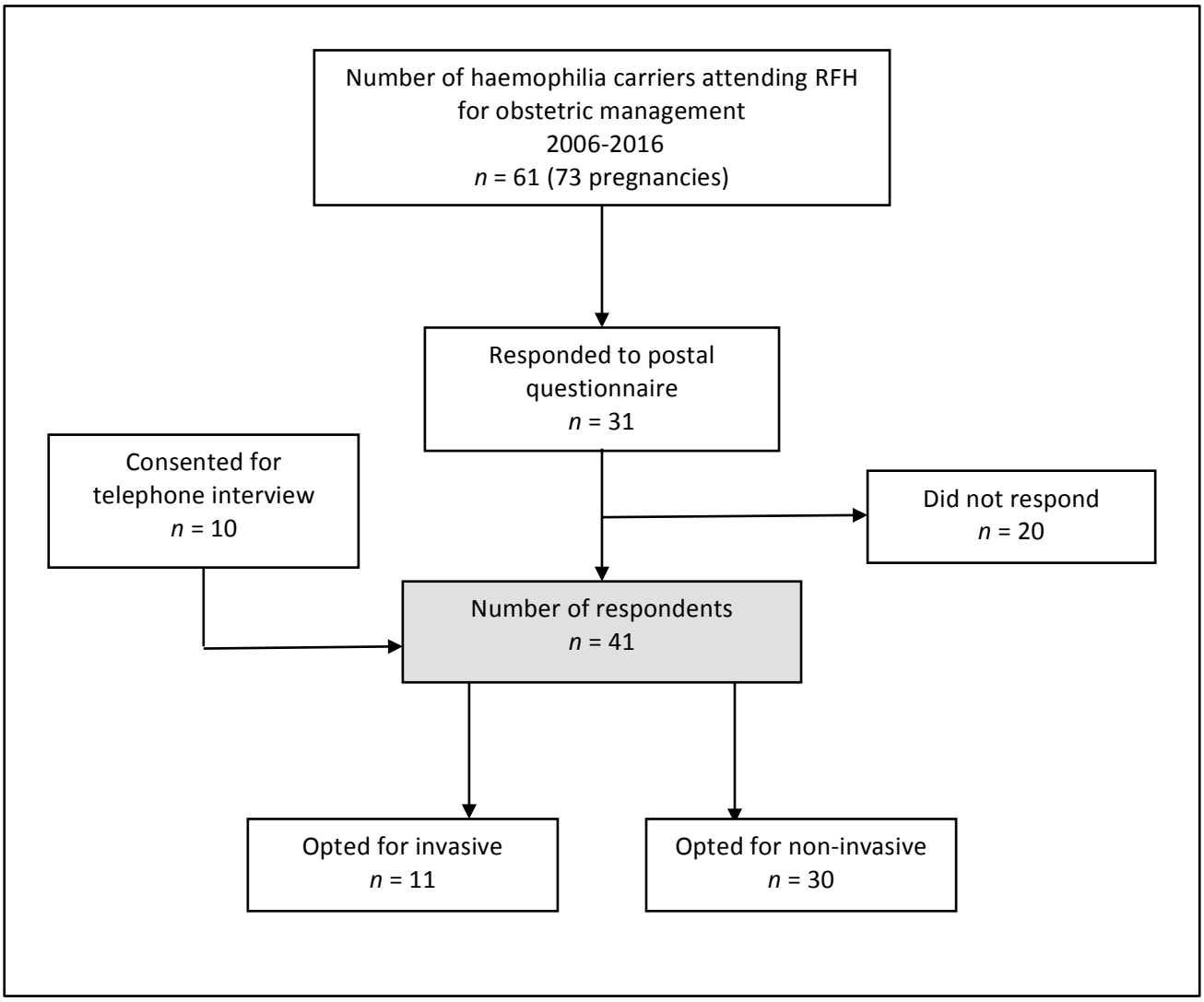
factors had a significant effect on the proportion of women who opted for invasive testing.

5.3 Results

Sixty-one carriers of haemophilia (44 haemophilia A and 17 haemophilia B) had obstetric care in 73 pregnancies at the Royal Free Hospital from January 2006 until January 2016 (Fig 5.1). Of the 73 pregnancies, 11 women received shared antenatal care between the Royal Free Hospital and their local maternity unit where they delivered. The median age at the time of pregnancy was 33 years (range 21-41). Eighteen pregnancies (25%) were in carriers of mild, twelve (16%) were in moderate, and forty-two (58%) were in severe haemophilia. The carriers' median baseline (non-pregnant) FVIII and FIX levels were 53 IU/dL (range 16-148 IU/dL), and 63 IU/dL (range 23-120 IU/dL), respectively.

Thirty-one out of 61 women responded to the postal questionnaire. A further ten questionnaires were completed by telephone interview following carriers' consent to be contacted by the haemophilia research team. Considering the nature of the questionnaire, the non-responders were not followed up with a telephone call without consent as this was felt to be too intrusive. The response rate was therefore 61% (41 out of 61) from those invited. Of the 41 women who responded to the questionnaire, 22 were carriers of severe haemophilia, seven were moderate, and twelve mild. Thirty were carriers of haemophilia A and eleven were carriers of haemophilia B.

Figure 5.1 Flowchart to show participants included in questionnaire study



RFH, Royal Free Hospital

5.3.1 Invasive prenatal diagnosis

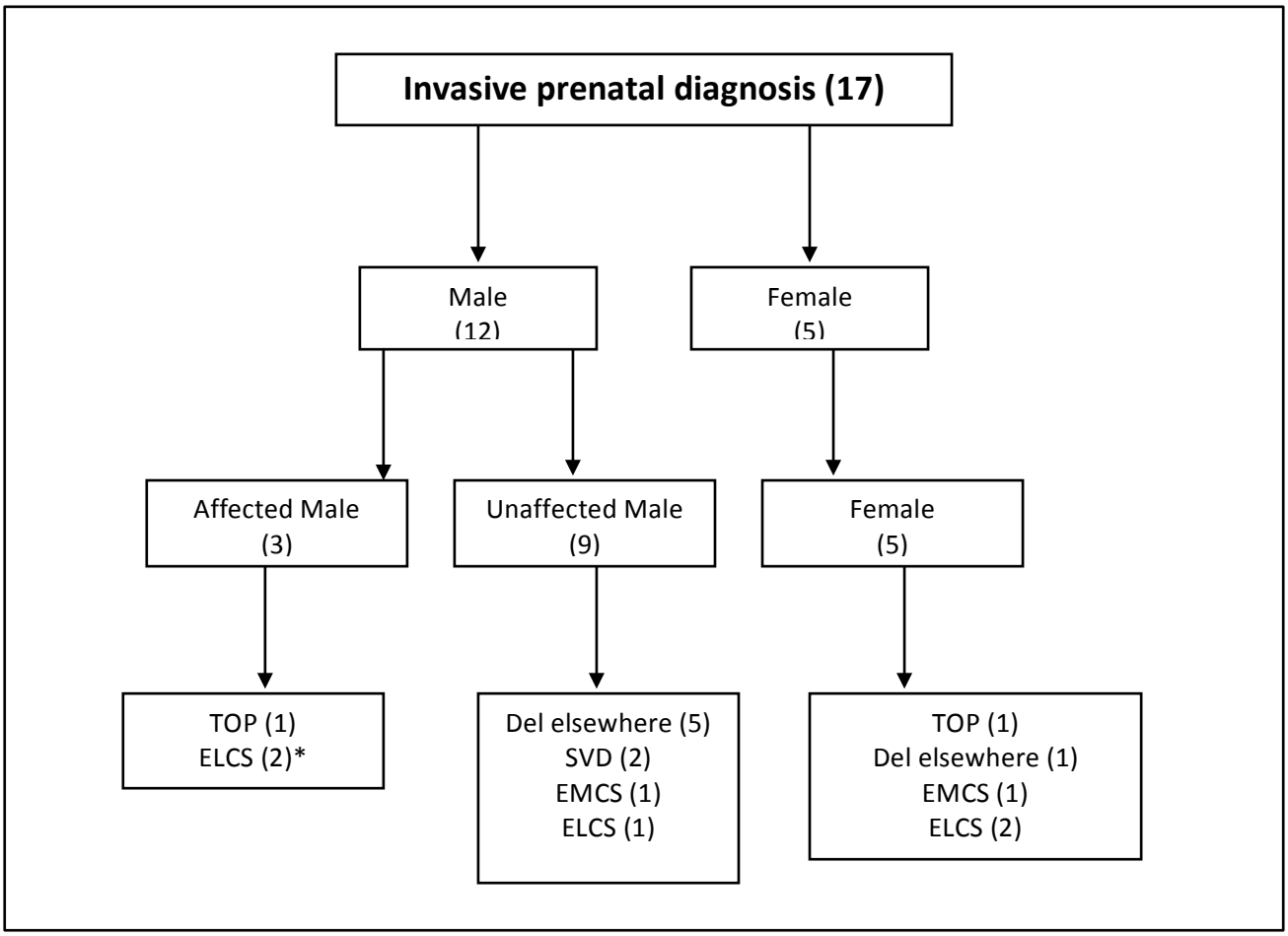
Invasive prenatal diagnosis was carried out in seventeen out of 73 pregnancies (23%), which was CVS in the majority (16 out of 17) of cases (Table 5.1). One patient underwent CVS due to a balanced translocation, which increased the risk for chromosomal abnormality. This was inconclusive and therefore she underwent amniocentesis, with successful karyotyping. In 15 out of 17 cases the woman was a carrier of severe haemophilia. In six pregnancies invasive testing was indicated for reasons other than haemophilia. One carrier of moderate haemophilia underwent CVS due to a previous pregnancy affected by trisomy 18. Another carrier of mild haemophilia underwent CVS due to a fetal anomaly diagnosed at the routine 11-13 week scan. One carrier of severe haemophilia underwent CVS in two pregnancies as she and her partner were both carriers of the cystic fibrosis gene mutation. CVS was undertaken to rule out chromosomal abnormalities following a high risk combined screening result in two carriers of severe haemophilia. Thus, the uptake for invasive prenatal diagnosis to rule out haemophilia solely was 15% (undertaken in 11 out of 73 pregnancies).

Early determination of fetal gender using ffDNA in maternal plasma, to confirm the presence or absence of Y chromosome specific sequences (Y-PCR), was introduced at the Royal Free Hospital as a research project in 2003 [151]. Following accurate confirmation of fetal gender using this method, it is now routinely offered to all pregnant carriers of haemophilia who book at the Royal Free. Non-invasive ffDNA Y-PCR was carried out prior to invasive testing from 8 to 12^{+6} week gestation in all cases. Repeat testing was required for an inconclusive result in three cases, when initial blood samples were obtained from 8-9^{+6} weeks gestation. Two repeat samples

obtained from 10-11^{+6} week gestation yielded a positive result. ffDNA Y-PCR was 100% accurate in all cases where a conclusive result was available. One case out of 17 (6%) was inconclusive following two samples, and confirmation was through CVS (Case No.7, Table 5.1).

Figure 5.2 shows the outcome following invasive prenatal diagnosis. Overall invasive testing was carried out in twelve male, and five female pregnancies. Invasive testing was undertaken in female pregnancies to rule out chromosomal abnormality or cystic fibrosis in four cases, and due to inconclusive ffDNA Y-PCR in one case (Table 5.1). In the twelve pregnancies where invasive prenatal diagnosis was performed on male fetuses, nine were unaffected, of which five women elected to deliver at a local maternity hospital. Three were affected and one woman elected to undergo termination of pregnancy (TOP). The two remaining affected male fetuses were delivered by elective caesarean section (ELCS) at the Royal Free Hospital. In the five pregnancies where invasive prenatal diagnosis was performed on female fetuses, one woman opted for TOP for fetal abnormality, one delivered elsewhere, and the remaining two were delivered by caesarean section for obstetric reasons at the Royal Free. Three carriers of haemophilia A, and one carrier of haemophilia B had FVIII/FIX levels of less than 50 IU/dL in the first trimester, and were administered factor concentrate or DDAVP for haemostatic prophylaxis prior to undergoing CVS. There were no complications associated with invasive prenatal testing (i.e. no bleeding/miscarriage) reported in the seventeen pregnancies.

Figure 5.2 Flowchart to show outcomes following invasive testing



TOP; termination of pregnancy, ELCS; elective caesarean section, EMCS; emergency caesarean section, SVD; spontaneous vaginal delivery

Table 5.1 Invasive prenatal diagnosis in pregnancies at risk of haemophilia

Case	Year	Non Invasive	Indication	Invasive (CVS)	Outcome
1	2006	ffDNA 10^{+2} Male	Carrier severe	46XY, unaffected	LB, unaffected male
2	2006	ffDNA 11^{+5} Male	Carrier severe	Amniocentesis 46XY unaffected	LB, unaffected male
3	2006	ffDNA 11^{+6} Female	Carrier moderate and fetal anomaly	46XX chromosomal abnormality	TOP
4	2006	ffDNA 13^{+0} Male	Carrier severe	46XY, unaffected	LB, unaffected male
5	2006	ffDNA 12^{+0} Male	Carrier severe	46XY, affected	LB, affected male
6	2007	ffDNA 12^{+2} Male	Carrier severe and high risk on combined screening	46XY, unaffected	LB, unaffected male
7	2008	ffDNA 8^{+3} and 10^{+0} inconclusive US 11^{+6} Male	Carrier severe	46XX	LB, female
8	2009	ffDNA 12^{+2} Male	Carrier severe	46XY, unaffected	LB, unaffected male
9	2009	ffDNA 12^{+0} Male	Carrier mild and previous trisomy 18	46XY, unaffected	LB, unaffected male
10	2010	ffDNA and US 11^{+6} Female	Carrier severe and cystic fibrosis	46XX, carrier of cystic fibrosis	LB, female
11	2011	ffDNA 9^{+1} Female	Carrier severe and high risk on combined screening	CVS inconclusive, Amniocentesis 46XX	LB, female
12	2012	ffDNA 9^{+6} Male	Carrier severe and cystic fibrosis	46XY, affected both haemophilia and cystic fibrosis	LB, affected male
13	2012	ffDNA 8^{+2} (repeated) 12^{+5} Female	Carrier severe and high risk on combined screening	46XX	LB, female
14	2012	ffDNA 11^{+2} Male	Carrier severe	46XY, unaffected	LB, unaffected male
15	2013	ffDNA 9^{+3} (repeated) 11^{+0} Male	Carrier severe	46XY, unaffected	LB, unaffected male
16	2015	ffDNA 10^{+4} Male	Carrier severe	46XY, unaffected	LB, unaffected male
17	2015	ffDNA 11^{+5} Male	Carrier severe	46XY, affected	TOP

CVS, chorionic villus sampling; ffDNA, free fetal DNA; LB, live birth; TOP, termination of pregnancy; US, ultrasound scan

Table 5.2 Factors affecting uptake of invasive prenatal diagnosis

	Invasive (%)	Non-invasive (%)	P value
Age ≥ 35 years	11 out of 17 (65)	19 out of 56 (34)	0.046
Year of pregnancy (pre 2012)	11 out of 17 (65)	16 out of 56 (28)	0.010
Severe haemophilia	15 out of 17 (88)	27 out of 56 (48)	0.004
Family history * (> 3 haemophilia complications)	9 out of 11 (81)	10 out of 30 (37)	0.012

* Data derived from questionnaire responses. *P* value determined by chi-squared or Fisher's exact test.

Age at the time of pregnancy was a significant factor with a higher proportion of women undergoing invasive prenatal testing if they were 35 years or older (Table 5.2). The year of pregnancy was another factor. The proportion of women undergoing CVS was significantly higher in the early half of the decade (2006-2011) compared to the latter half of the decade (2012-2016) ($p = 0.01$). The severity of haemophilia was a significant factor in determining uptake of invasive prenatal diagnosis ($p = 0.004$). A higher proportion of women who underwent invasive testing were carriers of severe haemophilia. A family history of haemophilia-related complications also impacted on rate of uptake ($p = 0.01$), with a higher proportion of women opting for invasive testing with a positive family history of three or more haemophilia-related complications (Table 5.2).

Eleven women who responded to the questionnaire opted for invasive testing in twelve pregnancies; ten were carriers of severe haemophilia, and one was a carrier of mild haemophilia. Five women responded that CVS had been indicated for reasons other than haemophilia.

The carriers were asked about their reasons for opting for invasive testing of haemophilia. Seven women who opted for this stated that they would have opted to undergo a TOP if the fetus was affected. Three women had wanted to know the haemophilia status to make a plan for delivery, and one woman did not state reasons for opting for invasive testing.

One woman stated:

> "I have had CVS in all four pregnancies, all very safe. I appreciated the early information from the blood test, not the 'non-invasiveness' of it. The real issue is taking the risk of having an affected child/termination."

Whilst another woman stated:

> "We would not terminate a baby with haemophilia, I only wanted to ensure a safe delivery."

Overall, third trimester amniocentesis was offered in thirteen out of 73 pregnancies (18%). This was usually offered in cases of severe haemophilia and was declined in all cases.

Carriers were asked in the questionnaire about whether they would consider amniocentesis in the third trimester of pregnancy, to avoid the risks associated with invasive testing in early pregnancy. Thirty-three out of 41 (80%) women who responded to the questionnaire stated they would not consider this as an option. Seven women (17%) said they were unsure, and one woman (2%) responded that she would consider this method of prenatal diagnosis in future pregnancies.

5.3.2 Non-invasive prenatal diagnosis

Non-invasive ffDNA was used to determine fetal gender in 56 pregnancies (77%). Figure 5.3 shows the outcome following non-invasive prenatal diagnosis. In four cases repeat testing was required to obtain a positive result. US only was used to determine the fetal gender in 15 cases. This was largely due to the fact that carrier

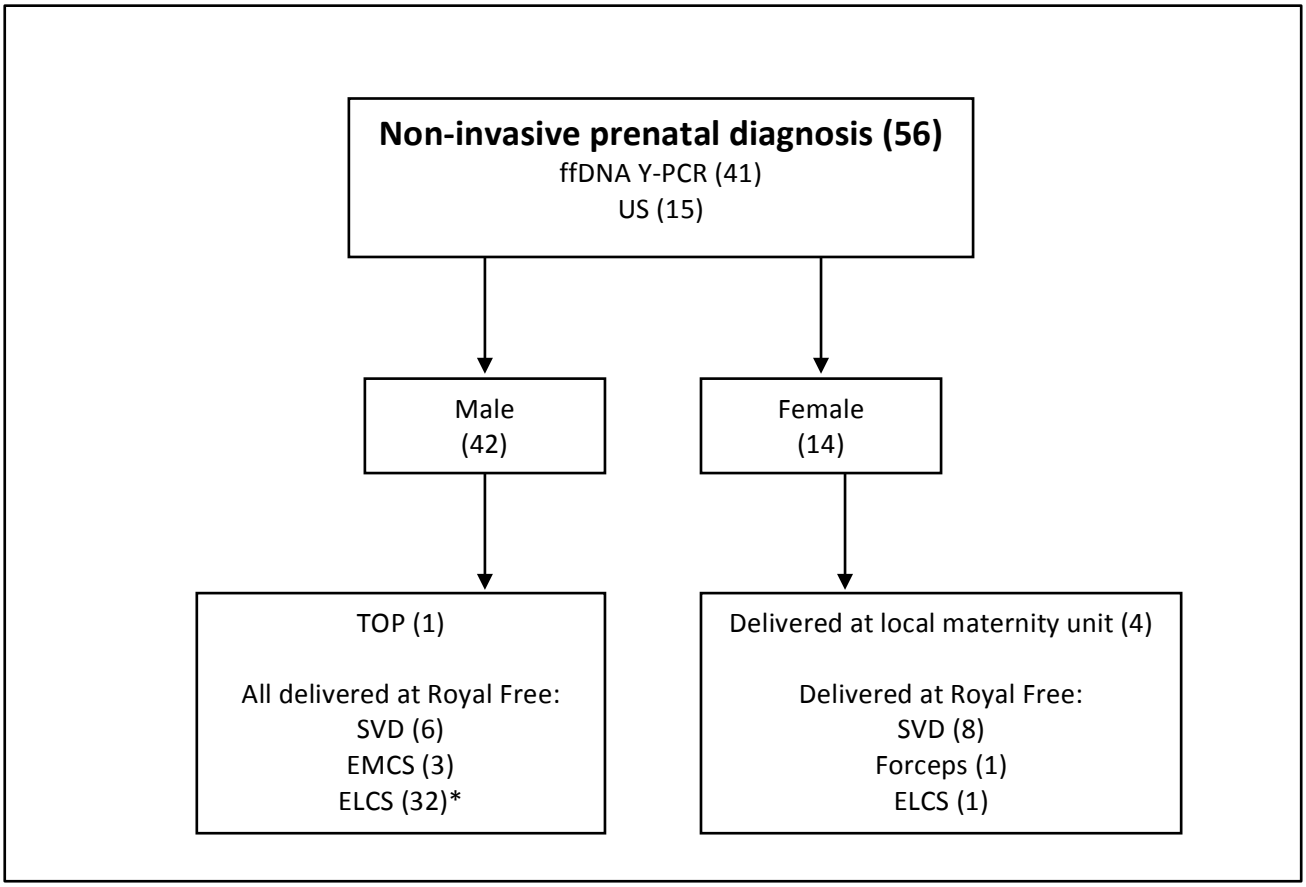
status was not confirmed until late in pregnancy, when the woman was referred to the Royal Free Hospital. The fetal gender had already been determined at the local hospital during the routine 18-23 week anomaly scan.

Women were also asked about reasons for *not* opting for invasive testing in the questionnaire. Eleven women out of 30 (37%) who opted for non-invasive testing stated 'fear of fetal loss from the procedure' as a reason not to undergo invasive testing. Eleven out of 30 (37%) felt that 'haemophilia was not a severe enough condition to warrant invasive testing', and 15 out of 30 (50%) women felt that 'they were prepared for the responsibility of having a child with haemophilia (i.e. accepting of the outcome either way)'.

One woman stated:

> "I only carry mild haemophilia and this would absolutely not put me off having children. Having the test (ffDNA) made me more comfortable and confident that I had the right people at hand to help with the delivery. I wish I could've been sure, as my son is not affected. I would have preferred not to have had a caesarean section, but I was happy to do so for his safety."

Figure 5.3 Flowchart to show outcomes following non-invasive testing



ffDNA Y-PCR; polymerase chain reaction detection of Y chromosome in free fetal DNA, EMCS; emergency caesarean section, ELCS; elective caesarean section, TOP; termination of pregnancy, SVD; spontaneous vaginal delivery, US; ultrasound scan

* In 7 out of 34 ELCS deliveries (total) there was another indication for ELCS (i.e. previous CS, breech presentation, previous myomectomy)

5.3.3 Impact of prenatal diagnosis on reproductive choice

Fourteen out of 41 (34%) respondents had made a conscious decision not to have children, or not to have any more children. Multiple reasons were given for this decision, however 9 out of 14 stated that this was due to fear of passing on haemophilia to their children/other children, 4 out of 14 stated that this was due to the stress of dealing with haemophilia, and 3 respondents (21%) stated this was due to the stress of undergoing invasive prenatal diagnosis in pregnancy.

When asked whether they would consider opting for a non-invasive blood test to diagnose the haemophilia status of the fetus during pregnancy, all 41 respondents (100%) stated that they would opt for this if it became available. However, when asked whether they thought having the availability of such a test would impact on their future reproductive choices, 12 out of 41 (29%) thought it would, 24 out of 41 (59%) thought it would not, and 5 out of 41 (12%) were unsure.

One woman stated:

> "Having a diagnosis through ffDNA would be far less stressful than having CVS. I always knew I wanted children, and my carrier status did not put me off this decision."

5.3.4 Prenatal diagnosis and mode of delivery

Of the 73 pregnancies managed initially at the Royal Free Hospital, three resulted in TOP; one was for severe haemophilia, one for a fetal abnormality and one for social

reasons. In the 70 ongoing pregnancies, 11 women delivered at their local maternity hospital. Fifty-nine deliveries (including 12 female and 46 live male births) were therefore managed at the Royal Free Hospital over the 10-year period.

Thirty-nine (66%) were ELCS fifteen (25%) were vaginal deliveries (VD), four (7%) were emergency caesarean (EMCS), and one (1.7%) was a forceps delivery. There was one case of a primary PPH (estimated blood loss 1000 mL following ELCS for previous myomectomy) among 59 deliveries, giving an incidence of 1.7%. The incidence of secondary PPH was not recorded.

A high proportion (41%) of the female and male pregnancies that were diagnosed as unaffected following invasive prenatal diagnosis were delivered locally. A higher proportion of live male births were managed at the Royal Free Hospital (79%). In addition, the majority of male babies were born by ELCS. The caesarean rate among carriers with male babies was 78% compared to 25% among carriers with female babies.

When asked about the estimated risk of intracranial haemorrhage (ICH) at delivery in babies with haemophilia; 5% considered the risk to be <1%, 60% of carriers considered the risk to be around 1-5%, 17.5% thought the risk was >10%, and 17.5% were unsure. When asked at what level of risk would they opt for ELCS, 27.5% responded that they would opt for a ELCS if the risk was <1%, 40% stated this to be around 1-5%, 15% responded if the risk was >10%, and 17.5% were unsure.

5.4 Discussion

In this study there was a high uptake of non-invasive prenatal diagnosis. Fetal sex determination was carried out using ffDNA in the first trimester in 58/73 (79%) pregnancies, or through routine second trimester US in 70/70 (100%) pregnancies. Invasive testing to rule out haemophilia was opted for in 11/73 (15%) of pregnancies. The rate of uptake for invasive testing to rule out haemophilia appears to be decreasing. In an early case series published in 1997 the rate of uptake for invasive testing was 17/48 (35%) in carriers of haemophilia managed at the Royal Free Hospital [2]. A follow up series published in 2008 showed the uptake to be 13/65 (20%) [1]. In addition, the proportion of women who opted for invasive testing was higher in the first part of the decade (Table 5.2). This downward trend in the uptake for invasive testing could be explained by the availability of an early non-invasive test to determine fetal gender using ffDNA, which was introduced at the Royal Free Hospital in 2003. Prior to this it was offered as part of a research project [151], and found to reduce the requirement for invasive testing in female pregnancies.

The rate of TOP for haemophilia was also lower in this series than reported in previous studies (1/73 [1.4%] compared to 5/90 [5.5%][1]). It is not easy to make any inference regarding the decreased TOP rate. From the questionnaire responses, severity of haemophilia, and family history of complications appear to impact on rate of uptake for invasive testing. This is similar to a Swedish study, where the rate of uptake of invasive testing (14% among 376 carriers) was influenced by family history, and a positive attitude towards TOP [283]. Although seven out of 11 women who responded to the questionnaire stated that they had opted for invasive testing to

undergo TOP if the fetus was affected, our data suggests that some women chose to continue with the pregnancy following a positive result from CVS. Improved haemophilia treatment and the prospect of gene therapy providing a cure may be important considerations, but the decision to continue with the affected pregnancy is clearly highly personal, and influenced by many complex factors. Attitudes towards PGD were not including in the survey. As rates of TOP for haemophilia are reducing, future research could address whether PGD would be considered more acceptable. However, currently its availability is limited in the UK, and it may be regarded as being too invasive for the woman, who has to undergo *in-vitro* fertilisation procedures.

Three out of 11 women (27%) had stated that they opted for invasive testing to make a plan for the delivery. Seven pregnancies where invasive testing demonstrated non-affected male and female pregnancies were subsequently referred back to their local maternity units. This highlights the advantage of having a definitive diagnosis about the haemophilia status of the fetus in advance of delivery.

Twenty seven per cent of carriers who responded to the questionnaire did not opt for invasive testing in pregnancy due to fear of fetal loss from the procedure. Many carriers (11/30) did not consider haemophilia to be sufficiently serious a condition to justify invasive testing. Three women stated that they had made a conscious decision not to have any more children due to the stress of undergoing prenatal testing. Having a safe, reliable non-invasive method of diagnosing haemophilia during the pregnancy would be highly welcome, and all carriers that completed our survey stated that they would opt for this if it became available in the future. Not only would this reduce the

psychological and emotional burden from invasive testing, its uptake would allow for more advanced planning of labour, and more options with regard to MOD. The majority of carriers (60%) correctly estimated the risk of ICH at birth in newborns with haemophilia to be around 1-5%, and 68% (28/41) would opt for ELCS if the risk was 5% or lower. Safely excluding a diagnosis of haemophilia in male fetuses will increase the birth options available to women. When the haemophilia status of the male fetus is unknown, the recommendation is to avoid any risk of cranial bleeding during labour and delivery by avoiding invasive monitoring, prolonged labour and pushing, as well as instrumental delivery. This leads to early resource to CS, possibly unnecessarily in those unaffected with haemophilia.

CS carries an inherent increased risk of bleeding for the carrier mother, and the risks are higher with multiple CS due to potential for abnormal placentation (placenta praevia/accrete) [284]. There is also increased risk of surgical complications such as cystotomy, bowel injury, ureteral injury, ileus and hysterectomy from multiple repeat CS. When prenatal diagnosis confirms the fetus to be unaffected with haemophilia, vaginal delivery without restrictions can be offered, thus avoiding unnecessary CS, and its potential risks for the mother and in future pregnancies. In addition, referral back to a local maternity unit closer to home can be planned, avoiding the inconvenience for the mother and her partner, of delivery far away from home.

Of note, the incidence of primary PPH was low (1.7%) among this cohort. The study from the previous decade reported a 19% incidence of primary PPH, although this was defined as an estimated blood loss >500 mL within 24h post delivery, regardless of MOD [1]. The case series from the preceding decade reported an incidence of 22%

[2]. The majority of women (66%) in this series underwent ELCS for a variety of indications, although the primary indication was for haemophilia (23/39 [59%] of deliveries). The estimated blood loss ranged from 300-1400 mL among women who delivered by ELCS, with a median of 500 mL. There was only one PPH with an estimated blood loss of 1400 mL in a woman with additional obstetric risk factors. This data supports the argument that ELCS with appropriate multidisciplinary input and careful consideration of the bleeding risk does not appear to be associated with excessive bleeding in carriers of haemophilia. All the women had a plan for labour and delivery in advance when they attended the Joint clinic at 30-32 weeks gestation. Bleeding risk was assessed and prophylaxis arranged with tranexamic acid, DDAVP or factor concentrate if appropriate. Active management of the third stage was implemented using uterotonic agents (intravenous syntocinon and per rectal misoprostol) for all women. Furthermore, women were instructed to take tranexamic acid until their lochial loss was minimal. Management guidelines were readily available and updated regularly for management of these patients. A copy of the management plan was given to the mother, and a copy was saved on her electronic records, which was circulated to the necessary care providers on the labour ward. Twenty-four hour contact with the team running the Joint clinic was made available. Thus, the incidence of PPH in this cohort (1.7%) was lower than the 3-6% incidence reported in the general population [197, 199].

This study has limitations. The sample size was small and a large proportion of respondents to the questionnaire were women who had delivered recently, and had undergone invasive testing. There may have been characteristics about respondents,

which resulted in bias, and their answers may not be representative of all haemophilia carriers who delivered at the Royal Free Hospital over the past decade.

5.5 Conclusion

The uptake for invasive prenatal diagnosis among haemophilia carriers is now low, and affected by factors such as severity of haemophilia, age, year of pregnancy and family history of haemophilia-related complications. These should be considered when counselling carriers, and each case should be managed individually. The rate of TOP of affected pregnancies is reduced compared to that reported in previous case series. This is may be due to the improved long-term management, and availability of treatment for patients with haemophilia.

Non-invasive prenatal diagnosis was able to confirm fetal gender in most cases. Introducing this method means that invasive testing for haemophilia can be avoided in in female fetuses. Thirty-seven per cent of carriers wished to avoid invasive testing due to fear of fetal loss from the procedure. The majority of carriers who do undergo invasive testing do not terminate the pregnancy, but opt for prenatal diagnosis to guide management decisions of labour and delivery. The majority of carriers (67.5%) stated that they would opt for ELCS if the risk of ICH at birth was ≤ 5%. A non-invasive test to confirm the haemophilia status of the fetus is of exceptional importance and required urgently. This will help to reduce the emotional impact and reduce fear associated with invasive testing.

CHAPTER 6

THE INCIDENCE OF CRANIAL BLEEDING IN NEWBORNS WITH INHERITED BLEEDING DISORDERS

6.1 Introduction

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6.1.2 Screening for intracranial haemorrhage

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Meta-analysis

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6.2.5 Conclusion

6.3 MRI screening for cranial bleeding at birth in newborns with inherited bleeding disorders

6.3.1 Aim

6.3.2 Methods

6.3.3 Results

6.3.4 Discussion

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6.1 Introduction

Cranial bleeding can have devastating consequences for newborns with IBDs. Cranial bleeding can occur during labour and delivery due to overlapping or moulding of the infant's cranial bones as it passes through the birth canal. Instrumentation causes further moulding and the sheering forces results in mechanical damage to the underlying blood vessels and dural sinuses. Intracranial haemorrhage (ICH), defined as an accumulation of blood within the cranial vault, can result in serious long-term neurological sequelae or death [285, 286]. Extracranial haemorrhage (ECH), defined as haemorrhage or haematoma occurring outside the cranial vault, can be rapid and life threatening due to persistent bleeding into the subgaleal space [287].

In the 1960s ICH accounted for over one-third of all deaths in patients with haemophilia [288]. Despite advances in treatment, mortality rates of over 20% are still reported in recent publications [289]. The risk of ICH and consequential long-term neurological sequalae is dependent on age, severity of the condition and inhibitor development. In 1978, Eyster *et al* reported a case series in an estimated population of 2500 haemophilia patients and found that 51 out of the 71 (72%) cases of ICH occurred in patients younger than 18 years of age [290]. Recent studies suggest that 15-33% of newborns present with initial bleeding manifestations in the neonatal period [252] and 41% of these early manifestations involve cranial bleeding [291]. A national survey conducted in the French haemophilia population demonstrated that the highest lifetime frequency of ICH occurred during the neonatal period (Fig 5.1) [289].

Figure 6.1 Age at the time of intracranial haemorrhage (ICH) in haemophilia patients. Main histogram: distribution of ICH by 2-year-age brackets; small histogram: distribution of ICH for children ≤ 2 years by 1-month-age brackets (reproduced from Stieltjes et al, 2005)

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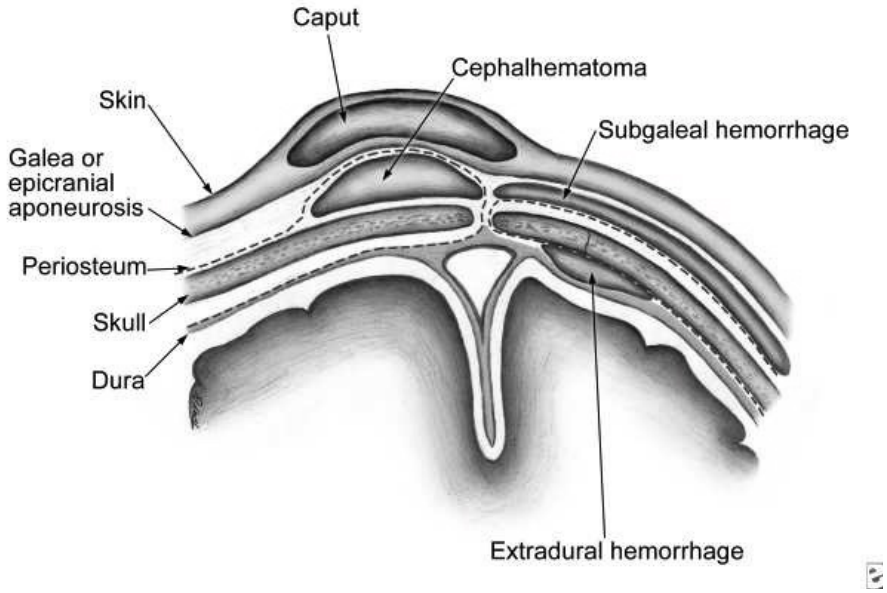
Although haemophilia A and B are the most prevalent IBD, other rare congenital bleeding conditions have also been associated with ICH in the newborn. These include severe cases of VWD, congenital afibrinogenaemia, and deficiencies of coagulation factors including factors II, V, VII, X and XIII (see Table 6.6) [292-295]. Fetal neonatal alloimmune thrombocytopaenia (FNAIT) results in severe bleeding, often recognised in the antenatal period. Severe fetal thrombocytopaenia develops due to maternally derived allo-reactive antibodies attacking human platelet antigens (HPA) inherited from the father. This condition is not classified as an inherited bleeding disorder and therefore not included in this thesis.

6.1.1 Classification of cranial bleeding

ECH is defined as haemorrhage or haematoma that occurs outside the cranial vault. They include cephalohaematoma and subgaleal haemorrhage (SGH). Cephalohaematoma arises from haemorrhage of the periosteum. This type of haemorrhage does not cross suture lines between the cranial bones, which classically differentiate cephalohaematoma from SGH (Fig 6.2). They occur in approximately 1% of live births and are more common with instrumental deliveries. They may increase in size slightly following delivery and take several weeks to resolve, usually with no long-term clinical sequelae [296].

Figure 6.2 Anatomical representation of extracranial haemorrhage in the newborn



SGH results from bleeding into the potential space inferior to the epicranium aponeurosis and the periosteum of the cranium. These collections usually increase in size during the first few days after delivery. They are more frequently encountered following difficult ventouse delivery [297]. Boo *et al* assessed obstetric factors that increased the likelihood of SGH associated with ventouse delivery [298]. Five factors where identified which included; failed ventouse extraction (adjusted odds ratio [OR] 16.4; 95% confidence interval [95%CI], 2.0-135.6), leading edge of vacuum cup < 3 cm away from the anterior fontanelle (suggestive of deflexion application) (adjusted OR 6.0; 95%CI, 1.7-21.0), cup marks on the sagittal suture (implicating a paramedian application) (adjusted OR 4.4; 95%CI, 1.7-21.0) and maternal nulliparity (adjusted OR 4.0; 95%CI, 1.6-10.0). Blood may tract down into the cervical regions below the attachments of the occipto-frontalis muscles and consequently blood loss can be massive. Neonates with coagulation disorders can develop hypovolaemic shock and exsanguinate due to SGH [297, 299].

ICH is defined as an accumulation of blood within the cranial vault. It is classified according to anatomical compartments and has six major types: subdural haematoma (SDH), subarachnoid haemorrhage (SAH), intraventricular haemorrhage (IVH), cerebellar haemorrhage, parenchymal (intracerebral) haemorrhage and epidural haemorrhage.

SDH and SAH are the most common types of ICH encountered in the term newborn and are classically associated with traumatic delivery. Improvements in obstetric care, and a global trend towards elective caesarean deliveries for fetuses presenting in the breech position at term, have resulted in an overall reduction in these haemorrhages in healthy term newborns [300]. SDH arises from excessive vertical molding and fronto-occipital elongation causing stretching of the dura mater, the falx and tentorium during prolonged and difficult labour. Haemorrhage into the subdural space may also occur due to tearing of bridging veins from the cortex to the superior sagittal sinus or from superficial cortical veins without tearing of the dura [296]. SDH are usually infratentorial (located inferiorly to the tentorium cerebelli), but can occur in a supratentorial location in association with more extensive bleeding and midline shift. SAH are typically clinically silent and benign, thus the exact incidence is unknown. Bleeding occurs from anastomoses between the penetrating leptomeningeal arteries or the bridging veins. More extensive SAH may be difficult to distinguish from SDH and the two may co-exist.

IVH are classically associated with preterm delivery. IVH arises from the germinal matrix, chorid plexus, or parenchyma. The germinal matix area is formed of primitive

neuroblast and glioblast cells surrounded by immature, fragile blood vessels. Fluctuations in cerebral blood pressure due to poorly regulated immature vasculature in the preterm infant cause rupture of the germinal matrix capillary bed [301]. By term gestation these vessels undergo rapid maturation with a greater continuity of the basement membrane to enhance vascular support. Thus, the incidence of germinal matrix IVH in term infants is low, and if encountered is attributed to trauma or severe asphyxia. Term infants with germinal matrix IVH are often asymptomatic and the prognosis is good. Long-term neurological sequelae are seen in infants with IVH and parenchymal involvement.

Cerebellar haemorrhages are associated with preterm delivery and have been reported in 20% of prospectively imaged preterm infants [302]. In the term infant cerebellar haemorrhage may be primary or related to venous infarction, or may complicate extensive IVH or SAH. The vermis is reported to be the initial site of haemorrhage in the majority of term infants. Lacerations of the cerebellum may result from traumatic vaginal breech deliveries [296]. Mortality from cerebellar haemorrhage is high in preterm infants. In term infants the mortality is lower, however in survivors there is a high incidence of residual motor and intellectual impairment with prominent cerebellar signs [303].

Parenchymal haemorrhages are rare in the full-term infant. The proposed aetiologies include asyphyxia, hypoxia, haemorrhagic infarction and birth trauma [304]. In addition they have been reported in association with structural anomalies such as arteriovenous malformation, aneurysm and cavernous malformation. Outcome varies

widely, but can be surprisingly good, even in patients with sizeable parenchymal haemorrhages.

In contrast to adults, newborns rarely develop epidural haemorrhage because the usual mechanism is related to skull fracture and tearing of the middle meningeal artery in its groove within the temporal bone. However, in newborns the process of moulding reduces skull fractures and the middle meningeal artery, which is not yet encased within the bone, moves freely away from displacement of the skull [305]. Skull fracture with epidural haemorrhage may result from a difficult forceps delivery, or pressure from the maternal symphysis pubis, sacral promontory, or ischial spines.

6.1.2 Screening for intracranial haemorrhage in neonate with IBD

As approximately one third of all cases of haemophilia arise from *de novo* mutations, many affected infants are born to women who are unaware that they are carriers of the condition. Therefore overt bleeding signs and symptoms such as ECH, persistent bleeding following invasive procedures, circumcision or a heel prick test may indicate the presence of an underlying IBD and prompt haematological investigation. Prolonged oozing from the umbilical stump is characteristic of a defect in fibrinogen production or function and FXIII deficiency.

ICH in the full term infant can present with diverse and often non-specific neurological symptoms (Fig 6.3). It is the second leading cause of fever (after infection), and the second leading cause of seizures (after asphyxia). It may produce ‘late jaundice’, peaking late in the first week of life, irritability, apathy, high-pitched

crying or abnormal muscle tone [306]. Once focal neurological signs have developed the bleed is often extensive therefore a high index of suspicion is required to prompt radiological investigations of non-specific symptoms.

Figure 6.3 Possible clinical signs of intracranial haemorrhage in newborns

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† Hypotension, tense fontanelle, pupillary/ocular changes, and apnoea.

* The most frequent *first* documented symptoms among children aged <2 years; often nonspecific.

To date there is a lack of consensus on whether to routinely screen for ICH in newborns diagnosed with IBD. In addition, the optimum imaging modality and timing is unknown. Cranial ultrasound scan (US) is often used as a first line investigation due to accessibility, low-cost, and absence of exposure to radiation. However, cranial US lacks sensitivity for detecting haemorrhages in the posterior fossa of the brain. Cerebellar haemorrhages can remain undetected using cranial US through the anterior fontanelle and result in cerebellar atrophy later in life [307]. The paediatric working party of the UK Haemophilia Centre Doctor's Organisation (UKHCDO) carried out a survey in 2002 to ascertain current practice with regard to management of neonates with IBD. The routine use of cranial US in all newborns with severe haemophilia was reported by 17/42 (41%) of responders. A further 16/42 (38%) would scan in specific circumstances where trauma at delivery may have been excessive. Nine out of 42 (21%) would only consider cranial US in the presence of clinical signs suggestive of ICH [308].

Cranial computerised tomography (CT) scanning is widely utilised in the emergency paediatric setting and has increased accuracy of diagnosis of ICH in the posterior fossa. However as CT exposes the particularly susceptible neonatal brain to ionising radiation it is not an acceptable modality for screening purposes. Magnetic resonance imaging (MRI) is non-invasive and non-ionising and produces excellent soft tissue differentiation, making it the modality of choice for screening for haemorrhages in the neonatal brain [309].

MRI appearances of haemorrhage vary over time and thus can be used to evaluate the age of a lesion. This is due to the different susceptibilities of oxyhaemoglobin,

deoxyhaemoglobin, and haemosiderin, which result in different signals on various pulse sequences. When haemorrhage is acute the signal is usually deeply hypointense with respect to brain tissue on T2-weighted images and intermediate on T1-weighted images; subacute haemorrhage is hyperintense on both T1- and T2-weighted images; and chronic haemorrhage becomes deeply hypointense on both T1- and T2-weighted images (Table 6.1) [310]. However, impaired coagulation may result in this sequence of changes in signal intensity to be disrupted due to aberrations in clot formation and clot dissolution, and therefore breakdown of haemoglobin. There is limited data on normal MR images of the neonatal brain in patients with impaired coagulation; if ICH is clinically suspected in a newborn with haemophilia or severe IBD, cranial CT is usually performed instead of MRI, as it is more rapidly available.

Table 6.1 MRI appearance of intracerebral haemorrhage (reproduced from Augilar & Brott, 2011 [311])

Stage	Age	Haemoglobin	T1-weighted	T2-weighted
Hyperacute	<24 h	Intracellular oxyhaemoglobin	Isointense	Slightly hyperintense
Acute	1-3 d	Intracellular deoxyhaemoglobin	Slightly hypointense	Very hypointense
Early subacute	>3 d	Intracellular methemoglobin	Very hyperintense	Very hypointense
Late subacute	>7 d	Extracellular methemoglobin	Very hyperintense	Very hyperintense
Chronic centre	>14 d	Extracellular hemichromes	Isointense	Slightly hyperintense
Chronic rim	>14 d	Intracellular hemosiderin	Slightly hypointense	Very hypointense

H, hours; d, days;

6.2 Mode of delivery and cranial bleeding in newborns with haemophilia: a systematic review and meta-analysis

6.2.1 Aim

The purpose of this systematic review was to compare overall incidence of both ICH and ECH in newborns with haemophilia and in the general population. A meta-analysis was performed to assess the impact of MOD on the incidence of ICH. The long-term outcome following ICH in both populations was reviewed.

6.2.2 Method

Methodology for this systematic review and meta-analysis were developed according to recommendations from Preferred Reporting Items for Systematic Reviews and Meta Analysis of Observational Studies in Epidemiology [312] statements.

Studies were included in the systematic review if they assessed incidence of cranial bleeding at birth, within a defined newborn period, and a denominator 'at risk' population (i.e. the total number of babies born with haemophilia in the referral region or centre or the total newborns delivered within a maternity unit, during a determined period of time). The incidence of ICH and ECH at birth was determined from retrospective review of hospital discharge notes (ICD-9 coding), parental interview or questionnaires completed by haemophilia treatment centres. Cases were excluded when cranial bleeding was iatrogenic, not related to the birth process, or the result of extreme prematurity (< 28 weeks gestation).

The studies included in the meta-analysis report MOD for each participant. Studies that reported the incidence of cranial bleeding, without sufficient information about MOD of newborns with ICH or the denominator population were excluded. In order to make comparison between MOD in babies with haemophilia, the delivery mode was recorded as either assisted vaginal delivery (AVD), which included both forceps and vacuum assisted deliveries, and caesarean section (CS), which included both emergency and elective CS. The MOD requires this grouping because not all studies define the type of instrumentation used (forceps or vacuum). In addition the studies report only 'caesarean delivery' without distinguishing between CS performed during labour or ELCS. The effect measure used for the meta-analysis was odds ratio (OR) of experiencing ICH. The odds of experiencing ICH at birth were evaluated in participants born by AVD and CS, compared to those born by SVD. The heterogenicity across studies was assessed using Cochrane's Q test and I^2 statistic and studies were assigned appropriate weight based on a fixed-effect model. A rare-events model utilising the Peto method was necessary to compare OR in babies born by CS compared to SVD [313].

All studies were observational cohort studies or prospective imaging studies. There were no randomised trials reporting the incidence of cranial bleeding in newborns either in the general population or in newborns with haemophilia.

Search strategy

A review of EMBASE (1980- October 2014) and MEDLINE (1950- October 2014) using the key search terms 'cranial bleeding', 'head bleeding', intracranial,

extracranial, intracerebral, ‘subgaleal hematoma’ OR cephalohematoma, cephalohaematoma, AND haemorrhag*, hemorrhag*, bleeding, AND newborn, neonat*, ‘at birth’, AND haemophilia, haemophilia, ‘bleeding disorder’ OR ‘coagulation disorder’ revealed the relevant studies. In addition to searching electronic databases the reference lists of review articles were scanned for relevant studies. Two investigators independently assessed titles and abstracts to identify studies that fitted the inclusion criteria.

6.2.3 Results

Figure 6.4 shows the flow chart for identifying eligible studies for the systematic review and meta-analysis. Seventeen studies report the incidence of cranial bleeding at birth in both newborns with haemophilia and in the general population. The characteristics of the 13 retrospective studies that assess incidence of ‘symptomatic’ cranial bleeding at birth are given in Table 6.2. The four prospective studies that report ‘asymptomatic’ cranial bleeding are outlined in Table 6.3. An overall incidence was calculated for ICH and ECH in both populations.

Only four studies were eligible to evaluate the extent of which MOD impacts on incidence of ICH. One study was available in the general population, which gave sufficient information regarding the MOD in each participant [251]. Three studies assessed the incidence of ICH by MOD in newborns with haemophilia [313-315] (Table 6.4).

Figure 6.4 Flowchart for identifying eligible articles in newborns in the general population (A) and newborns with haemophilia (B)

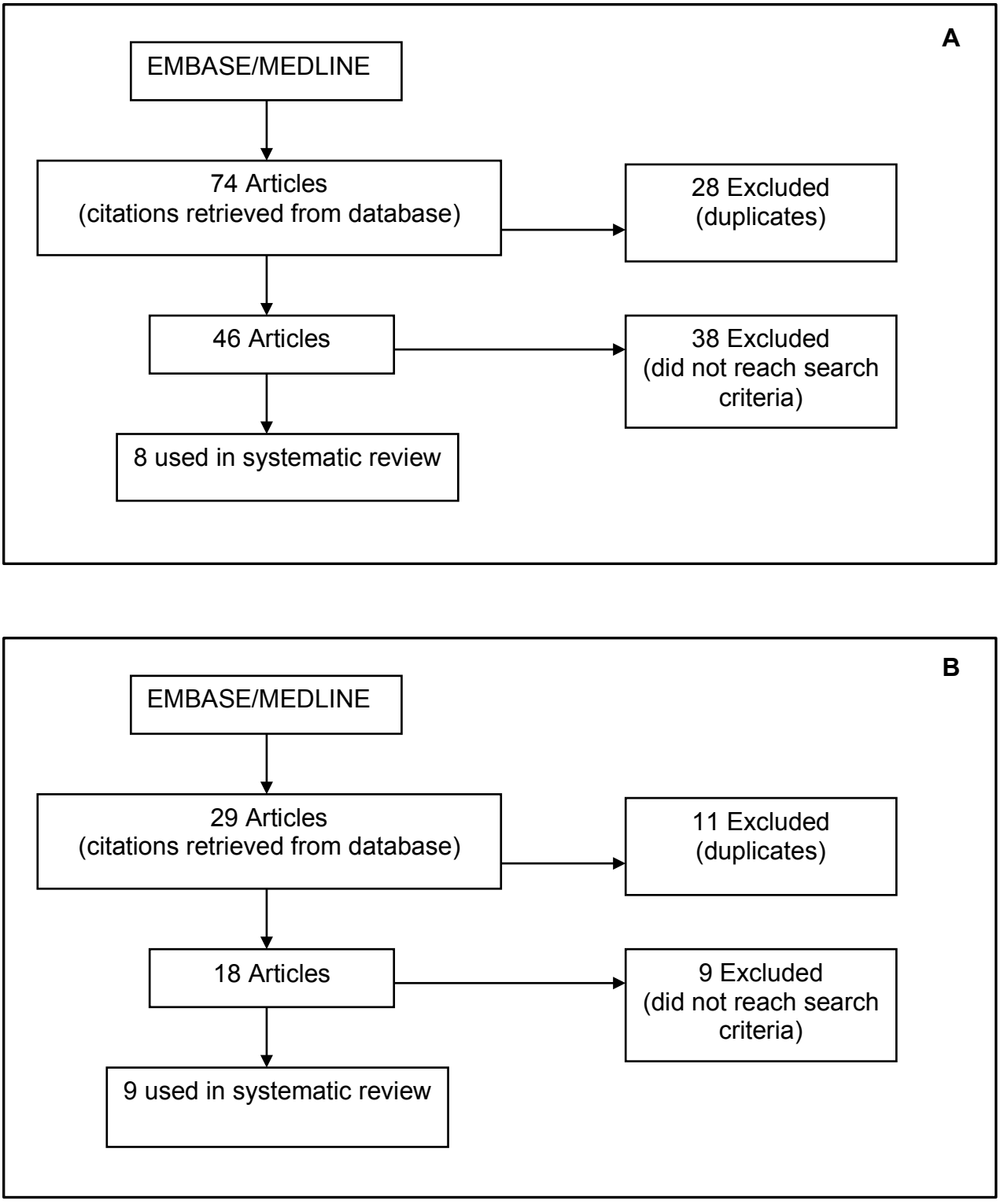


Table 6.2 Retrospective studies reporting incidence of symptomatic cranial bleeding

Author	Year	Type of study	Definition of neonatal period	No of ICH	No of ECH	Cohort	Incidence (%)
(A) In the general population							
Sachs *et al* [316]	1987	Descriptive	1-11 days	12	NS	23141	0.052
Hanigan *et al* [317]	1994	Retrospective cohort	0-9 days	28	NS	81000	0.035
Towner *et al* [251]	1999	Retrospective cohort	'All neonates'	361	NS	583340	0.062
Mosavat *et al* [318]	2008	Cross-sectional	'All neonates'	Nil	10	3340	0.299
Hughes *et al* [319]	1999	Case-control	'All neonates'	Nil	99	19901	0.497
Overall incidence				**401**	**109**	**687481** **23241**	**0.058 (ICH)** **0.469 (ECH)**
(B) In newborns with haemophilia							
Yoffe & Buchanan [320]	1998	Retrospective cohort	0 – 5 days	5	NS	150	3.4 (ICH)
Ljung *et al* [321]	1990	Retrospective cohort	'neonatal'	5	7	140	3.6 (ICH) 5.0 (ECH)
Klinge *et al* [286]	1999	Retrospective cohort	0 – 1 week	11	NS	742	1.5 (ICH)
Revel-Vilk *et al* [322]	2004	Case-control	0 - 1 week	11	NS	172	6.4 (ICH)
MacLean *et al* [323]	2004	Retrospective cohort	'neonatal'	1	6	73	1.4 (ICH) 8.2 (ECH)
Tarantino *et al* [292]	2007	Retrospective cohort	'neonatal'	17	NS	580	2.9 (ICH)
Kenet *et al* [315]	2010	Retrospective cohort	0-1 month	22	NS	633	3.5 (ICH)
Richards *et al* [313]	2011	Retrospective cohort	'neonatal'	4	14	508	0.8 (ICH) 2.8 (ECH)
Overall incidence				**76**	**27**	**2998** **721**	**2.5 (ICH)** **3.7 (ECH)**

ICH; intracranial haemorrhage, ECH; extracranial haemorrhage, NS; not-specified

Table 6.3 Prospective studies reporting incidence of asymptomatic cranial bleeding

Author	Year	Imaging modality	Time interval from delivery scan performed	No of ICH	No of ECH	Cohort	Incidence (%)
(A) In the general population							
Rooks *et al* [324]	2008	MRI and US	72 hours	46 (all SDH)	22	101	45.5 (ICH) 21.7 (ECH)
Whitby *et al* [325]	2004	MRI	48 hours	9 (all SDH)	NS	111	8.1 (ICH)
Looney *et al* [326]	2007	MRI	Up to 5 weeks	17	NS	88	19.3 (ICH)
Overall incidence				**72**	**22**	**300** **101**	**24 (ICH)** **21.7 (ECH)**
(B) In newborns with haemophilia							
Smith *et al* [327]	2007	US and/or CT	7 days	3	6	20	15 (ICH) 30 (ECH)

ICH; intracranial haemorrhage, ECH; extracranial haemorrhage, NS; not-specified

Systematic review of published literature reporting cranial bleeding in newborns with haemophilia and general population

Overall incidence of cranial bleeding in newborns in the general population

The five [251, 316-319] studies that reported symptomatic cranial bleeding are described in Table 6.2. Three retrospective studies reported 401 episodes of ICH in a total of 687,481 term newborns, giving an overall incidence of 5.8 per 10,000 deliveries. Sachs *et al* measured the incidence of ICH in a population of term newborns with birth weight over 2500g and found 12 cases of symptomatic ICH in 23,000 deliveries giving a similar overall incidence of 5.9 per 10,000 [316]. Hanigan *et al* performed a retrospective review of the cases of ICH in term newborns (gestational age >37 weeks and birth weight > 2500g) at a regional neonatal intensive care unit over a seven-year period. They found 28 cases of symptomatic ICH in an estimated population of 81,000 live births within the referral region [317]. Similarly, Towner *et al* calculated an incidence of symptomatic ICH of 7.3 per 10,000 births in live singletons born to nulliparous women with birth weight between 2500 – 4000g. This data was collected retrospectively from hospital discharge records over two years and included a total of 583,340 deliveries [251].

Two single-centred retrospective studies reported 109 cases of cephalohaematoma in 23,241 newborns giving an overall incidence of ECH of 47 cases per 10,000 [318, 319]. A single-centre, retrospective chart review of 3,340 live term neonates assessed the incidence of birth injuries and reported 10 cases of cephalohaematoma related to delivery, giving an incidence of 0.3%. Overall birth trauma (including clavicular fractures, brachial plexus injury and massive haematomas) occurred in 1.3% of

vaginal deliveries and 0.5% of caesarean deliveries ($p = 0.0001$). In this cohort there were no SGH reported and no episodes of ICH, although no radiological screening was performed [318]. Another retrospective study that assessed incidence of birth-associated head and neck trauma found 99 cases of cephalohaematoma in a cohort of 19,901 newborns during a 6 year period, giving an incidence of 0.5%. ICH was not reported in this cohort [319].

Three prospective imaging studies [324-326] reported the incidence of asymptomatic cranial bleeding in term newborns in the general population using various imaging modalities (Table 6.3). Only one study reported the incidence of ECH in addition to ICH with a high incidence 22/101 (22%) experiencing cephalohaematoma [324]. Rooks *et al* performed serial MRI and cranial US in 101 term asymptomatic neonates and found 46 neonates (46%) had supratentorial SDH on MRI performed within 72 hours of birth [324]. Twenty-two neonates had cephalohaematoma noted on initial MRI screening, 18 of which occurred in conjunction with SDH. Cranial US were performed after the initial screening MRI to monitor the natural evolution of bleeding. Only 11/17 (65%) of the posterior fossa SDH that were diagnosed on MRI were detected on cranial US.

A case-control study evaluated the prevalence of asymptomatic ICH and its relationship to obstetric and neonatal risk factors. Cranial MRI was performed prospectively on 88 neonates (>35 weeks) in the first few weeks of life. All newborns were categorised into two groups. The first group (n=19) were considered to be at risk for psychiatric or neurodevelopmental disorders which included 12 neonates with prenatal mild ventriculomegaly, and seven offspring born to mothers with

schizophrenia. The remaining controls (n=69) were considered to be without risk for psychiatric or neurodevelopmental disorders [326]. Both groups were included in the analysis as there is no evidence that prenatal mild ventriculomegaly, or being born to a mother with schizophrenia confers an increased risk of ICH. Seventeen (19%) out of 88 neonates had clinically silent ICH on MRI (15 control neonates, one neonate with mild ventriculomegaly, and one offspring of a mother with schizophrenia). The site of ICH included 16 posterior fossa SDH, one isolated case of IVH, five with co-existing parenchymal haemorrhages and two with SAH. All 17 cases of ICH occurred in newborns delivered vaginally which gave an overall prevalence of 26% of vaginal births.

Whitby *et al* found the incidence of clinically silent ICH in term newborns to be 8%. Nine babies out of 111 had SDH diagnosed on cranial MRI within 48 hours of delivery [325]. All cases were asymptomatic, did not require any specific intervention and resolved on MRI by four weeks of age. The overall incidence of clinically silent ICH in 300 healthy term newborns imaged prospectively was 24% (range 8-47%).

Overall incidence of cranial bleeding in newborns with haemophilia

Nine studies [286, 292, 313-315, 320, 322, 323, 327] were available that reported the incidence of cranial bleeding in newborns with haemophilia. In eight retrospective studies there were 76 cases of symptomatic ICH reported in 2,998 newborns with haemophilia giving an overall incidence of 2.5% [286, 292, 313-315, 320, 322, 323]. In three retrospective studies there were 27 episodes of ECH reported in 721 newborns with haemophilia giving an overall incidence of 3.7% (Table 6.2) [313, 321, 323].

Yoffe and Buchanan were the first authors to report on incidence of cranial bleeding in newborns with haemophilia in 1988. They reported five cases of ICH presenting within the first five days of life in a population of 150 boys with haemophilia [320]. All patients were delivered in different obstetric units in widely disparate geographical location over a 20-year time interval.

Two studies reported in the Swedish population collected retrospective data on incidence of symptomatic ICH in haemophilia neonates based on a national registry. Ljung *et al* reported 13 cases of head bleeding (five cases of symptomatic ICH) in 140 newborns over a 27-year period (1960-1987) [321] and at a later date, 16 cases of head bleeding (four of which were ICH) in 117 newborns over 20 years (1970-1990) [314]. The two studies have derived data from the same national registry and therefore the same cohort of patients. The larger cohort was included in this systematic review to assess cumulative incidence. The latter study, which reports rates of cranial bleeding in relation to MOD, was used in the meta-analysis.

A large cohort study was conducted using surveys sent to 17 HTC across Germany and Austria where a total of 742 newborns were included [286]. In this cohort, 11 cases of ICH occurred within the first week of life. Maclean *et al* explored the impact of unaware carriership on the clinical presentation of haemophilia by collecting data from medical records and parent interviews over two years (2002-2004). One episode of ICH and six episodes of ECH occurred in 73 deliveries [323]. A retrospective cohort study including 580 newborns with IBD recorded on the Nationwide Inpatient Sample was reported in the United States [292]. 17 cases of symptomatic ICH were

recorded in haemophilia newborns. However, in 11 out of 17 cases there were associated co-morbidities that increased the risk of ICH, such as prematurity, respiratory distress syndrome and sepsis.

In the United States, haemophilia patients are automatically enrolled in the Universal Data Collection (UDC) surveillance project that commenced in 2003, for the Centers for Disease Control and Prevention. In 2009, Kulkarni *et al* [49] and then subsequently in 2010, Kenet *et al* [315] published the results from this project, giving data on the frequency of ICH at birth in newborns with haemophilia. From the most recent report, a total of 633 patients were diagnosed with haemophilia within one month of age, and ICH associated with delivery occurred in 22 babies.

A recent European study evaluating the incidence of major bleeding in the neonatal period, reported 18 cases of head bleeding occurring within the first 28 days of life, four of which were ICH, and 14 were ECH, in a cohort of 508 newborns with haemophilia [313].

Only one prospective study assessed the incidence of asymptomatic cranial bleeding in newborns with haemophilia [327]. The sample size was limited to 20 newborns that underwent radiological screening within the first seven days of life. Of these 20 patients, 13 were diagnosed with haemophilia using cord blood testing or venepunture within 24 hours of delivery. The remaining seven cases were diagnosed following bleeding symptoms (circumcision, heel prick, ECH). Fifteen per cent (3/20) had SDH on cranial CT and 30% (6/10) had ECH; four with cephalohaematomas and two with SGH [327]. All three babies that had ICH were born to mothers with unknown carrier

status by vacuum extraction and diagnosed with haemophilia due to ECH and prolonged bleeding following circumcision. They were asymptomatic for ICH and radiological screening was performed within 24 hours of the diagnosis. One baby with moderate haemophilia A was diagnosed with ICH on CT following a normal cranial US scan.

The odds of experiencing symptomatic ICH at birth in newborns with haemophilia compared to the general population are 44 (95% CI 34.7-57.1) ($p < 0.01$). The odds of ECH in newborns with haemophilia compared to the general population are 8.2 (95% CI 5.38-12.6) ($p < 0.01$).

Meta-analysis to assess impact of mode of delivery on rates of cranial bleeding

Towner *et al* assessed the risk of ICH in term newborns according to MOD. ICH occurred in 1 per 1,990 infants delivered spontaneously, 1 per 860 infants born by ventouse delivery, 1 per 664 delivered by forceps, 1 per 907 delivered by CS during labour and 1 per 2750 delivered by ELCS [251]. Table 6.4 shows the studies that included data on MOD in both the general population and newborns with haemophilia. The relative risk (RR) of ICH by MOD comparing both cohorts is presented in the column on the right. Newborns with haemophilia delivered by AVD have a roughly 60 times increased risk (RR 61; 95%CI, 26-146) of ICH compared to the general population. Newborns with haemophilia born by SVD have a roughly 50 times increased risk (RR 51; 95%CI, 33-79) compared to the general population, and newborns with haemophilia born by CS have a 7 times increased relative risk (95% CI, 1.8-29.3) of ICH compared to the general population.

Figure 6.5 shows OR for ICH comparing all MOD to SVD. ELCS had reduced odds of ICH in the general population, although this did not reach statistical significance (OR 0.69; 95%CI, 0.39-1.24). All other MOD had an increased OR of ICH, with sequential instrumentation increasing the odds of ICH by more than seven fold (OR 7.45; 95%CI, 4.06-13.68).

Table 6.4 Intracranial haemorrhage by mode of delivery in newborns with haemophilia and in the general population

MOD	In the general population	In newborns with haemophilia				
	Towner *et al* [251]	Ljung *et al* [314]	Kenet *et al* [315]	Richards *et al* [313]	Total	RR
AVD	93/ 75299	1/17	2/15	2/34	5/66	61.3 (25.8 to 145.9)
SVD	204/ 387799	2/87	18/375	2/354	22/816	51.2 (33.2 to 79.1)
CS	114/ 117425	1/13†	1/182	0/89	1/283	7.28 (1.81 to 29.3)

Numbers in parentheses are 95% confidence intervals. MOD; mode of delivery, AVD; assisted vaginal delivery, SVD; spontaneous vaginal delivery, CS; caesarean section, RR; relative risk

† Delivered at 27 weeks gestation – case excluded from meta-analysis due to prematurity

Figure 6.5 Odds of experiencing ICH comparing spontaneous vaginal delivery with other modes of delivery in the general population

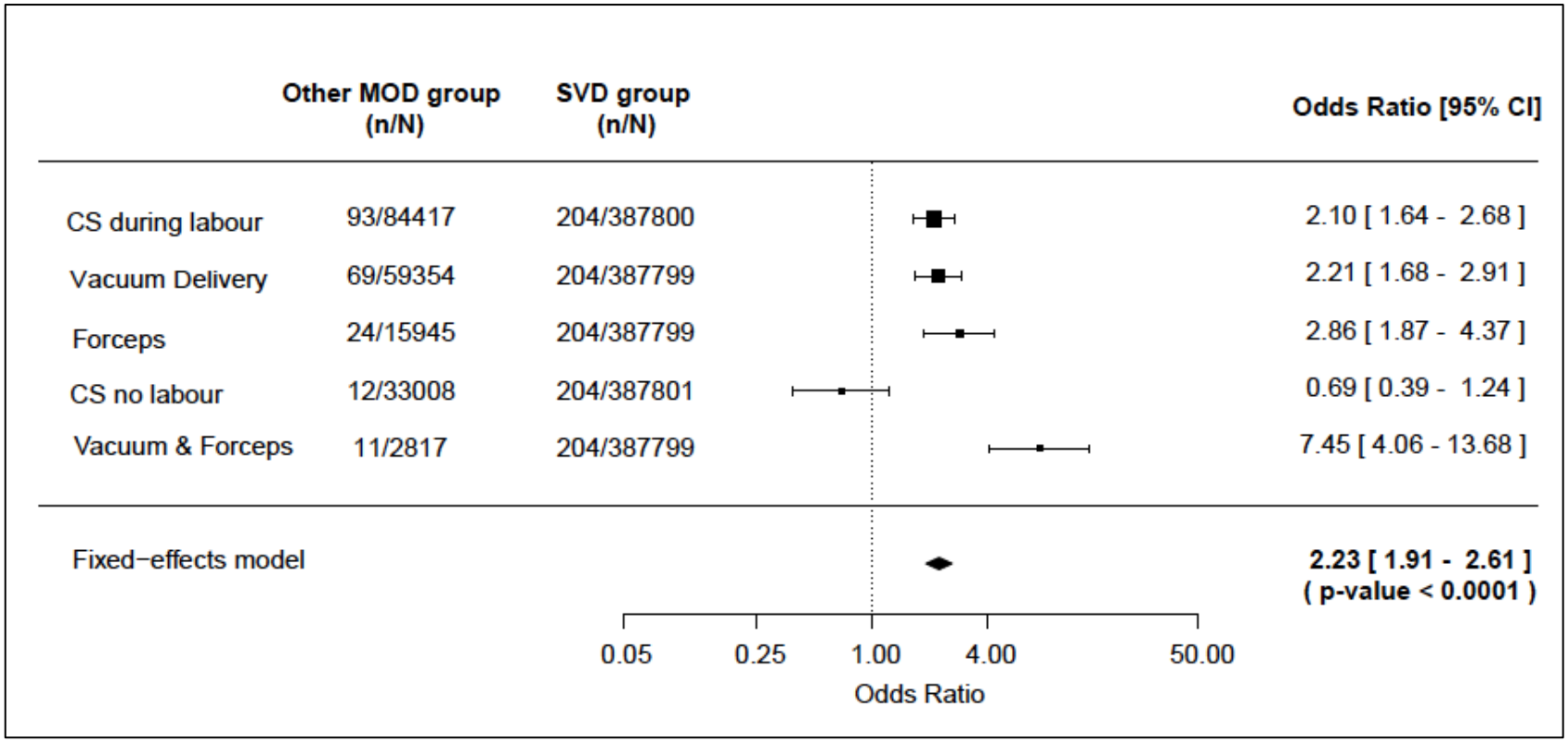


Figure 6.6 Odds of experiencing intracranial haemorrhage comparing spontaneous vaginal delivery (SVD) with assisted vaginal delivery (AVD) in newborns with haemophilia

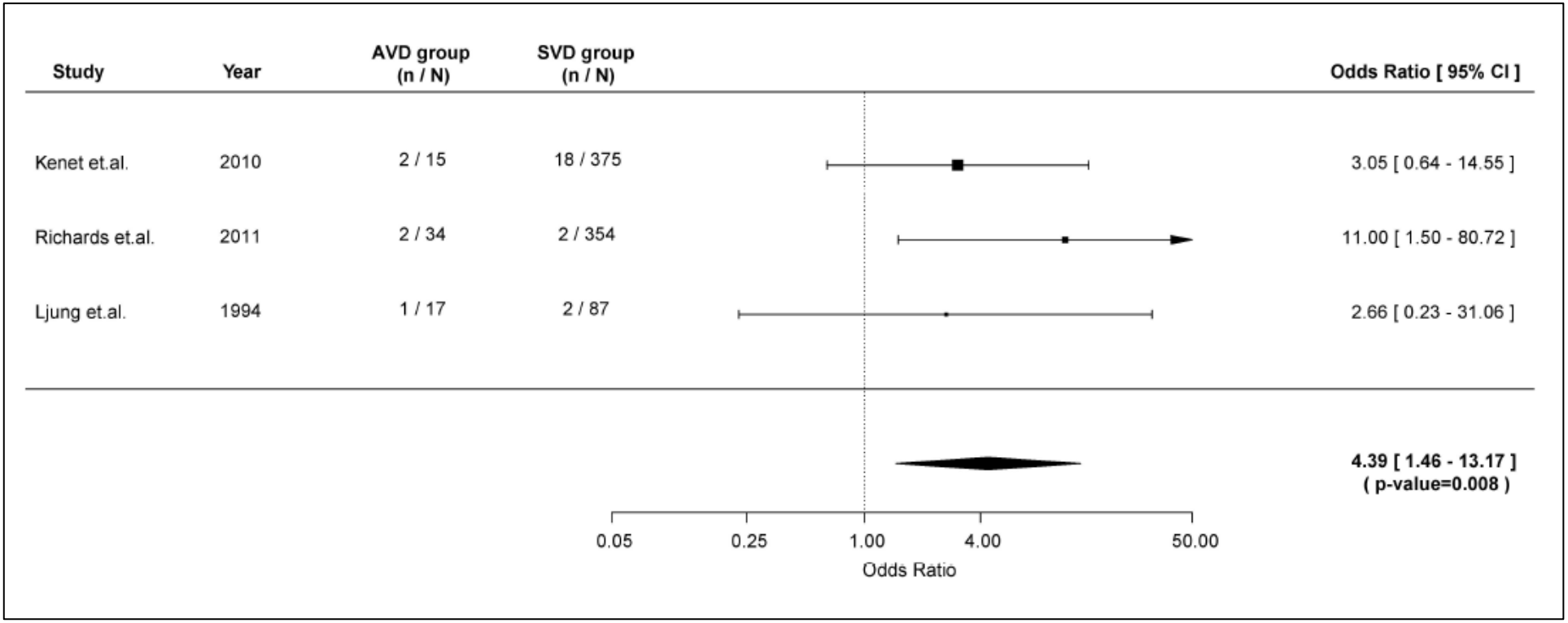
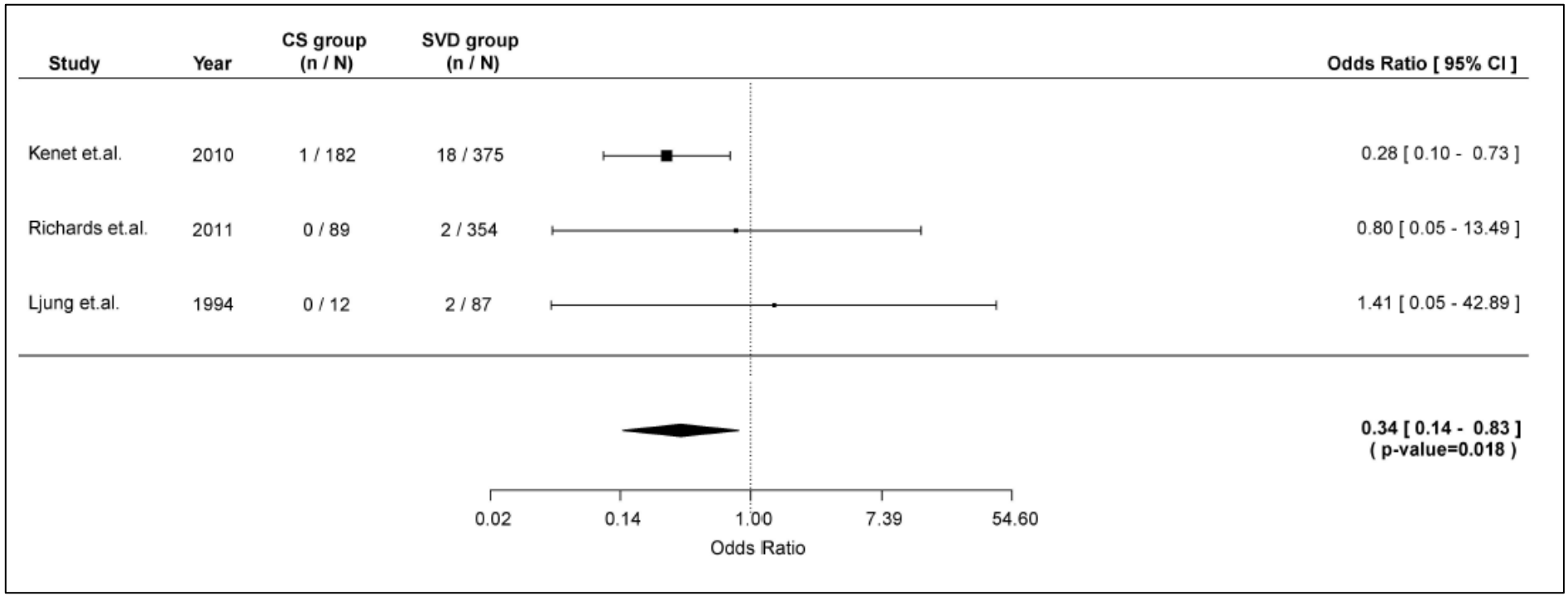


Figure 6.7 Odds of experiencing intracranial haemorrhage comparing spontaneous vaginal delivery (SVD) with caesarean section (CS) in newborns with haemophilia



The risk of symptomatic ICH differs significantly by MOD in newborns with haemophilia [313-315]. The odds of experiencing ICH are significantly higher in newborns born by AVD compared to SVD (OR 4.39; 95%CI, 1.46-13.7, $p = 0.008$) (Fig 6.6). The odds of experiencing ICH following CS are significantly reduced compared to SVD (OR 0.34; 95%CI, 0.14-0.83, $p = 0.018$) (Fig 6.7).

Neurological outcome in the general population

Data on the long-term neurological outcome following ICH in newborns in the general population was reported in two studies (Table 6.5 [A]). The developmental outcome at a median age of 3.4 years (range 1 to 6.5 years) was described in 33 term neonates with symptomatic ICH [317]. Five cases were excluded, as ICH was iatrogenic. In the remaining 28 cases of spontaneous ICH at birth, one infant died, giving a mortality rate of 3.6%. In the remaining survivors 14/27 (52%) experienced severe developmental delay, and in one case there was mild developmental delay.

Conversely, in the prospective imaging studies that assessed asymptomatic ICH in term newborns the long-term neurological outlook was excellent. In the screening MRI study performed by Looney *et al*, all 46 cases with ICH were clinically asymptomatic with normal neurological examination, repeat imaging showed spontaneous resolution and clinical follow up at 24-months showed no gross motor developmental delay [326].

Neurological outcome in newborns with haemophilia

Data on the long-term neurological outcome following ICH in newborns with haemophilia was reported in eight studies (Table 6.5 [B]). Two fatalities were reported in the eight studies, one as a direct result of intracerebral bleeding, and

another as a result of pneumococcal meningitis at the age of 4. Overall neurological morbidity was variable ranging from 9-100%. The trend was for the older studies to report a higher frequency of neurological morbidity in survivors of ICH. The neurological outcome reported included seizures, focal neurological deficit, global developmental delay and psychomotor retardation.

In a Canadian case-control study comparing the health, physical function and quality of life in boys with and without a history of ICH, 4 cases of symptomatic ICH were diagnosed during the first week of life, in a cohort of 172 boys with haemophilia. The controls were matched to their cases by age, type and severity of haemophilia. Neurological examination, physical function and quality of life were measured using standardised quantitative assessments, which demonstrated that boys with haemophilia and ICH had a worse clinical outcome. Neurological examination was abnormal in 44% of boys with ICH versus 28% of boys without ICH. The mean physical function was lower (82% ± 25% vs 93.5% ± 12%, p=0.045) and quality of life scores were significantly reduced (6.8 ± 3.2 vs 8.5 ± 1.4, p=0.02) in boys with ICH compared to controls [322].

Table 6.5 Neurological outcomes following intracranial haemorrhage

	Mortality (%)	*Morbidity (%)*	*Seizure disorder*	*Focal Neurological deficit*	*Developmental delay*	*Unspecified*
(A) In the general population						
Sachs *et al* [316]	8.3	?	-	-	-	-
Hanigan *et al* [317]	7.1	50		1/28	14/28	
(B) In newborns with haemophilia						
Richards *et al* [313]		11	-	-	-	2/18
Kenet *et al* [315]		9	2/11	2/11	-	-
Smith *et al* [327]		66	1/3	-	1/3	-
Revel-Vilk *et al* [322] †		44	-	7/16	-	-
Klinge *et al* [286] ‡	3.4	100	-	13/29 (cerebral palsy)	-	17/29 PMR 15/29 SMR
Yoffe & Buchanan [320]	12.5	63	5/8	-	-	5/8 PMR

PMR; psychomotor retardation, SMR; statomotor retardation

† Data on morbidity for ICH occurring in all boys aged 0-18 years

‡ Data on morbidity for all cases of ICH in paediatric patients up to 3 years of age

The long-term outcome in boys with haemophilia, who had an ICH before three years of age, was assessed in a case-control study [328]. This group found lower intellectual functioning, reduced visual-spatial and fine motor skills in the group with ICH compared to their aged-matched controls. In addition the boys in the ICH group had reduced language and mathematical skills.

6.2.4 Discussion

This systematic review of the literature shows the risk of ICH and ECH at birth in newborns with haemophilia is 44 and 8 times higher than in newborns in the general population, respectively. A meta-analysis is performed to determine the odds of experiencing ICH by MOD in newborns with haemophilia, comparing AVD and CS delivery to SVD. The current literature is limited to only three retrospective studies that report MOD and outcomes in this population. In the general population only one large cohort study was available for comparison. In this study the lowest risk of ICH at delivery was in ELCS and the highest risk was in sequential instrumental delivery.

In newborns with haemophilia the odds of experiencing ICH at birth following AVD were over four fold increased compared to SVD (OR 4.39; 95%CI, 1.46-13.7). The odds of experienced ICH following CS are reduced compared to SVD (OR 0.34; 95%CI, 0.14-0.83). This combines both elective and emergency (following labour) CS. The studies included in this analysis did not distinguish between elective and emergency CS. The latter was associated with a greater risk of ICH compared to SVD in the general population [251].

Relying on retrospective case review to determine the incidence of cranial bleeding has limitations. This method relies on historic records, where the accuracy of recording clinical diagnoses and the sensitivities of available investigations at the time could be unreliable. ICH diagnosed through the onset of neurological symptoms often indicates extensive bleeding. Smaller episode of intracranial bleeding may go undetected but result in adverse neurological outcome later in life [329]. Fatal cases may not have been included in the retrospective data collection, unless reported from post-mortem examination. In addition, around 50% of newborns with haemophilia have no positive family history and around 30% of cases are 'sporadic' due to *de novo* mutation in asymptomatic female carriers [314]. Thus, it is not always feasible to safeguard these babies from bleeding complications at the time of delivery. Indeed, mothers that are unaware of their carrier status are more likely to undergo an instrumental delivery, which is associated with a higher risk of cranial bleeding [323].

The high incidence of subclinical bleeding detected in the prospective imaging studies performed on healthy term newborns in the general population is concerning. It is not known how frequently this occurs in newborns with haemophilia due to a lack of data in this cohort. For early diagnosis the optimum imaging modality and timing is unknown, and no current consensus exists with regard to mode of screening in patients with IBDs. Whilst cranial US is inexpensive and readily available, it lacks sensitivity to detect bleeding in the posterior fossa. In the three prospective imaging studies in the general population [324-326], cranial CT and MRI were more sensitive than US at detecting posterior fossa bleeds. CT exposes the neonatal brain to ionising radiation making it an unsuitable imaging modality for screening purposes. As mentioned previously, neonatal MRI has superior diagnostic potential and considered

to be the safest imaging modality available, making it the optimum choice for screening for ICH in newborns with IBDs [309].

The risk of cranial bleeding with AVD is well known and guidelines recommend against instrumental delivery for potentially affected fetuses [151]. The risk reduction in ICH of up to two-thirds with caesarean delivery has not been fully appreciated. ELCS is recommended in other obstetric situations where the risk of ICH is increased. The National Institute for Health and Clinical Excellence (NICE) recommends that a ELCS is offered to women with fetuses in a breech presentation [330]. The reported risk of ICH in breech vaginal deliveries is 0.2%, and the relative risk of serious birth injury is 3.17 (95%CI, 1.77-5.73) compared to CS delivery [331].

The UKHCDO guideline on O&G management of women with IBDs state that spontaneous labour should be allowed where possible, but recommends against induction of labour, which is associated with a higher incidence of prolonged labour and instrumental delivery [250]. Likewise, the Canadian Haemophilia Society guidelines state that an IBD is not an indication per se for CS; a decision to proceed with CS should be based on obstetric indications [332]. The American Medical and Scientific Advisory Council (MASAC) of the National Hemophilia Foundation state that the outcome of labour cannot be predicted, and spontaneous (non-operative) vaginal delivery cannot be guaranteed therefore all women with an affected, or potentially affected fetus should be given the option of ELCS [333]. All guidelines advise against fetal scalp sampling for blood gases and fetal scalp electrodes and vacuum extraction and forceps deliveries are to be avoided. The problem with this recommendation is that as the fetus descends into the maternal pelvis during labour,

there is a ‘point of no return’, beyond which CS is more difficult [334]. The course of labour is unpredictable and 20% of women who commence labour spontaneously without any risk factors have prolonged labour or fetal distress that require an assisted delivery during the second stage [335].

Until further prospective data is available to assess the risk of cranial bleeding and MOD the findings of this review should be considered when counselling expectant mothers. Carriers of haemophilia with a potentially affected fetus, especially if severe haemophilia is expected, should be offered ELCS. On the other hand, it has been argued that CS is associated with adverse maternal morbidity, especially where multiple repeat CS are expected. The risk of severe PPH with an ELCS has been shown to be lower in nulliparous women and women with previous CS compared to intended vaginal delivery [336, 337]. A frank discussion should take place in the multidisciplinary clinic in advance of the expected delivery date to help the mother make an informed decision. This practice has been adopted by some centres and has led to an increased CS delivery rate in carriers of haemophilia [1]. However this has not correlated with an increase in blood loss at delivery or rates of PPH, especially when a multidisciplinary approach is adopted [1]. On the contrary, the carriers who delivered at the Royal Free over the past 10 years had a reduced incidence of PPH (1.7%) compared to the general population (see Chapter 5.4).

This study has limitations. The available published data on MOD and cranial bleeding in newborns with haemophilia is small and of limited quality, from retrospective cohort studies only, and therefore subject to bias and inaccuracy. Another important

limitation of retrospective data is the inability to control for other factors such as knowledge of carrier status and severity of haemophilia.

6.2.5 Conclusion

Cranial bleeding can result in a devastating outcome that further adds to the challenges faced by families with haemophilia. Although often cited as being a rare event, the risk of symptomatic ICH and ECH is 2.5% and 3.7%, respectively. This is 44 and 8 times higher than the risk in the general population. CS is the safest MOD that is associated with a reduced risk of ICH compared to SVD with an OR of 0.34 (95%CI, 0.14-0.83) in newborns with haemophilia. The highest risk is with AVD, which increases the odds of ICH by over four fold (OR 4.39; 95%CI, 1.46-13.7) and this MOD should be avoided in the potentially affected fetus.

The information should be discussed with expectant mothers and appropriate MOD should be assessed individually in each case by a multidisciplinary team including the mother, the haemophilia team and the obstetrician. An ELCS should be offered to mothers expecting a baby with potentially moderate/severe IBD.

6.3 Prospective cranial magnetic resonance imaging screening at birth in newborns at risk of inherited bleeding disorders

6.3.1 Introduction

The systematic review of the literature presented in Chapter 6.2 highlighted the lack of studies assessing asymptomatic ICH in newborns with IBDs. Prospective screening studies in newborns in the general population have demonstrated a high rate of clinically insignificant ICH (8-46%) [324-326]. There is very little data on the actual incidence of asymptomatic ICH in neonates with IBDs, in whom the consequences could be devastating. Only one prospective study assessed the incidence of asymptomatic cranial bleeding in newborns with haemophilia. ICH was diagnosed in 3 out of the twenty newborns who underwent radiological screening within the first week of life [327]. There are numerous case reports of ICH in neonates with rare IBDs (Table 6.6), however there are no prospective screening studies that assess the incidence in these patients.

Cranial MRI is a non-invasive, safe method of screening for ICH in neonates with a higher sensitivity than other imaging modalities. Recent advances in MRI technology have led to better image quality and faster exam times. The potential for acquiring adequate images of the newborn brain, to exclude an acute haemorrhage, is therefore more feasible [338].

6.3.2 Aim

The aims of this study was to:

1) Determine the feasibility of undergoing MRI imaging within 72 hours of birth, to rule out asymptomatic ICH in newborns at risk of moderate/severe IBD
2) Assess prevalence of asymptomatic cranial bleeding in neonates with moderate/severe IBD at birth
3) To assess affect of MOD on rate of asymptomatic cranial bleeding

6.3.3 Methods

Participants

Cranial MRI was undertaken within 72 hours of birth in term newborns with confirmed IBDs from July 2013 until May 2016. All pregnant women carrying a fetus with a potentially moderate or severe IBD were invited to participate. These included moderate and severe haemophilia, severe VWD, and heterozygotes with rare IBD. A member of the multidisciplinary team including an obstetrician, haematologist, paediatrician and a specialist haemophilia nurse provided both verbal information and a written information sheet about the study and the women and her partner were given time to consider participation. They were approached again in the second trimester and written consent was obtained prior to delivery.

The diagnosis was confirmed either by chorionic villus sampling in early pregnancy, or at delivery by cord blood sample. Newborn participants underwent cranial MRI if they had a confirmed diagnosis of a moderate/severe IBD. Any uncertainty regarding

the diagnosis in relation to the cord blood sample (i.e. mild haemophilia B) was resolved when the infant was investigated, usually by peripheral blood sample within 2 weeks of age. The newborn underwent cranial MRI, but details of their case were excluded from the final analysis if the newborn was found to be unaffected. All parents were informed of the scan result immediately after. The paediatric haemophilia team were informed directly if any acute haemorrhage was detected.

Data collection

Birth data obtained included onset of labour, use of oxytocin augmentation, duration of each stage of labour, MOD, gestational age at delivery and Apgar scores. Following delivery, a paediatrician examined for any signs or symptoms of ICH or bleeding. The outcome of the MRI was recorded. Details of any necessary treatments including factor replacement therapy were recorded. In addition, the paediatric follow-up outcome at 12 months of age, including any neurological impairment, further bleeds and inhibitor development was recorded.

Table 6.6 Case reports of intracranial haemorrhage occurring in term newborns with VWD and rare bleeding disorders

Inherited bleeding disorder	Case reports	Number of reported cases
VWD	Wetzstein *et al.* 2006 [339]	1 (severe type 3)
	Mullaart *et al.* 1997[340]	1 (type 2A) diagnosed in antenatal period
Fibrinogen deficiency or abnormality	Vorstman *et al*. 2003 [341]	1 (afibrinogenaemia)
	Ataoglu *et al*. 2010 [295]	1 (afibrinogenaemia)
	Tavil *et al*. 2016 [342]	1 (afibrinogenaemia)
	Hariharan *et al*. 2010 [343]	1 (afibrinogenaemia, diagnosed antenatally)
FII deficiency	Pasmant *et al*. 2011 [344]	1 (severe)
FV deficiency	Salooja *et al*. 2000 [293]	1 (severe)
	Totan *et al*. 1999 [345]	1 (severe)
	Ellestad *et al*. 2007 [28]	1 (diagnosed antenatally)
FV and FVIII deficiency	No reported cases	
FVII deficiency	Wong *et al*. 2000 [346]	1 (severe)
FX deficiency	Ermis *et al*. 2004 [347]	2 (siblings, both severe)
	De Sousa *et al*. 1988 [348]	1 (severe)
	Sumer *et al* 1986 [349]	1 (severe) diagnosed antenatally)
		1 (severe)
FXI deficiency	No reported cases	
FXIII deficiency	Abbondanzo *et al*. 1988 [350]	1

Procedures

The newborns underwent cranial MRI scan without sedation within 72 hours of delivery. A locally agreed scanning protocol was formulated by the radiology and research team, and followed for each participant. The newborns were transported to the radiology department with their parents in a mobile cot. Imaging was timed to occur following a morning feed. Once sleeping the newborn was swaddled and gently transferred to the MRI table, under an 8-channel head coil. The head was secured with foam sponges to minimise motion. Ear protectors were placed to minimise noise distraction.

MRI was carried out with a high-field (1.5 Telsa) Philips Achiever (Philips Healthcare, Guildford, UK) using the following sequences: 1) axial, sagittal, and coronal T1-weighted spin echo 2) axial T2-weighted turbo spin echo, fast field echo and diffusion weight imaging. Slices were 5 mm thick with a 2.5 mm gap. All MR images were independently reviewed on a PACS (Centricity; GW Healthcare) by a board-certified neuroradiologist. Any abnormalities reported on MR imaging were discussed immediately with the haemophilia team and consultant paediatrician. If the initial MRI image was normal no further cranial imaging was arranged and the neonate underwent routine clinical follow up. In the event of an abnormality the follow up scan was arranged as determined by the paediatrician.

6.3.3 Results

Table 6.7 shows the characteristics of the initial eight participants, and Table 6.8 provides the details of labour and delivery. MRI screening did not demonstrated asymptomatic ICH in any newborn with IBDs in this cohort.

The majority of women (6/8) made an informed decision to have ELCS following counselling regarding MOD at the Joint clinic. The mothers opted for ELCS to avoid the risk of ICH; of these six women, one went into spontaneous labour five days prior to her scheduled ELCS date, and two women had pre-labour rupture of membranes, and underwent EMCS the same day.

The woman who went into spontaneous labour prior to her scheduled CS date was a parous woman; her firstborn was affected with severe haemophilia (Case No.3). Although she had opted for ELCS to avoid the risk of ICH, she attended the labour ward contracting strongly and her cervix was five centimetres dilated. In addition, there was a pathological pattern on the fetal cardiotocograph indicating fetal distress. Although the progress of labour was not prolonged, the emergency CS was carried out for presumed fetal compromise. The neonate was found to have cephalohaematoma at birth.

Table 6.7 Characteristics of newborns that underwent MRI screening at birth

Case	Disorder	Mutation*	Maternal ethnicity	Cord factor level (%)	Gestational age (weeks)	Birth Weight (grams)
1	Severe HA	Intron 22 inversion	White Caucasian	< 1	38	3444
2	Severe HA	Intron 22 inversion	Mixed –Afro-Caribbean	< 1	38	2930
3	Severe HA	Intron 22 inversion	Black African	< 1	38	3110
4	Moderate HB Leyden	c.-35G>A promoter	Black African	2 (6 months)	39	3222
5	Severe HB	g.300117T>A (p.Cys222Ser)	Asian	< 1	38	3500
6	Severe HA	Intron 22 inversion	White Caucasian	< 1	39	3100
7	Severe HB		Mixed – Asian	< 1	39	3020
8	Severe HA	Intron 22 inversion	White Caucasian	< 1	36	2500

* Mutations determined through carrier testing. Mutation nomenclature is based on the guidelines of the Human Genome Variation Society [351].

Table 6.8 Labour details of neonates born with inherited bleeding disorder

Case	Parity	Onset of labour	Duration of 1st stage	Duration of 2nd stage	Final Cervical dilatation	MOD	EBL at delivery
1	0	No labour	-	-	0 cm	ELCS	500
2	0	No labour	-	-	1 cm	EMCS	300
3	3	Spontaneous	90 mins	-	5 cm	EMCS	600
4	2	Spontaneous	27 mins	5 mins	10 cm	SVD	300
5	2	No labour	-	-	0 cm	ELCS	700
6	0	No labour	-	-	0 cm	ELCS	500
7	0	No labour	-	-	0 cm	ELCS	500
8	0	No labour	-	-	1 cm	EMCS	500

ELCS, elective caesarean section; EMCS emergency caesarean section; MOD, mode of delivery; SVD, spontaneous vaginal delivery; EBL, estimated blood loss

Table 6.9 shows the follow up outcomes for each participant. The newborn with cephalohaematoma diagnosed at birth had severe haemophilia A with inversion 22 mutation and cord blood FVIII activity level < 1% (Case 3). Cephalohaematoma was diagnosed clinically and on cranial CT undertaken immediately to rule out ICH. There was no evidence of ICH on both CT and MRI performed on day 0 and 3, respectively. The infant was administered recombinant FVIII at a dose of 50 IU $kg^{-1}$for the first two weeks of life. He continued with on demand treatment, where he was administered factor concentrate (Advate®) to provide cover for medical interventions (i.e. circumcision and insertion of a portacath®), or for treatment of bleeding episodes. He had a spontaneous SDH at four months of age, which has resulted in a poor

neurological outcome and his treatment was compromised by the development of a high titre inhibitor.

Another infant in our cohort (Case 6) experienced a spontaneous SDH at 5 months of age. Again, this boy was affected with severe haemophilia A from an inversion 22 mutation. He presented with high-pitched, irritable crying and his mother, who was the daughter of a severe haemophilic, was aware to monitor for signs and symptoms of ICH, and promptly sought medical attention. The infant was receiving 'on demand' treatment but has now converted to regular prophylaxis since the diagnosis of first bleeding episode. He had normal neurological development at his last assessment.

Table 6.9 Outcome at one year of age

Case	Bleeding episodes	Events requiring Prophylaxis	Current Treatment	Inhibitor development	Neurological outcome
1	Joint and muscle bleeds at 10 month	Circumcision Portacath® insertion	Regular prophylaxis rFVIIa (Nova7)	Low titre at 6 months	Normal
2	Nil	Portacath® insertion	On demand FVIII (Advate®)	Transient at 9 months	Normal
3	Spontaneous SDH at 4 months	Evacuation of ICH Portacath® insertion	Regular prophylaxis FVIII (Advate®)	High titre	Global developmental delay, speech and language delay
4	Superficial bruising lower limbs	Circumcision Portacath® insertion	On demand FIX	Nil	Normal
5	Umbilical stump bleed on day 10	Circumcision and hernia repair	On demand FIX	Nil	Normal
6	Spontaneous SDH at 5 month	ICH and Portacath® insertion	Regular prophylaxis FVIII (Advate®)	Nil	Normal
7	Superficial bruising only	Nil	Received TXA only	Nil	Normal
8	Nil*	Nil	Nil	Nil	Normal

ICH; intracranial haemorrhage, SDH; subdural haematoma, FIX; factor IX, FVIII, factor VIII, TXA; tranexamic acid, rFVIIa, activated recombinant FVII

* Assessed at 2 months of age

6.3.4 Discussion

This is a pilot study to assess the feasibility of performing cranial MRI to rule out ICH on asymptomatic newborns with severe IBD. All participants were successfully imaged within 72 hours of age, allowing diagnostic discrimination for acute intracerebral haemorrhage at birth. One infant out of 8 (12.5%) experienced an ECH that was apparent clinically and treated promptly. No episodes of asymptomatic ICH were detected.

One infant (Case 3) experienced cephalohaematoma following EMCS. The cephalohaematoma likely occurred when pressure from uterine contractions forced the fetal head into the maternal pelvis, resulting in rupture of blood vessels in the periosteum. Cranial bleeding may have occurred as a result of the labour process. This case supports the argument for ELCS (without labour) in fetuses at risk of severe IBDs. The two babies who developed ICH at 4-5 months of age provide a strong argument for repeat MRI screening in the first few months of life. The bleeds may have occurred at the time of delivery and were too small to be detected on cranial MRI carried out within 72 hours of delivery. Serial MRI screening at weekly intervals for the first few months may be indicated, especially where the delivery has been traumatic, as in case No. 3. This would ensure earlier detection and treatment of ICH with improved neurological outcome. The optimum timing for screening for asymptomatic ICH in newborns with severe/moderate IBDs is still not known, but clearly further research would be valuable.

EMCS performed at full cervical dilatation for obstructed labour is a risk factor for skull fracture and ECH/ICH, thought to occur when the fetal head is disimpacted from the maternal pelvis [325] [352]. Although the evidence for reduced neonatal morbidity and mortality is limited to one, non-randomised clinical trial, recently NICE have endorsed the use of a balloon devise "fetal pillow", to elevate the fetal head and reduce trauma [353]. Five per cent of the 117 newborns experienced ECH in the later study reported by Ljung. There were 12 cases of subgaleal or cephalic haematoma, all with severe haemophilia; 10 were delivered by ventouse, 1 by SVD, and 1 by CS [314]. In the European cohort study there were 14 cases of cephalhaemoatoma in 508 neonates with haemophilia. Five out of 34 (15%) occurred following AVD, 2 out of 53 (4%) occurred following CS, and two occurred out of 316 (2%) following SVD. In both studies there were cases of prematurity (<36 weeks gestation) and the breakdown of ECH by MOD and gestational age were not specified [313]. In addition there was no distinction between elective and emergency CS.

All newborns in our cohort were born to mothers who were known carriers, in a maternity unit affiliated with a haemophilia centre. Expectant mothers booked at the Royal Free Hospital received multidisciplinary care with an individualised plan However in both studies the labour details are not provided (emergency or elective CS, prolonged labour) and there is no data on the gestational ages of these cases.drawn up in advance of labour. All mothers expecting a newborn potentially at risk of moderate or severe IBD were offered ELCS. If the mother was keen for vaginal delivery this was documented in the plan with recommendations such as avoiding fetal blood sampling, scalp electrode, ventouse delivery and difficult (mid-cavity) forceps with early recourse to EMCS, if any signs of obstructed labour.

Adopting this approach resulted in no ICH diagnosed at birth. However, due to the limited number of cases in this series it is not possible to comment on the frequency of asymptomatic ICH in newborns with IBD. This case series does highlight the high incidence of cranial bleeding seen in infants with severe haemophilia (one episode of ECH, and two episodes of ICH among 8 children within the first 6 months of age).

Unpublished data on all deliveries of newborns with haemophilia were recorded at another UK haemophilia centre (Alder Hay Children's Hospital, NHS Foundation Trust, Liverpool). The carriers delivered at their local hospital and the infants were brought to the centre for an MRI scan within the first week of life. Two out of six infants (33%) imaged had asymptomatic ICH diagnosed on cranial MRI. One infant was delivered by SVD following a prolonged second stage (3 hours and 55 minutes) at 39 weeks gestation, weighing 3000 grams to a mother that was a known carrier of severe haemophilia A. Neonatal MRI demonstrated bilateral SDH in the posterior fossa. Another neonate delivered by SVD prematurely at 36 weeks weighing 2500 grams to a mother with an unknown carrier status. Investigations for a bleeding disorder were carried out following prolonged bleeding from the heal prick examination and neonatal jaundice. This demonstrated a prolonged APTT and FVIII level < 1%. Although asymptomatic for ICH, the neonate had an anterior IVH on MRI scan. Both infants received factor concentrate treatment followed by prophylaxis from birth, had repeat MRI scans at six months of age that showed complete resolution of the haemorrhages, and subsequently normal neurological outcome.

These cases highlight the benefit of early screening for ICH in asymptomatic newborns diagnosed with moderate/severe IBD. The birth process results in

mechanical forces that are likened to a traumatic head injury, which would ordinarily necessitate urgent radiological imaging in a child with moderate/severe IBD. In addition, the clinical signs and symptoms of ICH in a neonate are vague and a failure to diagnose ICH and administer factor replacement therapy early in neonates with moderate/severe IBD is associated with a poorer neurological outcome [289, 320, 354].

One potential side effect of routine MRI screening for ICH in asymptomatic neonates with moderate/severe IBDs is the risk of early exposure to factor concentrate administration and potential for inhibitor development. Roughly 25% of children with severe haemophilia A develop inhibitory antibodies to infused FVIII resulting in significant challenges with treatment later in life [355]. Patients with high-titre inhibitors have increased risk of potentially life-threatening bleeds and treatment of these patients is more complex and costly [356]. In the study by Richards *et al* inhibitor development occurred in 5 patients with neonatal bleeding complications [313]. The relative risk (RR) of inhibitor development was 1.9 (95% CI 0.9-4.5) in children with neonatal bleeding, although the authors stated that this figure should be interpreted with caution because the risk did not adjust for other cofounders. Chalmers *et al* reported on FVIII exposure and subsequent inhibitor development and found no significant difference in children treated at different time points during the first year of life [357]. The results of the multicentre Concerted Action on Neutralising Antibodies (CANAL) study also support these findings; the incidence of inhibitors appeared to be associated with age of first treatment, however, after adjustment for treatment intensity this association largely disappears. Regular prophylaxis was associated with a 60% lower risk of inhibitor development than on

demand treatment (RR 0.4; 95%CI, 0.2-0.8) [358]. The risk of developing inhibitors was 2.8 (95%CI, 1.5-5.0) times higher in high-risk compared to low-risk mutations. The risk of developing inhibitors was 3-fold higher in patients with a positive family history of inhibitors. In the recently reported Survey of Inhibitors in Plasma-Product Exposed Toddlers (SIPPET) study, male patients (< 6 years of age) with severe haemophilia A treated with plasma-derived FVIII containing von Willebrand factor had a lower incidence of inhibitors than those treated with recombinant FVIII (hazard ratio, 1.87; 95%CI 1.17-2.96) [359].

The UKHCDO recommend primary prophylaxis to be initiated from the time of first joint bleed as standard of care in infants with severe haemophilia A. The Research of Determinants of INhibitor Development (RODIN) group reported that a second-generation recombinant FVIII (rFVIII) was associated with a higher incidence of inhibitor development [360]. High-dose intensive FVIII treatment was associated with increased inhibitor risk (adjusted hazard ratio [aHR] 2.00; 95%CI, 1.3-3.0), whilst regular prophylaxis, especially in patients with low-risk *f8* mutations, had a reduced risk of inhibitor development (aHR 0.61; 95%CI, 0.19-2.0 and aHR 0.5; 95%CI, 1-1.4, respectively) [360]. Some clinicians advocate prophylaxis from birth, a concept that has been gaining popularity among paediatric haemophilia treatment providers for many years [361]. The notion of preventing haemorrhages rather than treating them once they occur was first recommended in 1994 as optimal management by the MASAC of the National Hemophilia Foundation [362]. Whether routine MRI screening for intracerebral bleeding at birth would lead to increased factor exposure and subsequent inhibitor development requires consideration. However, these studies suggest that the risk of inhibitor development is more associated with genetic factors,

family history, and treatment intensity, than age of first exposure. Further research and careful interpretation of the data is required prior to making recommendations for appropriate treatment decisions [363].

6.3.5 Conclusion

This prospective pilot study provided evidence to support the feasibility of MRI imaging within 72 hours of delivery. Imaging was possible in eight cases, to exclude acute asymptomatic ICH. All neonates underwent cranial MRI scan without sedation and no adverse effects according to the protocol.

Cranial MRI carried out within 72 hours of birth did not detect any asymptomatic ICH in this case series of eight newborns with haemophilia. The majority of infants (6 out of 8) were delivered by ELCS, or semi-elective CS without labour. A high frequency of cranial bleeding within 6 month of age was reported; one infant experienced cephalohaematoma following an EMCS, and later suffered a spontaneous ICH, another infant experienced a spontaneous SDH at 5 months of age. Whether these bleeds were truly spontaneous, or delayed onset secondary to the birth process is not known.

Recruitment for this study is ongoing. Currently there is insufficient data to allow for any meaningful interpretation as to the benefit of MRI screening of asymptomatic neonates with IBD. Detection and prompt treatment of any asymptomatic ICH could prevent serious long-term neurological sequelae or even death. This would provide a strong argument for cranial MRI screening at birth to be standard care in

asymptomatic neonates diagnosed with an IBD. Due to the high frequency of delayed 'spontaneous' ICH reported in this cohort, repeat MRI at weekly or monthly intervals in the first six months of age would provide additional evidence to guide optimal timing of cranial MRI screening.

Only a large multi-centred collaborative study with national, or multi-national participant recruitment will provide sufficient evidence to properly assess the effect of MOD on rates of cranial bleeding. In addition maternal outcome, such as incidence of PPH, or complications from repeat ELCS should be recorded. Long-term neurological outcome data and the incidence of inhibitor development in neonates undergoing radiological screening should be recorded. Data on asymptomatic cranial bleeding at birth and how this evolves in neonates with IBDs is still lacking. An evidence-based approach would lead to better outcomes, if serious cranial bleeding can be detected and treated at the earliest opportunity.

CHAPTER 7

THE CHANGES IN ROTATIONAL THROMBOELASTOMETRY IN OBSTETRIC PATIENTS WITH FXI DEFICIENCY

7.1 Introduction

Women with FXI deficiency are at an increased risk of bleeding complications at delivery. FXI levels are unaffected by pregnancy [157, 158] and the persistence of low factor levels in the third trimester is common in women with this condition [49, 175, 182]. The risks of bleeding do not correlate well with the underlying FXI level [364] and relying on this alone may result in inappropriate assessment of the bleeding risk.

In normal pregnancy a hypercoagulable state is achieved due to increased levels of coagulation factors, and a reduction in anticoagulant free protein S. This is considered to be a physiological mechanism that prevents excessive bleeding at parturition. Rotational thromboelastometry (ROTEM®) is sensitive to hypercoagulable changes in normal pregnancy [365-367] that result in a shortened clotting time (CT) and clot formation time (CFT), an increase in the alpha angle and maximum clot firmness (MCF) [367, 368].

The aim of this case-control study was to determine whether ROTEM® detects hypercoagulable changes in pregnant women with FXI deficiency, by comparing parameters with non-parturient controls. In addition, any hypercoagulable changes detected in pregnant women with FXI deficiency were compared with healthy parturient controls. Bleeding tendency was measured with a bleeding score and FXI activity level (FXI:C). Correlation between with ROTEM® parameters, bleeding score, and FXI:C was also assessed.

7.2 Materials and methods

7.2.1 Study participants

ROTEM® was performed on 60 women in three distinct groups. The first group constituted 27 pregnant women with FXI deficiency in the third trimester of pregnancy. Nineteen women had mild FXI deficiency with FXI:C level 20 – 70 IU/dL and 8 women had severe FXI deficiency with FXI:C level < 20 IU/dL. The second group constitute 20 age-matched parturient control women with uncomplicated pregnancies who were in the third trimester of pregnancy. The third group constituted 14 non-parturient healthy control women. The exclusion criteria was age <18 years or > 45 years, weight < 50kg or > 100kg, current use of anticoagulant/antiplatelet medication, blood transfusion within the previous 28 days, abnormal full blood count and history of smoking or concurrent medical condition (cardiovascular, renal, malignancy, or hypertension). Parturient and non-parturient controls had no personal or family history of a coagulation disorder.

7.2.2 Blood sampling and laboratory analysis

Blood samples were obtained following consent during antenatal visits for parturient women in the third trimester of pregnancy (28 weeks + 0 days until 42 weeks gestation). Blood was obtained from non-parturient control women following consent and ensuring there were no exclusion criteria. Following an initial discard, 4.5 mL of venous blood was collected from the upper limb without venous occlusion using vacutainer tubes containing 0.106 M trisodium citrate (1 part citrate: 9 parts blood, Sarstedt, Leicester, UK). Bloods samples were analysed by ROTEM® within 30-120 minutes of collection. 300μL of citrated whole blood was re-calcified using 20 μL

$CaCl_2$ in HEPES buffer pH 7.4 and 0.1% sodium acid (Star-TEM® reagent, Pentapharm GmbH, Munich, Germany). The non-activated thrombelastometry test (NATEM) was used. Full details of the ROTEM® methodology and analysis are provided in Chapter 3.3. FXI:C was measured using a one-stage APTT-based assay as described in Chapter 3.1.

7.2.3 Data collection

The following data were collected from each participant: 1) demographic information (age, parity and BMI at booking), 2) baseline and third trimester FXI:C level (cases only), 3) ROTEM® parameters, 4) gestational age when ROTEM® was performed 5) bleeding score. A qualified haemophilia specialist obtained bleeding scores using the condensed MCMDM-1VWD bleeding questionnaire (see Appendix 4) [369, 370].

7.2.4 Statistical analysis

ROTEM® parameters, FXI:C levels, bleeding scores and demographic data were reported as mean and standard deviations (SD) where normally distributed, and median and interquartile ranges (IQR) where data was skewed. The differences between ROTEM® parameters between groups were compared using student's unpaired t-test for parametric data, and Mann-Whitney U test for non-parametric data. A p value of <0.05 was considered statistically significant. Comparison of FXI:C levels and bleeding scores with ROTEM® parameters was performed using Pearson's correlation coefficient (r).

7.3 Results

Complete ROTEM® and demographic data was available for all 27 case participants. One non-parturient participant was excluded due to an error when processing the sample. Table 7.1 shows the demographic details of each group.

Table 7.1 Demographic characteristics of the three groups

	Non-parturient controls (n = 12)	Parturient controls (*n* = 20)	FXI deficiency (*n* = 27)	*p* value
Age at delivery (years)	30 (4.0)	31 (6.0)	33 (4.0)	0.108
Parity at delivery	-	1.0 (1.0)	1.0 (2.0)	0.645
BMI at booking (kg/m^2)	23 (3.0)	23.5 (3.2)	23.6 (4.2)	0.682
Gestation at time of ROTEM® (weeks)	-	30 (8.0)	31 (3.0)	0.592

BMI, body mass index; ROTEM®, rotational thromboelastometry; FXI, factor XI
Numbers presented are means and one standard deviation (BMI, gestation) or median and interquartile range (age, parity). *P* value determined by student's t-test or Mann-Whitley U test depending on distribution of data.

The median age of parturient women with FXI deficiency was 30 (range 21 to 39 years). The median age of parturient control women was 31 (range 20 to 38 years) and among non-parturient controls was 33 years (range 26 until 39 years). There were no significant differences in median age between the groups (p = 0.12). Likewise the mean BMI at booking among women with FXI deficiency was 23.6 (range 17.4-35.2 kg/m^2), among parturient controls was 23.5 (range 19.0-28.6 kg/m^2), and among non-parturient controls was 23.0 (22.0-27.0 kg/m^2), with no statistical difference seen between groups (p = 0.68). ROTEM® was performed between 28 and 37 weeks gestation in parturient women with FXI deficiency with a median gestational age of 30 weeks. In parturient controls ROTEM® was performed between 28 weeks and 38 weeks gestation with a median gestational age of 31 weeks. There was no statistically significant difference detected in the gestational age when ROTEM® was performed in the women with FXI deficiency compared to parturient controls (p = 0.59).

7.3.1 Comparison of ROTEM® between groups

Table 7.2 shows the mean and SD of ROTEM® parameters of women with FXI deficiency and both control groups. All parameters (CT, CFT, alpha angle and MCF) were statistically different with $p < 0.05$. Figure 7.1 shows the box and whisker plot for the means, SD range and p value to show difference in ROTEM® parameters between FXI deficiency patients and the two control groups. Figure 7.2 shows the thromboelastogram for the different groups. CT was significantly shorter (p <0.001) in both parturient (375 ±SD 55.8 seconds) and non-parturient controls (555 ±SD 85.5 seconds) compared to patients with FXI deficiency (617 ±SD 194 seconds). CFT was significantly shorter (p <0.001) in parturient controls (87 ±SD 23.0 seconds) compared to patients with FXI deficiency (136 ±SD 44.8 seconds) and significantly

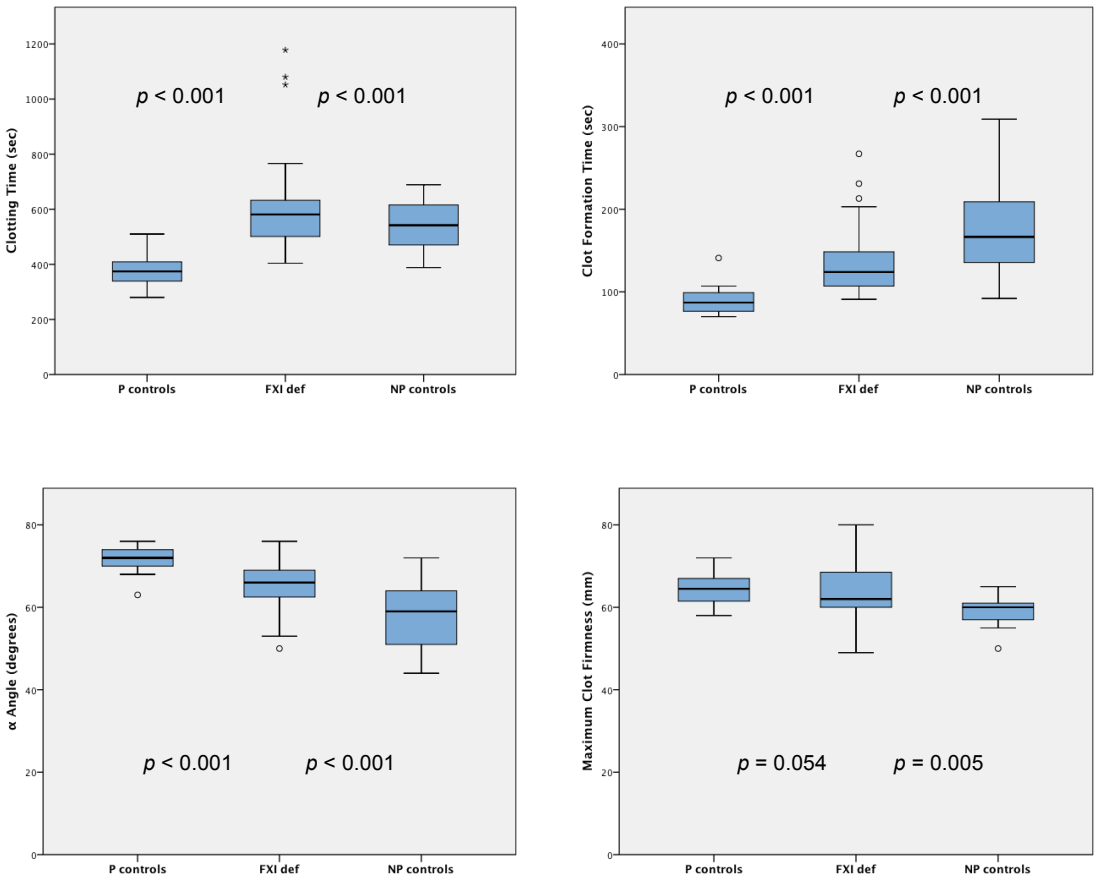
shorter (p <0.001) in patients with FXI deficiency compared to non-parturient controls (184 ± 56.3 seconds). Alpha angle was significantly higher (p <0.001) in parturient controls (72 ±SD 3.0 degrees) compared to patients with FXI deficiency (65 ±SD 6.2 degrees) and significantly higher (p <0.001) in patients with FXI deficiency compared to non-parturient controls (57 ± 7.6 degrees). No significant difference (p = 0.542) in MCF was seen between parturient controls (64 ± SD 3.7 mm) and patients with FXI deficiency (63 ±SD 6.5 mm). However MCF was significantly higher (p = 0.005) in patients with FXI deficiency compared to non-parturient controls (60 ±SD 5.0 mm) (Table 7.2).

Table 7.2 Comparison of ROTEM® parameters between groups

	Parturient controls (n = 20)	p value	FXI deficiency (n = 27)	p value	Non-parturient controls (n = 12)
CT (secs)	375 ± 55.8	< 0.001	617 ± 194	< 0.001	555 ± 85.5
CFT (secs)	87 ± 23.0	< 0.001	136 ± 44.8	< 0.001	184 ± 56.3
α angle (°)	72 ± 3.0	< 0.001	65 ± 6.2	< 0.001	57 ± 7.6
MCF (mm)	64 ± 3.7	0.542	63 ± 6.5	0.005	60 ± 5.0

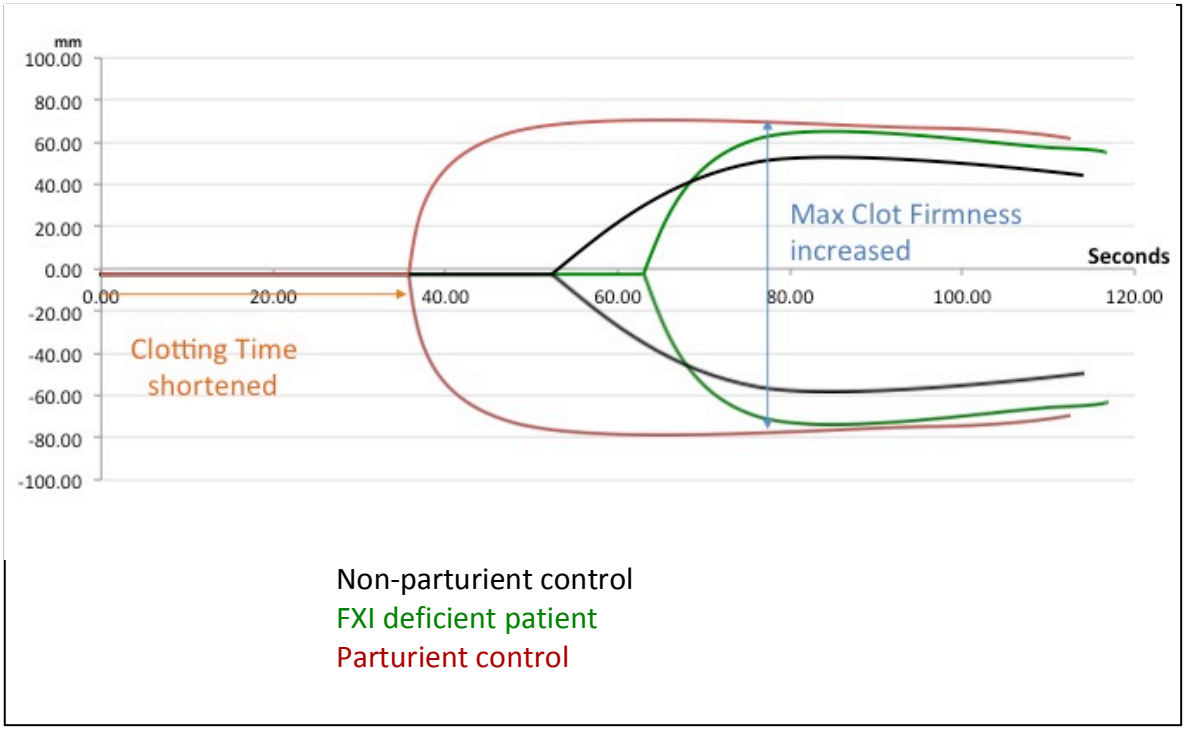
CT; clotting time, CFT; clot formation time, MCF; maximum clot firmness. Values are mean and one standard deviation (SD) or median and interquartile range (IQR). Parameters highlighted in grey are statistically different between groups (p < 0.05).

Figure 7.1 Comparison of ROTEM® between groups



P controls; parturient controls, FXI def; factor XI deficiency, NP controls; non-parturient controls. Participants divided into three groups for comparison. The data is presented in a box-and-whisker plot. The box spans the interquartile range (from the 25th to the 75th percentiles), and the line within the box denotes the median. The whiskers extend from the maximum and minimum 1.5 IQR.

Figure 7.2 Thromboelastogram demonstrating mean ROTEM® differences between groups



7.3.2 Comparison of ROTEM® between parturient and non-parturient controls

Table 7.3 demonstrates the mean and SD of ROTEM® parameters in both parturient and non-parturient controls. All parameters (CT, CFT, alpha angle and MCF) were statistically different with $p < 0.05$. Comparison of ROTEM® parameters between both control groups demonstrated that parturient controls had significantly shorter CT and CFT ($p < 0.001$) compared to non-parturient controls. The alpha angle ($p<0.001$) and MCF ($p = 0.003$) were significantly increased in the parturient compared to non-parturient controls.

7.3.3 Comparison of ROTEM® between mild and severe FXI deficiency patients

Table 7.4 demonstrates the mean and SD of ROTEM® parameters in both mild and severe FXI deficiency. The parameters highlighted in grey (CT, CFT and MCF) were statistically significant with a $p < 0.05$. Figure 7.3 shows the box and whisker plots for the mean, standard deviation, range and *p* values in ROTEM® parameters between mild and severe FXI deficiency patients. Figure 7.4 shows the thromboelastogram demonstrating the difference between mild and severe FXI deficient patients. CT (531 ±SD 70.3 seconds) and CFT (120 ±SD 19.8 seconds) were significantly shorter ($p <0.001$ and $p = 0.002$, respectively) in mild compared to severe FXI deficiency patients (820 ±SD 245 seconds and 176 ±SD 62.7 seconds, respectively). Alpha angle was higher in mild (66.5 ±SD 3.4 degrees) compared to severe FXI deficiency patients (61.3 ±SD 9.6 degrees) although this difference was not statistically significant ($p = 0.15$). MCF was significantly lower ($p = 0.02$) in mild (62.0 ±SD 5 mm) compared to severe FXI deficiency patients (69.0 ±SD 10 mm). A wider variation in MCF was seen in parturient patients with FXI deficiency (49-80 mm) compared to parturient (58-72 mm) and non-parturient controls (55-62 mm).

Table 7.3 Comparison of ROTEM® parameters between parturient and non-parturient controls

	Parturient controls (*n* = 20)	*p* value	Non-parturient controls (*n* = 12)
CT (secs)	375 ± 55.8	< 0.001	555 ± 85.5
CFT (secs)	87 ± 23.0	< 0.001	184 ± 56.3
α angle (°)	72 ± 3.0	< 0.001	57 ± 7.6
MCF (mm)	64 ± 3.7	0.003	60 ± 5.0

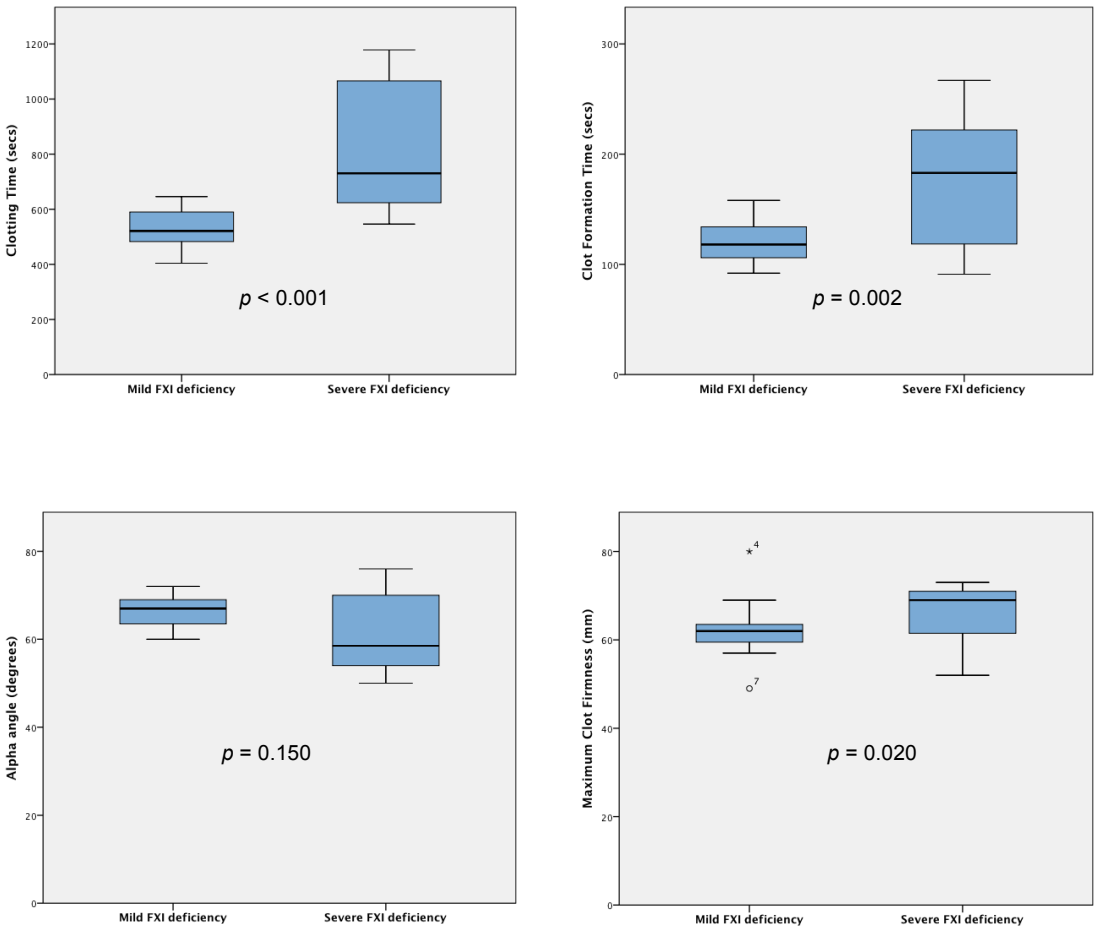
CT; clotting time, CFT; clot formation time, MCF; maximum clot firmness. Values are mean and one standard deviation (SD) or median and interquartile range (IQR). Parameters highlighted in grey are statistically different between groups ($p < 0.05$).

Table 7.4 ROTEM® analysis between mild and severe FXI deficiency patients

	Mild FXI deficiency (*n* = 19)	*p* value	Severe FXI deficiency (*n* = 8)
CT (secs)	531 ± 70.3	< 0.001	820 ± 245
CFT (secs)	120 ± 19.8	0.002	176 ± 62.7
α angle (°)	66.5 ± 3.4	0.150	61.3 ± 9.6
MCF (mm)	62.0 ± 5	0.020	69.0 ± 10

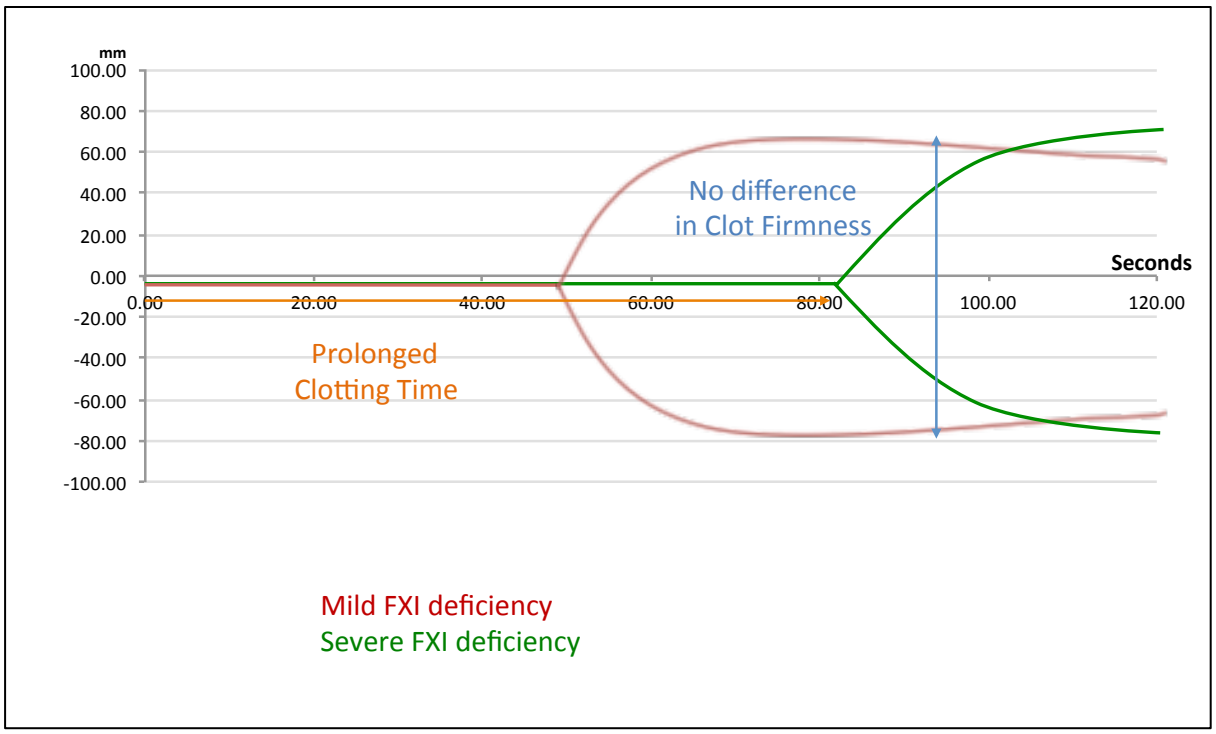
CT; clotting time, CFT; clot formation time, MCF; maximum clot firmness. Values presented are mean and one standard deviation (SD) or medians and interquartile range (IQR). Parameters highlighted in grey are statistically different between groups ($p < 0.05$).

Figure 7.3 Comparison of ROTEM® between mild and severe FXI deficiency patients



FXI deficiency patients divided into two groups. Those with mild FXI deficiency had FXI:C level 20-70 IU/dL, those with severe had FXI:C < 20 IU/dL. The data is presented in a box-and-whisker plot. The box spans the interquartile range (from the 25th to the 75th percentiles), and the line within the box denotes the median. The whiskers extend from the maximum and minimum 1.5 IQR.

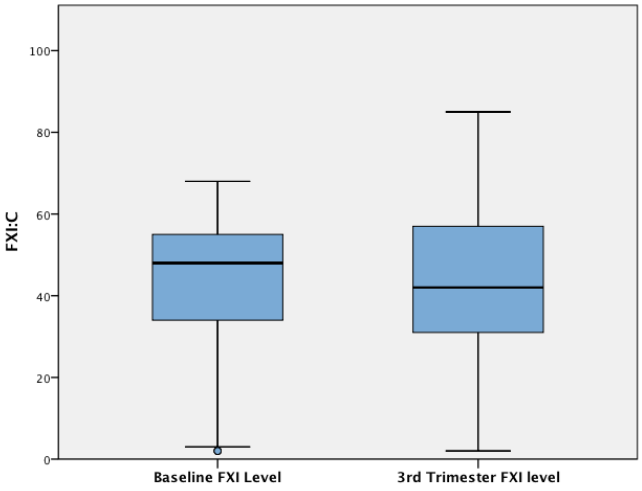
Figure 7.4 Thromboelastogram demonstrating mean ROTEM® differences in mild and severe FXI deficient patients



7.3.4 Correlation of ROTEM® with FXI:C levels

Figure 7.5 demonstrates the change in FXI levels in 46 pregnancies where the baseline and third trimester FXI:C level were recorded. There was no significant difference (p = 0.152) between baseline FXI:C level (median 47 IU/dL) and third trimester FXI:C level (median 46 IU/dL). Table 7.5 demonstrates the correlation coefficients between ROTEM® parameters and FXI:C level. When comparing ROTEM® parameters with FXI:C levels, a significant negative correlation was detected between CT (r= -0.65, p<0.001) and CFT (r = -0.59, p = 0.002). No significant correlation was detected between FXI:C levels and alpha angle (r = 0.37, p = 0.06) and MCF (r= -0.23, p=0.24).

Figure 7.5 Changes in factor XI level during pregnancy (n = 46)



FXI:C; factor XI activity level. The data is presented in a box-and-whisker plot. The box spans the interquartile range (from the 25th to the 75th percentiles), and the line within the box denotes the median. The whiskers extend from the maximum and minimum 1.5 IQR.

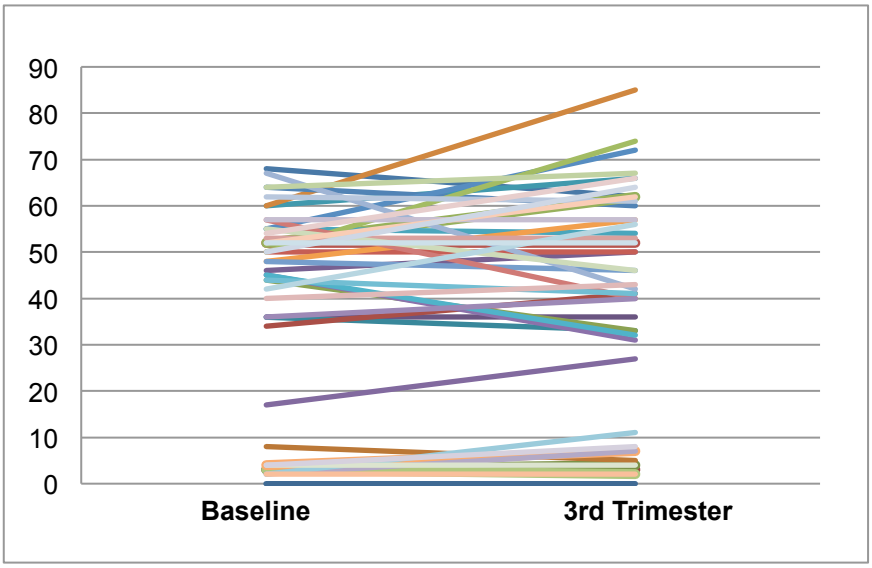


Table 7.5 Correlation between ROTEM® parameter and FXI level

	r	r^2	*p* value
CT (secs)	-0.65	0.42	< 0.001
CFT (secs)	-0.59	0.35	0.002
α angle (°)	0.37	0.14	0.057
MCF (mm)	-0.23	0.05	0.241

Values are Pearson's correlation coefficient (*r*) and r^2

Table 7.6 Correlation between ROTEM® parameters and bleeding score

	r	r^2	*p* value
CT (secs)	0.58	0.34	< 0.001
CFT (secs)	0.42	0.18	0.003
α angle (°)	-0.45	0.21	0.001
MCF (mm)	0.03	0.00	0.831

Values are Pearson's correlation coefficient (*r*) and r^2

7.3.5 Correlation of ROTEM® with bleeding score

The bleeding score ranged from -2 to 7 (median 3) in women with FXI deficiency, and from -1 to 3 in controls (median 0). Table 7.6 demonstrates the correlation coefficients between ROTEM® parameters and the bleeding score. CT had a significant positive correlation (r = 0.58, p<0.001) with bleeding score. A positive correlation was detected between CFT and bleeding score (r = 0.42, p = 0.003) and a negative correlation was detected between alpha angle and bleeding score (r = -0.45, p = 0.001). There was no significant correlation between MCF and bleeding score.

7.4 Discussion

This is the first study to report ROTEM® changes in parturient women with FXI deficiency compared to parturient and non-parturient controls. On average, parturient women with FXI deficiency took longer to clot (prolonged CT) compared to both parturient and non-parturient controls. This was more pronounced in patients with severe FXI deficiency. The role of FXI in clot initiation is still not fully understood. FXI activation occurs initially via the extrinsic pathway, with the resultant activated FIX contributing to sustained thrombin generation through a feedback loop [45]. In the consolidation phase of coagulation, a small amount of thrombin generated in the initiation phase is sufficient to activate FXI, which then activates FIX to augment the thrombin burst [48]. Clot consolidation and clot strength was increased in parturient patients with FXI deficiency compared to non-parturient controls. On average, the increase in clot consolidation was not as pronounced as in parturient controls. An explanation for the increase in clot consolidation and clot strength seen in FXI deficient women could be due to the progressive rise in coagulation factors (F)VII, FVIII, FX, FXII, fibrinogen and VWF [155-157] (Chapter 2.3.2). The rise in coagulation factors, especially FVIII and VWF, are most pronounced in the third trimester. The change in coagulation factor levels is accompanied by a reduction in physiological anticoagulants, most significantly a reduction in protein S activity levels and an acquired protein C resistance [160]. These changes could account for the increase in clot consolidation and clot strength in pregnant women with FXI deficiency compared to non-parturient controls.

In non-parturient patients with FXI deficiency those with reduced clot stability and a reduction in fibrin network density have an increased bleeding tendency [242]. There was a strong correlation between CT and CFT with bleeding score but no correlation with MCF. In addition, the range of MCF differed greatly in parturient patients with FXI deficiency compared to control participants, reflecting the unpredictability of changes in clot strength. Pregnancy induced haemostatic changes; mainly the significant rise in fibrinogen may have led to improved or corrected overall clot strength in some women with FXI deficiency. These findings are important and should be considered when assessing the bleeding risk in parturient women with FXI deficiency. Women with prolonged CT and CFT in the third trimester of pregnancy may be at increased risk. ROTEM® analysis should be incorporated into the clinical assessment during the third trimester of pregnancy. The following chapter in this thesis aims to assess the role of ROTEM® in assessment of bleeding risk in pregnant women with FXI deficiency.

7.5 Conclusion

Measurement of whole blood viscoelasticity with ROTEM® provides a global assessment of haemostasis during pregnancy in women with FXI deficiency. Pregnancy causes an increase in clot consolidation and clot strength in women with FXI deficiency compared to non-parturient controls. Women with FXI deficiency had shorter CFT, (p<0.001), increased alpha angle (p<0.001) and increased MCF (p = 0.005). However, they had significantly prolonged CT (p<0.001) compared to non-parturient controls.

Overall, pregnancy induced hypercoagulation in women with FXI deficiency, although these changes were not as pronounced as in the normal parturient population. Women with FXI deficiency had prolonged CT and CFT (p <0.001), and reduced alpha angle (p <0.001), compared to parturient controls. No difference was detected in clot strength (MCF p=0.054). Prolonged CT and CFT were associated with higher bleeding scores in women with FXI deficiency, and these women could have an increased risk of bleeding at delivery.

CHAPTER 8

THE ROLE OF ROTATIONAL THROMBOELASTOMETRY IN ASSESSMENT OF HAEMOSTASIS AND RISK OF BLEEDING AT DELIVERY IN WOMEN WITH FXI DEFICIENCY

8.1 Introduction

Pregnancy and delivery in women with FXI deficiency pose a particular challenge for the obstetrician. The incidence of both primary and secondary postpartum haemorrhage (PPH) is increased in women with FXI deficiency, as demonstrated in numerous studies [49, 175, 182, 211]. The bleeding phenotype is highly variable and does not correlate well with the underlying FXI activity (FXI:C) level. Relying on FXI:C levels alone may result in inappropriate assessment of the bleeding risk and overuse of FXI concentrates, which are associated with a risk of thromboembolic complications, and the potential for viral transmission. In addition, regional anaesthesia may be withheld in a woman with FXI deficiency due to concerns over bleeding complications resulting in spinal injury.

In the previous chapter of this thesis, ROTEM® parameters during pregnancy in women with FXI deficiency were compared to non-parturient and parturient controls. Women with prolonged CT, and CFT had significantly higher bleeding scores, and were considered to be at increased risk of bleeding. The ability of ROTEM® to assess bleeding tendency in FXI deficiency has been controversial. In a recent study a significantly prolonged CT was the only parameter to differentiate between bleeders and non-bleeders in patients with severe FXI deficiency [241].

The main objective of this prospective cohort study was to evaluate the use of ROTEM® in assessment of bleeding risk and provision of haemostatic cover during delivery in women with FXI deficiency.

8.2 Materials and Methods

8.2.1 Study participants

The cohort constituted pregnant women with FXI deficiency who attended the multidisciplinary joint obstetric haemophilia clinic at the Royal Free Hospital from October 2004 until October 2014. A management plan was made in the third trimester for labour and delivery to determine the haemostatic prophylaxis required to provide safe regional anaesthesia and to reduce the risk of PPH. A haemophilia specialist obtained bleeding scores using the condensed MCMDM-1VWD bleeding questionnaire [369]. A score of ≥3 was indicative of increased risk of bleeding [371].

ROTEM® analysis during the third trimester of pregnancy was introduced as part of the bleeding risk assessment in women with FXI deficiency attending the clinic from 2009. Subsequently, all deliveries were managed using this additional investigation. The decision was individualised for each patient and took into account her previous bleeding history and FXI level. In addition, obstetric factors such as provision of regional anaesthesia and mode of delivery (MOD) were important considerations for type of treatment.

In order to assess the impact of ROTEM® analysis on clinical management, women with FXI deficiency were divided into two groups for comparison: i) women who delivered pre-2009 (without ROTEM® analysis) and, ii) women who delivered post-2009 (with ROTEM® analysis). The latter group were further subdivided according to the ROTEM® findings.

8.2.2 Data collection

The following clinical data were collected: 1) demographic information (age, parity and BMI at delivery), 2) bleeding score; 3) baseline and third trimester FXI activity level; 4) ROTEM® parameters in the third trimester; 5) haemostatic cover for labour and delivery; 6) mode of delivery; 7) estimated blood loss (EBL); 8) haemorrhagic complications at delivery; 9) obstetric risk factors for PPH; 10) use of regional anaesthesia with any documented complications.

Primary PPH was defined as an estimated blood loss ≥500 mL within 24 hours following vaginal delivery and ≥1000 mL following caesarean delivery. Secondary PPH was defined as heavy or excessive lochia occurring after 24 hours to six weeks following delivery. The bleeding score was obtained using the condensed MCMDM-1VWD bleeding questionnaire [369, 370].

8.2.3 Statistical analysis

Chi-squared or Fisher's exact test was used to test statistically significance of proportional data. A p value of <0.05 was considered statistically significant.

8.3 Results

8.3.1 *Pregnancy outcomes*

From 2004 until 2014 a total of 57 deliveries occurred in 37 women with FXI deficiency. The median gestation at delivery was 40 weeks (range 35-42 weeks). The MOD was 31/57 (54%) vaginal deliveries, 11/57 (19%) emergency caesarean, 9/57 (16%) elective caesarean and 6/57 (11%) instrumental deliveries (all forceps).

The diagnosis of mild FXI deficiency was unknown at the time of delivery in 8 cases. Twenty-two deliveries were managed prior to 2009, before the introduction of ROTEM®. Of these, 18 deliveries were in women with mild FXI deficiency and the remaining 4 were in women with severe FXI deficiency. Twenty-seven deliveries were managed following the introduction of ROTEM®. Of these, 19 were in women with mild and 8 were in women with severe FXI deficiency. The women were placed into three categories depending on how many ROTEM® parameters were within the range of parturient controls (Table 8.1). Category 1 comprised of 10 women (5 mild, 5 severe) who had 1 or no parameters in keeping with normal pregnancy. Category 2 comprised of 13 women (10 mild, 3 severe) who had 2 or 3 parameters in keeping with normal pregnancy. Category 3 comprised 4 women with mild FXI deficiency who had four ROTEM® parameters in keeping with normal pregnancy.

Table 8.1 Normal pregnancy ROTEM® parameters

ROTEM® parameters	Range of parameters in normal pregnancy
CT (secs)	312 - 498
CFT (secs)	74 - 135
Alpha angle (degrees)	69 - 76
MCF (mm)	60 - 72

CT; clotting time, CFT; clot formation time, MCF; maximum clot firmness.

This table demonstrates the reference range for 'normal' parturient NATEM ROTEM® parameters. The figures are determined by the 95% reference limits (two standard deviations from the mean), from control parturients in the third trimester of pregnancy, reported in Chapter 7.

8.3.2 Haemorrhagic complications

Three primary PPH and one secondary PPH occurred in eight deliveries where the diagnosis of FXI deficiency was unknown, leading to investigations that revealed an underlying bleeding disorder (cases 1-4, Table 8.2). In pregnancies with known FXI deficiency, the overall incidence of primary PPH was 12% (6/49) and secondary PPH 2% (1/49) (cases 5-11, Table 8.2). No significant correlation was demonstrated between ROTEM® parameters and the EBL at delivery among women with FXI deficiency. In addition, there was no significant difference in the mean and standard deviation of ROTEM® parameters in women who experienced PPH (n = 6) and those who did not (n = 21).

Table 8.2 Cases of postpartum haemorrhage in women with FXI deficiency

Case No	FXI level	ROTEM	Mode of delivery	Haemostatic cover	Primary or secondary	EBL (mL)	Obstetric factors
Diagnosis of FXI deficiency not known at delivery							
1	46	-	ELCS	None	Secondary	2400	ELCS for multiple pregnancy
2	42	-	SVD	None	Primary	1000	Nil
3	55	-	Forceps	None	Primary	900	Retained placenta
4	29	-	EMCS	None	Primary	2000	Uterine atony requiring B-lynch suture
Diagnosis of FXI deficiency known at delivery							
5	36	Pre- 2009	SVD	FXI and TXA	Primary	1000	Episiotomy and bilateral vaginal lacerations
6	45	Pre- 2009	EMCS	TXA	Primary	1400	Uterine atony
7	2	Category 1	SVD	rFVIIa and TXA	Secondary	-	Represented to A&E with heavy lochia
8	2	Category 1	ELCS	FXI and TXA	Primary	3000	Grade IV placenta praevia
9	50	Category 2	EMCS	TXA	Primary	1300	Delivered at 35 weeks for severe IUGR
10	52	Category 2	Forceps	TXA	Primary	1000	Episiotomy
11	36	Category 3	EMCS	TXA	Primary	2000	Uterine atony and lateral extension of uterine excision

FXI; factor XI, ROTEM®; rotational thromboelastometry, EBL; estimated blood loss, EMCS; emergency caesarean, ELCS; elective caesarean, SVD; spontaneaous vaginal delivery, TXA; tranexamic acid

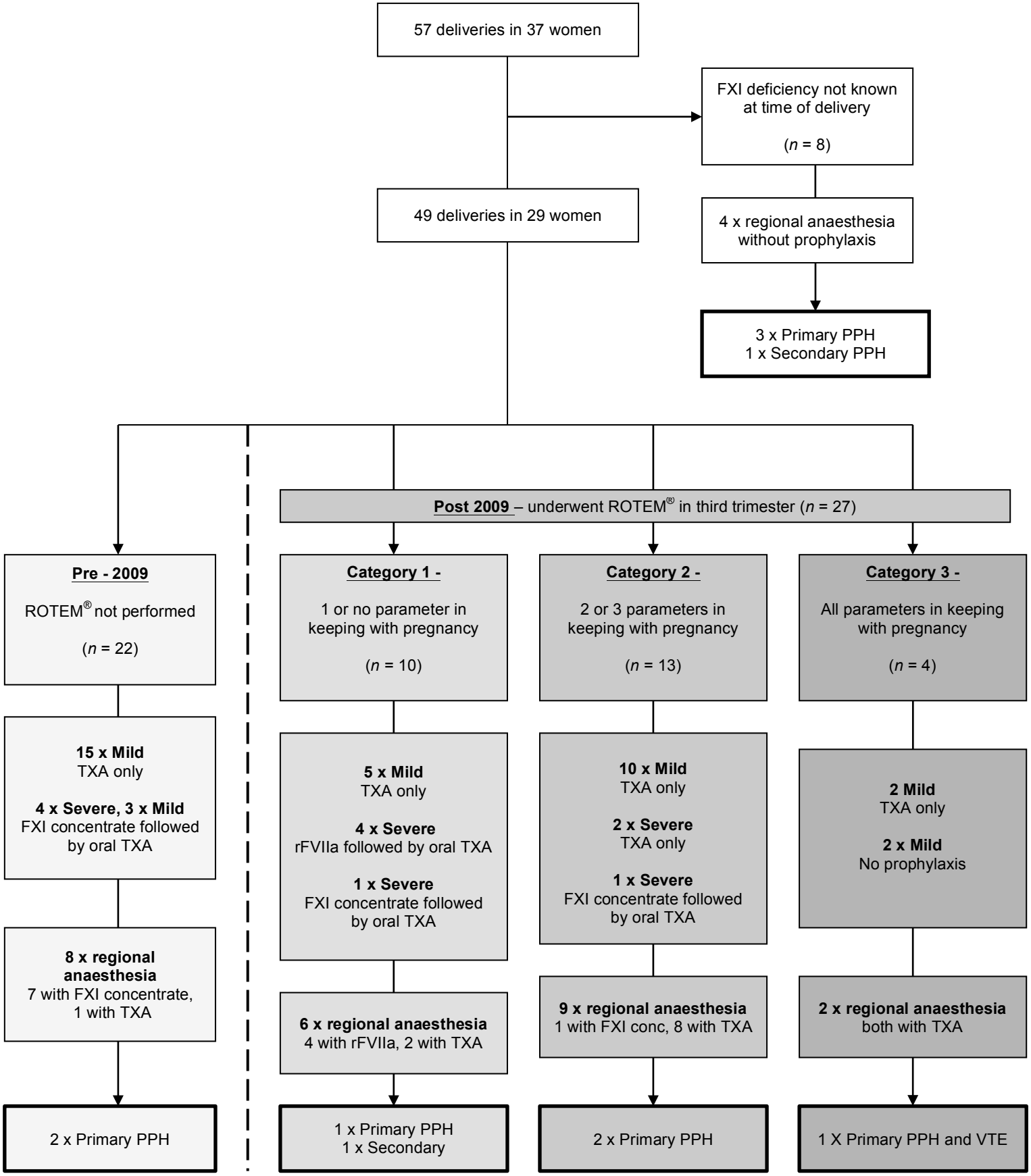
A primary PPH occurred in two out of 22 (9%) deliveries in women with mild FXI deficiency managed pre-2009, prior to the introduction of ROTEM®. Both cases were considered to be secondary to obstetric causes (cases 5 and 6). Four primary PPH (14.8%) and one secondary PPH (4%) occurred in two women with mild and three with severe FXI deficiency among 27 deliveries managed post-2009 following the introduction of ROTEM® (Cases 7-11, Table 8.2). All cases of primary PPH had coexisting obstetric risk factors including placenta praevia, uterine atony, and trauma. One secondary PPH occurred in a woman with severe FXI deficiency who presented with excessive lochia two weeks following an uncomplicated vaginal delivery. This is the only case that may have occurred solely due to FXI deficiency. There was no significant difference in the rate of PPH between both groups (0% pre-2009 vs 4% post-2009, $p = 1.0$).

Figure 8.1 shows the treatment and pregnancy outcomes according to third trimester ROTEM® parameters in women with FXI deficiency. FXI concentrate was administered to cover labour and delivery (4 severe and 3 mild FXI deficiency) in seven out of 22 (32%) deliveries managed pre-2009 prior to the introduction of ROTEM®. FXI concentrate was administered on the basis of FXI level and previous bleeding history. Among 27 deliveries managed post-2009 following the introduction of ROTEM®, haemostatic cover with rFVIIa or FXI concentrate was provided in six (22%) cases. Thus, the introduction of ROTEM® reduced the overall use of blood product administered, although this finding was not statistically significant (32% pre-2009 vs 22% post-2009, $p = 0.45$). Two women with severe FXI deficiency were treated with tranexamic acid only.

Regional anaesthesia was administered in 29 deliveries (21 with mild, 8 with severe FXI deficiency) without bleeding complications. The diagnosis of mild FXI deficiency was not known at the time of delivery in four cases where regional anaesthesia was sited. In the cases managed pre-2009 without ROTEM® analysis, regional block was sited in eight out of 22 (36%) deliveries (5 with mild, 3 with severe FXI deficiency). Seven women received FXI concentrate and one woman received tranexamic acid prior to siting regional anaesthesia.

In the group managed post-2009 with ROTEM® analysis, regional anaesthesia was sited in 17 out of 27 (63%) deliveries (12 with mild, 5 with severe FXI deficiency). Four women with severe FXI deficiency had rFVIIa and one had FXI concentrate to cover regional anaesthesia. Twelve patients with mild FXI deficiency were administered tranexamic acid to cover regional anaesthesia. Thus overall there was significantly reduced blood product usage for regional anaesthesia in the patients managed post-2009 with ROTEM® analysis (88% pre-2009 vs 29% post-2009, p = 0.01).

Figure 8.1 Treatment and pregnancy outcomes according to third trimester ROTEM® parameters in women with FXI deficiency



8.4 Discussion

Assessment of coagulation with ROTEM® in women with FXI deficiency has been carried out as part of routine care at the Royal Free Hospital since 2009. Prior to this, assessment of bleeding risk and the considered administration of rFVIIa/FXI concentrates was primarily based on a woman's bleeding history and FXI:C level [49]. One study published in Israel evaluated 164 deliveries in 62 women with severe FXI deficiency (FXI:C < 17u/dL). They concluded that as bleeding did not occur in 43 out of 62 women, plasma replacement therapy was not always necessary [211]. However, the rate of primary PPH (22% of all deliveries) in this population was still considerably higher than in the general population. Hence the need for an additional mechanism to assess bleeding risk in FXI deficient patients.

A lower proportion of patients received FXI concentrate or rFVIIa in the group managed with ROTEM® analysis. In addition, the use of ROTEM® enabled treatment decisions for providing haemostatic cover for regional anaesthesia. The findings of the ROTEM® analysis provided confidence to avoid the use of blood product that would otherwise have been administered [250]. After performing ROTEM® two cases of severe FXI deficiency were managed successfully with tranexamic acid. Thus these patients were able to avoid unnecessary exposure to blood product and the associated risk of thrombotic complications.

The lack of any correlation between ROTEM® parameters and EBL, and no discernible differences in the ROTEM® parameters of women who had a PPH, more likely reflects the practice of providing haemostatic cover to women with abnormal

ROTEM® parameters. From this data it was not possible to predict likelihood of PPH in women with FXI deficiency based on ROTEM® analysis solely. It is also not possible to determine whether using ROTEM® analysis to guide therapy has any effect on the overall rate of PPH. An interventional study with prospective randomisation would be more appropriate to properly evaluate this.

The cases with abnormal ROTEM® parameters in the third trimester were corrected with either rFVIIa/FXI administration or tranexamic acid. Four women out of 23 still experienced a PPH (Cases 7 - 10, Table 8.2). Whilst three of the four cases were due to obstetric causes, one secondary PPH occurred after the completion of a postnatal course of tranexamic acid. This highlights the increased risk of bleeding during times of high fibrinolytic activity that occurs in patients with FXI deficiency. An extended course of antifibrinolytic treatment may be necessary in such cases. Further research is required to assess serial FXI:C levels, or global haemostatic changes measured through thromboelastometry, in FXI deficient women during the postnatal period. This will help to determine how quickly they normalise, and therefore the duration of therapy that is required to prevent secondary PPH.

There was one case of pulmonary embolus in a woman with mild FXI deficiency who had significant VTE risk factors including prolonged labour, emergency caesarean and severe PPH. This woman had ROTEM® parameters in keeping with normal pregnancy and no significant bleeding history. Tranexamic acid was administered to provide cover for regional anaesthesia. It could be argued that as her ROTEM® parameters were in keeping with normal pregnancy this intervention was not necessary, and increased her risk for developing VTE. In addition,

thromboprophylaxis was withheld for 24 hours due to potential bleeding risk because of FXI deficiency. Although FXI deficiency may be protective against venous thromboembolism [372], it is important that risk factors are assessed and thromboprophylaxis is given appropriately.

8.5 Conclusion

Women with FXI deficiency have an unpredictable bleeding risk at delivery that is challenging to predict and manage. They require an individualised risk assessment incorporating the bleeding history or score, FXI level, and ROTEM® analysis to help determine bleeding risk and enable treatment decisions regarding use of haemostatic cover. The use of ROTEM® results in less overall blood product usage to cover delivery and for regional anaesthesia. Further research is required to evaluate the role of ROTEM® in this setting.

CHAPTER 9

EVALUATING THE EFFECT OF PROTHROMBOTIC MARKERS ON BLEEDING PHENOTYPE IN WOMEN WITH INHERITED BLEEDING DISORDERS

9.1 Introduction

Bleeding tendency in women with IBDs is highly variable and in clinical practice is often challenging to predict. Consideration of the bleeding risk prior to any haemostatic challenge such as the onset of menses, childbirth or anticipated surgery is an important management aspect. A better appreciation of the overall bleeding risk is important when considering the need for haemostatic prophylaxis.

The aim of this study was:

1) To quantify bleeding tendency (phenotype) in women with IBDs using the condensed MCMDM-1VWD bleeding questionnaire (Appendix 4) [369].

2) To assess the correlation between the phenotype and haemostatic variables

3) To assess if underlying prothrombotic variables, or the presence of thrombotic genotype modified the overall bleeding phenotype.

9.2 Method

9.2.1 Study participants

This was a collaborative study with women recruited from the Royal Free Hospital in North London, and the Haemophilia Treatment Centre (HTC) in Duisburg, Germany. A total of 68 women were recruited from the UK centre and 35 women were recruited from the HTC in Germany. Women with a confirmed diagnosis of von Willebrand disease (VWD) or carriers of haemophilia A or B who attended the joint multidisciplinary gynaecology haemophilia clinic were invited to participate. Data collected on each participant included age, diagnosis, baseline factor level.

The bleeding phenotype was described using the modified, condensed MCMDM-1 bleeding questionnaire developed by Tosetto *et al* (Appendix 4). This has been validated in the clinical setting to discriminate severity of bleeding symptoms [373]. It has reasonable inter-observer reliability, where 24 subjects (of a total 259) were administered the questionnaire by two different observers three months apart (inter-observers Spearman's Rho 0.72 and inter-class correlation of 0.8) [370].

9.2.2 Laboratory methods

50 mL of venous blood was collected from each participant using a 19-gauge butterfly with minimal occlusion of the antecubital fossa vein into blood collection tubes containing 106 mol/L sodium citrate [Sarstedt monovettes, Sarstedt, Leicester, UK]. After centrifugation for 10 minutes at 2000*g*, plasma and white blood cells were separated. High molecular weight DNA was isolated from the white blood cell fraction by standard methods. The presence of mutations in the *factor V* gene (1691,

G>A) and the prothrombin gene (20210, G>A) were determined as previously described [374, 375]. Protein C and protein S activities were measured on ACL TOP coagulometer [Instrumentation Laboratory] using the PROCHROM assay [Instrumentation Laboratory], and the protein S free latex assay [Instrumentation Laboratory] respectively. Antithrombin III (ATIII) was measured on an ACL 300R as previously described [376]. The laboratory methods used to measure coagulation factors (VWF:Ag, VWF:RCo, VWF:CB, FVIII:C and FIX:C) are described in Chapter 3.1.

9.2.3 Statistical analysis

Pearson's correlation coefficient was used to analyse the association between bleeding scores and haemostatic variables (VWF:Ag, VWF:RCo, FVIII:C, FXI:C). As multiple variables were recorded for each patient, multilinear regression analysis was used to determine if any thrombotic variable, or the presence of a thrombotic gene mutation, altered the bleeding score.

Correlation of bleeding score with VWF:Ag, VWF:RCo and VWF:CB was for patients with VWD only. The results from the carriers of haemophilia were separated from this part of the analysis. Correlation of bleeding scores with FVIII and FXI levels included both VWD patients and women who were carriers of haemophilia A and B.

9.3 Results

A total of 103 women with IBDs were recruited for this study; 91 women had VWD, 12 were carriers of haemophilia. Only carriers of haemophilia with FVIII/FIX level < 70 IU/dL were included. Of the 91 women with VWD, 75 women had type 1 VWD, 11 women had type 2 VWD, and 5 women had type 3 VWD. The median age of all participants was 35 (range 18-59). The median bleeding score was 5 (range -1 to 25). Age was an independent variable that affected the bleeding score with a significantly increased score seen in older women (r = -0.44, r^2 = 0.19; $p < 0.001$), [Fig 9.1]).

Seventeen women out of 103 (17%) tested positive for a thrombotic marker, which was a deficiency in anticoagulant activity level in eleven women, or prothombotic gene mutation in five women (Table 9.1). Three women tested positive for heterozygous factor V (FV) Leiden gene mutation, one woman for heterozygous prothrombin 20210 gene mutation, and one for lupus anticoagulant. The mean protein C activity level (protein C:Ac) was 107 IU/dL (standard deviation [SD]±21) with a range of 10-150 IU/dL. The mean protein S activity level (protein S:Ac) was 86 (SD±19) with a range of 32-145 IU/dL. The mean antithrombin III activity level (ATIII:Ac) was 100 (SD±11) with a range of 55-130 IU/dL. There was no significant correlation between anticoagulant activity levels and bleeding score.

Figure 9.1 Correlation of bleeding score with age

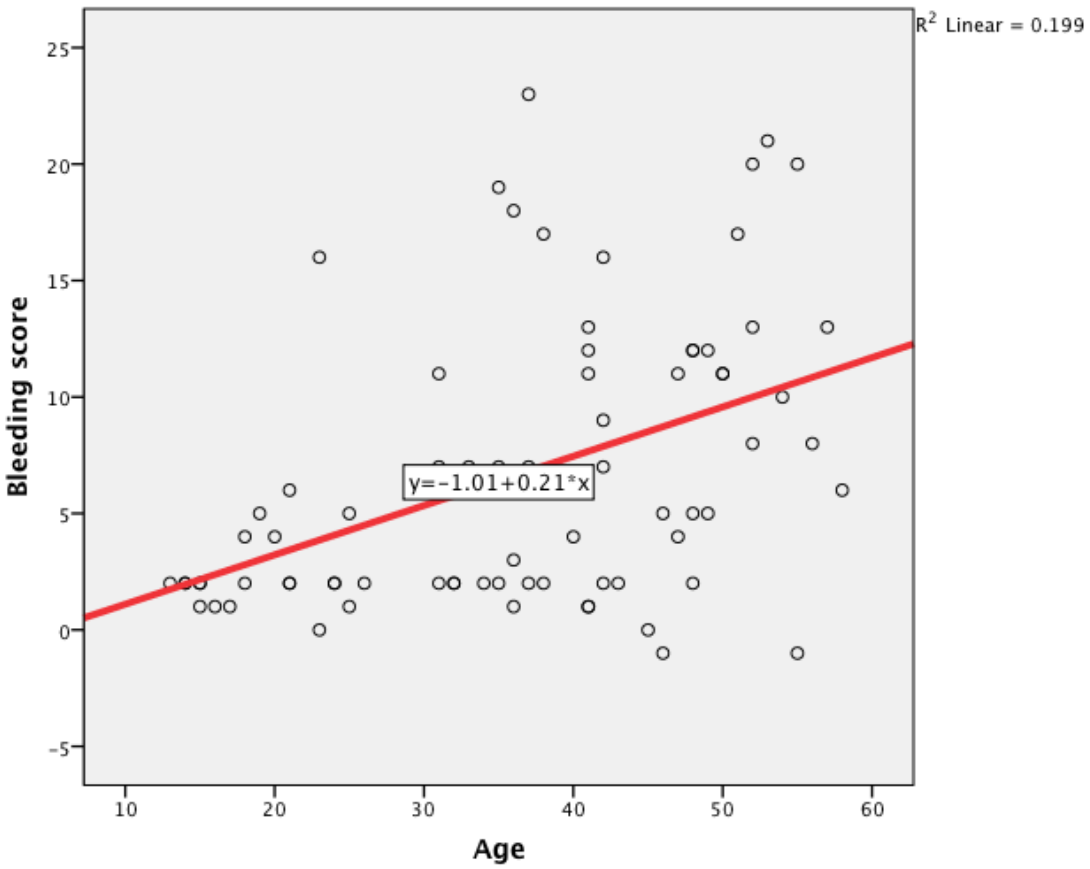


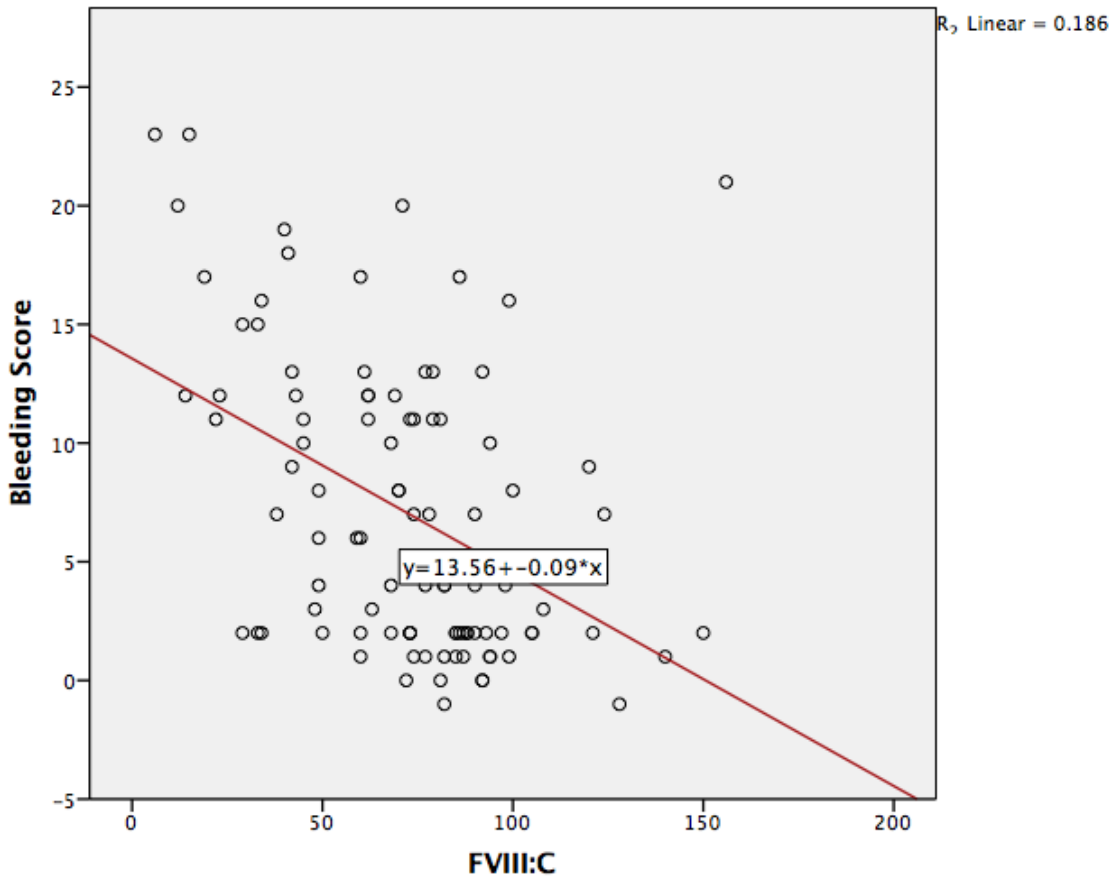
Table 9.1 Anticoagulant deficiency or presence of prothrombotic marker in women with inherited bleeding disorder

Thrombotic marker	Normal lab reference range	No of women (n)	Activity level (IU/dL)
Protein C deficiency	70-140 IU/dL	1	10
Protein S deficiency	71-130 IU/dL	10	32, 45, 52, 53, 59, 61, 61, 62, 64
Antithrombin III deficiency	86-114 IU/dL	2	72, 55
FV Leiden gene mutation	Heterozygote	3	
Prothrombin 20210 gene mutation	Heterozygote	1	
Lupus anticoagulant		1	

9.3.1 Correlation of bleeding score with haemostatic variables

VWF:Ag, VWF:RCo and FVIII:C had a statistically negative correlation with bleeding score. Table 9.1 shows the mean and standard deviation and correlation coefficient with bleeding score for each variable. FVIII:C had the strongest negative correlation with bleeding score (r = -0.43, r^2 0.19; $p < 0.001$ [Fig 9.2]). VWF:Ag and VWF:RCo also had negative correlation with bleeding score (r = -0.23, r^2 = 0.06; p = 0.023 [Fig 9.3] and (r = -0.21, r^2 = 0.051; p = 0.042 [Fig 9.4], respectively). There was no correlation demonstrated with bleeding score and VWF:CB and FIX:C (Fig 9.5 and 9.6).

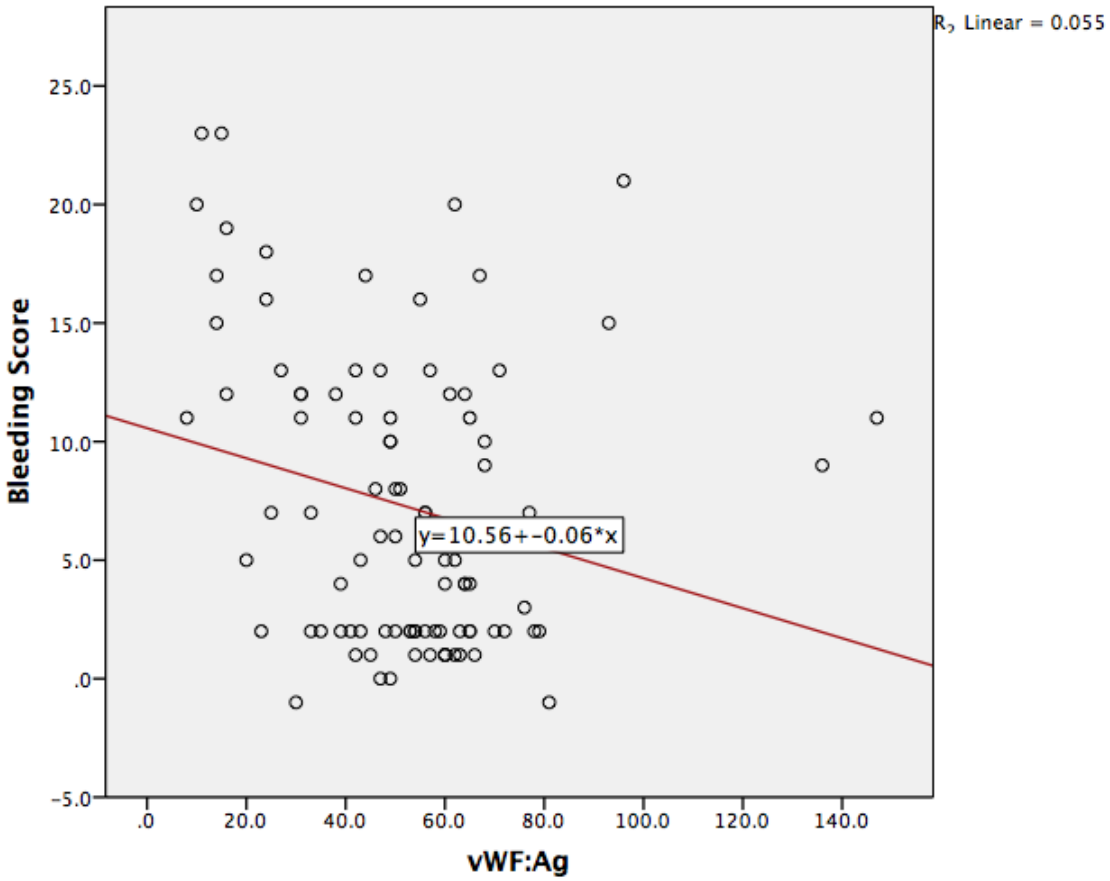
Figure 9.2 Correlation of bleeding score with FVIII activity level



FVIII:C, factor VIII activity level

r = -0.43, r^2 0.19; $p < 0.001$

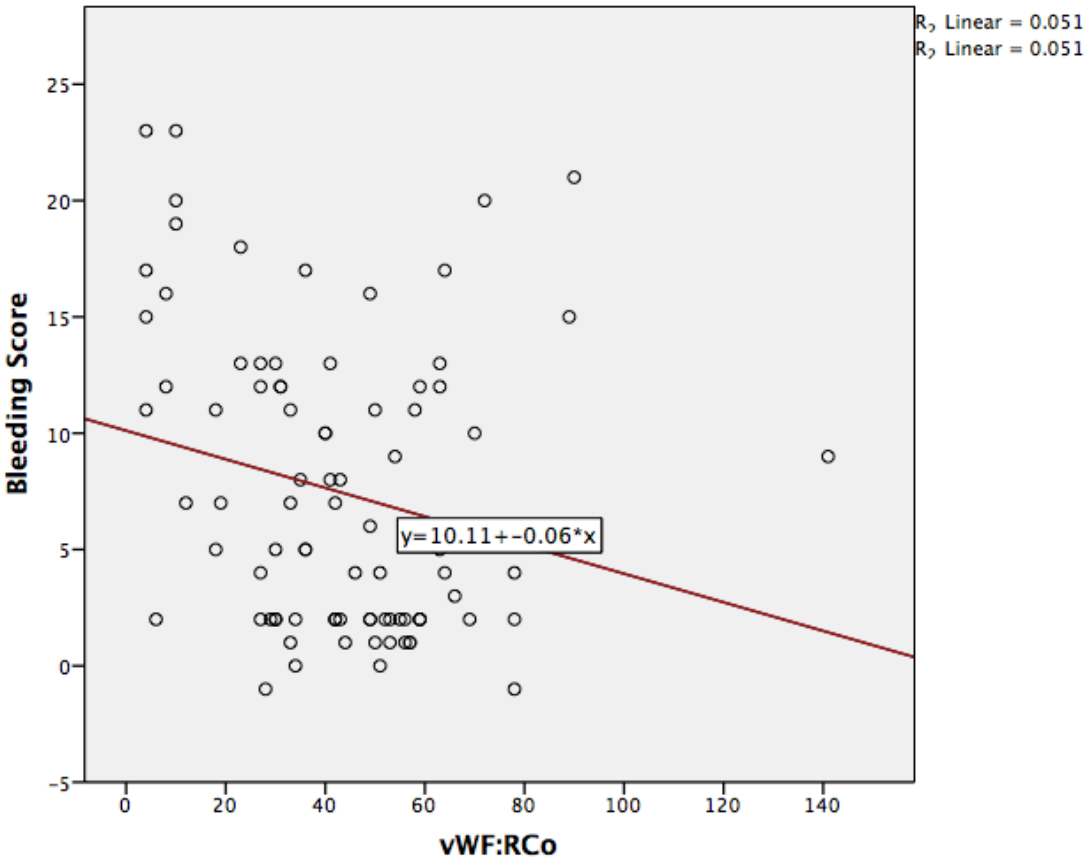
Figure 9.3 Correlation of bleeding score with VWF:Ag level



VWF:Ag; von Willebrand factor antigen

r = -0.23, r^2 = 0.06; p = 0.023

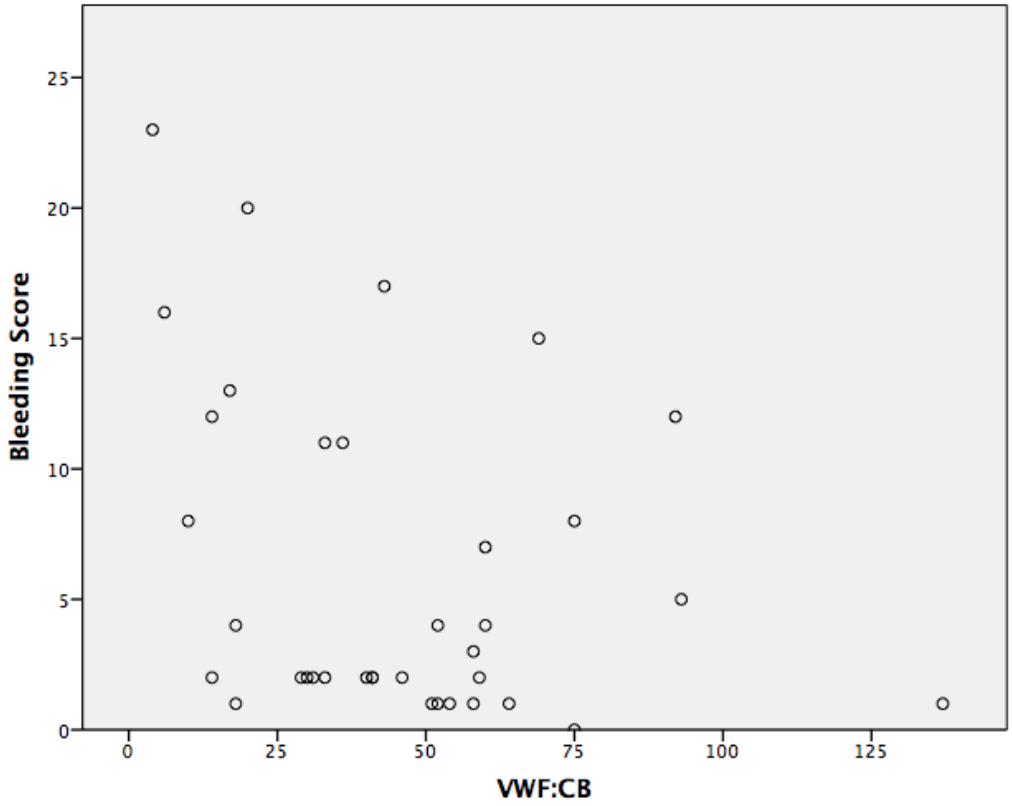
Figure 9.4 Correlation of bleeding score with VWF:RCo level



VWF:RCo; von Willebrand factor ristocetin cofactor

r = -0.21, r^2 = 0.051; p = 0.042

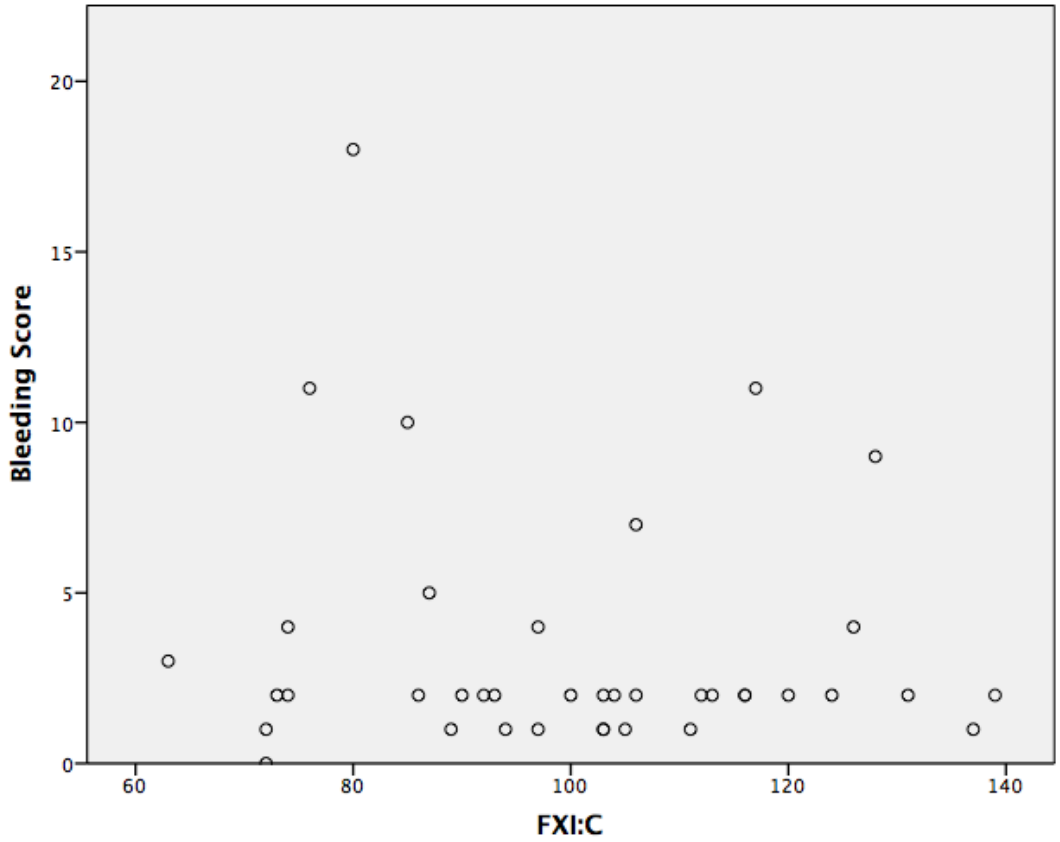
Figure 9.5 Correlation of bleeding score with VWF:CB activity level



VWF:CB, von willebrand factor collagen binding assay

r = -0.32, r^2 = 0.1, p = 0.06

Figure 9.6 Correlation of bleeding score with FXI activity level



FXI:C, factor XI activity level

r = -0.12, r^2 = 0.01, p = 0.46

Table 9.2 Correlation of bleeding score and haemostatic variables

	Mean ± Standard deviation	Correlation coefficient (*r*)	r^2	*p* value
VWF:Ag	51.7 (±22.8)	- 0.234	0.055	0.023
VWF:RCo	43.0 (±22.8)	- 0.226	0.051	0.034
VWF:CB	44.5 (±30)	- 0.315	0.099	0.07
FVIII:C	72.3 (±29)	- 0.431	0.186	< 0.001
FIX:C	100.4 (±19)	- 0.121	0.015	0.464

VWF:Ag, von Willebrand factor antigen; VWF:RCo, von Willebrand factor ristocetin activity level; VWF:CB, von Willebrand factor collagen binding activity level; FVIII:C, factor VIII activity level; FIX:C, factor IX activity level. Areas highlighted in grey are statistically significant ($p < 0.05$).

9.3.2 Effect of thombotic variables on bleeding score

No significant correlation was detected between anticoagulant variables (protein C, protein S and ATIII activity levels) and bleeding score. Table 8.3 shows the mean and standard deviation and correlation coefficient with bleeding score for all variables.

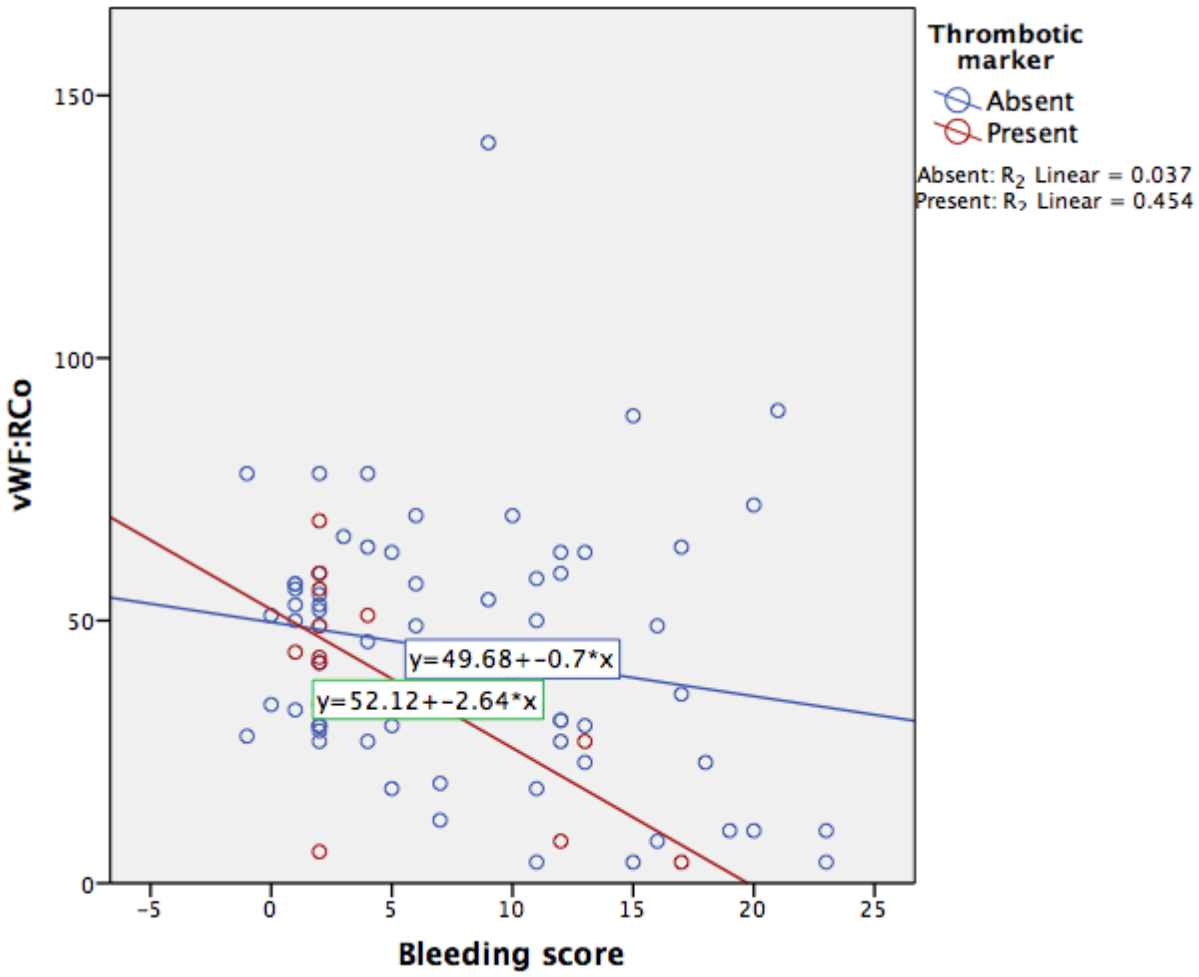
Table 9.3 Correlation of bleeding score and thrombotic variables

	Mean ± Standard deviation	**Correlation coefficient (*r*)**	***r*²**	***p* value**
Protein C:Ac	107.6 (21)	-0.064	0.004	0.526
Protein S:Ac	85.6 (18.9)	0.168	0.028	0.093
ATIII:Ac	100.4 (11.0)	0.172	0.030	0.086

Protein C:Ac, Protein C activity level; Protein S:Ac, Protein S activity level; ATIII:Ac, antithrombin III activity level

In the multiple regression analysis the presence of a thrombotic marker (either prothrombotic gene mutation or deficiency in protein C:Ac, protein S:Ac or ATIII:Ac) altered the correlation between VWF:RCo and bleeding score ($p = 0.015$) (Fig 9.5). The presence of a thrombotic marker caused a shift to the left in bleeding score. No other relationship between haemostatic variable and bleeding score was affected by the presence of a thrombotic marker.

Figure 9.7 Presence of a thrombotic marker alters relationship between VWF:RCo and bleeding score



VWF:RCo, von Willebrand factor ristocetin cofactor activity level

9.4 Discussion

Patients with severe haemophilia can have a milder bleeding tendency than expected [377]. Severe *f*8/*f*9 gene mutations, referred to as null mutations, are usually associated with virtually undetectable factor activity. The non-null mutations are usually associated with a variable factor level. However, different clinical phenotypes have been reported in patients with the same mutation [31, 32]. The basis for this heterogeneity in clinical expression of severe haemophilia is poorly understood.

Inherited thrombophilia, notably FV Leiden have been postulated to alter the bleeding tendency in patients with severe haemophilia. FV Leiden occurs with a relatively high frequency (3-7%) in the general population [378]. The mechanism by which FV Leiden affects the clinical phenotype of haemophilia involves increased thrombin generation by reducing thrombin downregulation through the activated protein C pathway [379]. A systematic review reported that co-inheritance of other thrombophilia, such as protein C and protein S deficiency, and prothrombin gene mutation, may affect the clinical phenotype and even result in thromboembolic disease in patients with severe haemophilia [380].

The clinical expression of VWD is very heterogeneous with a large variability in bleeding frequency and severity seen between patients. The bleeding phenotype is strongly associated with type of VWD, and the VWF:Ag. VWF:RCo and FVIII:C levels [381]. Tosetto *et al* developed and validated a bleeding score for patients with type 1 VWD, to quantify the number and severity of bleeding symptoms [369]. Bowman *et al* developed a condensed version of this bleeding score and applied it to

42 patients in whom VWD was previously diagnosed, including all types of VWD, and found a significant difference in bleeding score (with type 3 >> type 2 >> type 1, ANOVA p = 0.001) [370]. The effect of co-inheritance of thrombophilia on clinical expression of VWD patients has not been previously evaluated.

The findings of this study demonstrate that FVIII:C level is the strongest predictor of bleeding risk in women with VWD and haemophilia A carriers (Fig 9.2) as this variable had the strongest correlation with bleeding score. In haemophilia, this finding is expected as bleeding severity consistently correlates well with factor levels [34, 37, 382], however, in VWD the relationship is less clear. The fact that higher FVIII levels in women with VWD may be protective against bleeding requires further consideration and may have important implications in its clinical management.

Poor correlation was seen between bleeding score and VWF:CB assay (Fig 9.5). This is likely due to fact that the majority of women recruited to this study had type 1 VWD, without a functional impairment. Due to the limited sample size, all participants with VWD were analysed together. Poor correlation was also seen between bleeding score and FIX levels (Fig 9.6). The majority of patients included in this analysis had VWD, which does not affect FIX levels. Only a very small number (n = 2) of participants were haemophilia B carriers and therefore it was not possible to interrogate the correlation between bleeding tendency and factor level in these patients. Future studies that explore bleeding tendency in women IBDs would be more informative if the different types, and subtypes of VWD and haemophilia are analysed separately.

The presence of a co-inherited thrombophilia appears to influence the bleeding phenotype that is milder than expected in women with IBDs. The presence of a thrombotic marker alters the relationship between VWF:RCo level, with a lower than expected bleeding score seen in these patients (Fig 9.7, p = 0.015). This is an important finding, which may help to explain the variability in bleeding phenotype seen in women with IBDs.

A better understanding of bleeding phenotype is important when anticipating the bleeding risk and the requirement for prophylactic cover prior to a haemostatic challenge (the onset of menses in adolescence, in pregnancy and at delivery, or prior to undergoing any surgical procedure).

Women with VWD or haemophilia who have a lower than expected bleeding score should undergo thrombophilia screening as this may be of clinical significance. Not only is this important to gain a better appreciation of bleeding risk, but also could identify women who may be at increased risk for thromboembolic disease later in life. Patient with type 1 VWD experience age-related increase in VWF:Ag and VWF:RCo, which can result in normalisation of VWF levels over time [383]. It is also likely that carriers of haemophilia experience an age-dependent increase in FVIII:C level, which has been confirmed in patients with mild haemophilia [384]. A prothrombotic marker detected in these patients should result in consideration of thrombotic risk, depending on other factors. The use of the oral combined contraceptive pill or hormone replacement therapy (HRT), which is associated with increased thrombotic risk in the general population, may be contraindicated in these women. In addition, this has important implications in the management of women with coinheritance of IBDs and

thrombophilia during pregnancy. Thrombotic risk should be considered due to the pro-thrombotic state of pregnancy. Thromboprophylaxis should be administered especially if additional venous thromboembolism risk factors arise at delivery (i.e. operative delivery, postpartum haemorrhage, infection, immobility).

In this study, age resulted in higher bleeding scores. This finding is expected as patients are exposed to more haemostatic challenges (pregnancy, surgery) throughout their lifetime. In addition, women who are approaching menopause may experience heavy menstrual bleeding (HMB) related to gynaecological causes (endometrial pathology, fibroids). Further research should be untaken to assess bleeding phenotype, including thrombin generation studies, in women with co-inherited bleeding disorders and thrombophilia. International collaboration may be required to recruit sufficient number of women with rare IBDs. Prospective measurement of factor levels, bleeding score and frequency of thromboembolic complications with increasing age should be assessed in women with IBDs.

9.5 Conclusion

The presence of a thrombotic marker causes the bleeding score to be lower than expected for a given VWF:RCo level. This indicates that the co-inheritance of thrombophilias may be protective (lowers the bleeding tendency) in patients with low VWF:RCo levels. In addition, these patients may also be at increased risk of thromboembolism, especially as FVIII:C and VWF normalises with age, or during pregnancy, or with oral contraceptive medication. Thrombophilia testing should be considered in cases were there is as a discrepancy in phenotype and VWF:RCo level, to gain a better appreciation of bleeding and thrombotic risk in these patients.

CHAPTER 10

CONCLUSION AND FUTURE STUDIES

10.1 Overall conclusion

10.2 Suggestions for future research

10.1 Overall conclusion

Women with IBDs have increased obstetric and gynaecological morbidity due to increased risk of bleeding. Endometriosis is associated with a higher frequency of platelet aggregation defects to one agonist ($p = 0.005$), multiple agonists ($p < 0.05$) and epinephrine ($p = 0.02$). Women with severe (stage IV) endometriosis have reduced VWF activity levels. These findings could be explained by increased retrograde menstruation resulting in higher rate of 'seeding' within the pelvic cavity. Impaired local haemostasis within endometriotic implants result in recurrent cyclical internal bleeding, exacerbating the spread of the condition throughout the pelvic cavity. This could have important implications in the management of endometriosis. Targeted screening for platelet aggregation defects in women with a positive bleeding history could reduce the risk of perioperative bleeding. Women found to have endometriosis and a PFD should be advised to avoid anti-platelet analgesics.

The rate of uptake for invasive prenatal diagnosis and termination of affected pregnancies is decreasing among carriers of haemophilia. Severity of haemophilia and family history of haemophilia-related complications affect the uptake for invasive testing. Women are choosing to have non-invasive methods such as ffDNA to determine fetal gender, which is more acceptable and associated with less psychological stress. Invasive testing may be carried out in order to make a plan for delivery, and in cases where the fetus is unaffected the woman may be referred back to her local maternity unit. A non-invasive method using ffDNA in maternal plasma to rule out haemophilia in the fetus is highly welcomed by carriers of haemophilia and

the uptake is likely to be high. It will provide women with more birth options, and has the potential to reduce the caesarean section rate among carriers of haemophilia.

Systematic review of the current literature demonstrates that newborns with haemophilia have 44 fold-increased risk of symptomatic ICH at birth (OR 44; 95% CI 34.7-57.1, $p < 0.01$) compared to the general population. The odds of ECH in newborns with haemophilia compared to the general population are 8 (95% CI 5.38-12.6) ($p < 0.01$). In the meta-analysis the odds of experiencing ICH at birth following an AVD are over four fold increased compared to SVD (OR 4.39; 95%CI, 1.46-13.7) in newborns with haemophilia. The odds of experienced ICH following CS are reduced compared to SVD (OR 0.34; 95%CI, 0.14-0.83).

The incidence of asymptomatic ICH in the general population is around 24%. The incidence of asymptomatic ICH in newborns with IBDs and how this evolves is not known. The current literature is limited to a small number of retrospective case review studies that report incidence of symptomatic ICH and extracranial haemorrhage only. There is only one published study that assesses incidence of asymptomatic ICH through radiological screening in neonates with haemophilia [327]. All women who are pregnant with a potentially affected fetus should have a frank discussion within a multidisciplinary antenatal setting, where the risks of cranial bleeding and risks associated with CS are discussed. Women who are pregnant with a fetus potentially affected by a moderate/severe IBD should be offered a ELCS to reduce the risk of bleeding complications at delivery.

Cranial MRI carried out within 72 hours of birth in eight newborns with severe/moderate IBD proved feasible without sedation, to exclude asymptomatic ICH. In this small series none of the affected neonates showed evidence of asymptomatic ICH occurring at birth on MRI screening. There were three episodes of cranial bleeding reported in two infants by 6 months of age. One male infant was born with cephalohaematoma following an EMCS. He later suffered a spontaneous ICH with high titre inhibitor development by 4 months of age. The detection and treatment of any asymptomatic ICH at birth has potential to reduce serious long-term neurological sequelae or even death in neonates with IBDs. This would provide a strong argument for MRI screening at birth to be standard care. Larger collaborative studies are required to properly assess the incidence, outcome and effect of MOD of asymptomatic ICH in neonates with IBDs.

Pregnancy induces a hypercoagulable status in the third trimester of pregnancy in women with FXI deficiency, due to increase in other coagulation factors. Pregnancy causes an increase in clot consolidation (reduced CFT), and an increase in clot strength (increased MCF) in pregnant women with FXI deficiency compared to non-parturient controls. On average, women with FXI deficiency take longer to clot (prolonged CT) compared to both parturient and non-parturient controls. This was more pronounced in women with severe FXI deficiency. If hypercoagulable changes are not achieved by the third trimester of pregnancy (i.e. prolonged CT and CFT, reduced alpha angle and MCF), these women should be considered as having an increased risk of bleeding.

Women with FXI deficiency have an unpredictable bleeding risk at delivery that is challenging to predict and manage. They require an individualised risk assessment incorporating their bleeding history or score, FXI level and ROTEM® analysis. Using ROTEM® analysis in the third trimester evaluation of bleeding risk in pregnant women with FXI deficiency enables treatment decisions for providing haemostatic cover for labour and delivery, and results in less blood product usage. Woman with severe FXI deficiency can be managed safely with tranexamic acid, including provision of regional anaesthesia, if ROTEM® parameters indicate hypercoagulable status. These patients can avoid the risk of thrombotic complications that are associated with blood product usage. Using ROTEM® analysis to guide therapy had no discernable effect on the overall rate of PPH, which is still high (12% and 2% for primary PPH and secondary PPH, respectively) compared to rates in the general obstetric population (3-6% and 1%, respectively). Larger prospective, randomised trials may provide more evidence to support the role of ROTEM® on bleeding risk in pregnant women with FXI deficiency.

The presence of a thrombotic marker (anticoagulant deficiency or prothrombotic gene mutation) alters the bleeding score for a given VWF:RCo level in women with IBDs ($p = 0.015$). This indicates that co-inheritance of thrombophilia may be protective (i.e. reduce bleeding phenotype). Bleeding score increases with age with positive correlation seen ($r = -0.44$, $r^2 = 0.19$; $p < 0.001$). However, factor levels (VWF and FVIII) also increase with age, and thus the risk of thromboembolic disease should be considered in these patients.

10.2 Suggestions for future research

The finding of increased frequency of platelet aggregation defects in women with endometriosis, and reduced VWF activity level in women with more severe rASRM laparoscopic staging requires further investigation. Platelet testing in a larger cohort of women, including control subjects who have no laparoscopic evidence of endometriosis, should be undertaken.

The effect of administering haemostatic therapy in addition to hormonal treatments, to women with a bleeding disorder and endometriosis should be assessed in a clinical trial. Oral tranexamic acid or DDAVP (i.e. intranasal desmopressin) can be administered prior to or during menses to assess if this affects endometriosis symptoms or rate of endometriosis progression.

Further research is required to develop a non-invasive prenatal diagnostic test using ffDNA to detect haemophilia mutations, specifically the intron 22 mutation, which causes about half the cases of severe haemophilia A. This will provide a universal test that can be used worldwide for a significant number of severe cases that are more likely to opt for and benefit from prenatal diagnosis. Future studies should evaluate the impact of a non-invasive prenatal diagnostic test. Having a non-invasive test available could enable treatment decision in early pregnancy, and has the potential to provide more birth options for carriers of haemophilia. Whether the availability of such a test impacts on reproductive choices and CS rates in carriers of haemophilia should be assessed. A qualitative study should be undertaken to properly assess

anxiety levels of women who undergo invasive procedures to ensure that appropriate levels of psychological support are available. This would provide a stronger argument to make a non-invasive test widely available.

Cranial MRI within 72 hours of birth was proven to be feasible and safe and able to exclude asymptomatic ICH in newborns with IBDs. There is now an urgent need to encourage other haemophilia centres to adopt this protocol. Only a larger, collaborative, multi-centred study will properly assess the incidence of asymptomatic ICH in newborns with moderate/severe IBDs. Where possible, screening should be carried out at multiple time points during the first few weeks of life to determine the optimum timing and highest detection rate for asymptomatic head bleeds. Large numbers of participants are required to assess the effect of MOD on cranial bleeding and provide better evidence on optimum management. In addition, maternal outcomes such as frequency of PPH and complications resulting from multiple CS could be evaluated in women with IBDs who opt for ELCS to reduce the risk of neonatal bleeding. This will only be achieved through multi-centre and multinational collaboration. Long-term developmental studies should be undertaken in neonates with moderate/severe IBDs who have undergone MRI screening at birth. In addition, treatment regimes, bleeding episodes, and rates of inhibitor development should continue to be reviewed in infants with haemophilia.

An interventional study with prospective randomisation will help to evaluate the effect of ROTEM® analysis on rates of PPH in women with FXI deficiency. Postpartum ROTEM® analysis and measurement of clot lysis using additional ROTEM® assays (FIBTEM and EXTEM) should be undertaken in women with FXI

deficiency. This would provide useful data to guide the optimum duration of postpartum haemostatic therapy, and reduce risk of secondary PPH that may occur once other clotting factors return to pre-pregnancy levels.

Bleeding phenotype and correlation with factor levels should be interrogated separately in women with different types and subtypes of VWD, and in haemophilia carriers. Women found to have a lower bleeding score than expected should be assessed for an underlying thrombophilia. Further large collaborative studies should assess whether the presence of a thrombotic marker influences the bleeding score in women with different types of IBDs, including rare IBDs. Bleeding phenotype should be assessed with thrombin generation studies or thromboelastometry in women with co-inherited bleeding disorders and thrombophilia to gain a better appreciation of bleeding and thrombotic risk. Prospective measurement of factor levels, bleeding score and frequency of thromboembolic complications with increasing age should be assessed in women with IBDs.

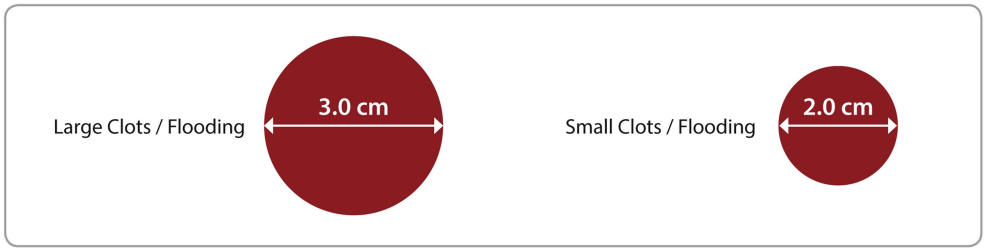
APPENDIX 1

Pictorial Blood Assessment Chart

Day 1 of Menstruation:

D D M M M 2 0 Y Y Y Y

Score	Towels	1	2	3	4	5	6	7
			No bleeding ☐	No bleeding ☐	No bleeding ☐	No bleeding ☐	No bleeding ☐	No bleeding ☐
1								
5								
20								
	Tampons							
1								
5								
10								
1	Small Clots / Flooding							
5	Large Clots / Flooding							



APPENDIX 2

PAIN IMPACT QUESTIONNAIRE (PIQ-6)

If you are not sure about a question, please give the best answer you can. There are no right or wrong answers to these questions. Thank you for completing this survey! For each of the following questions, please select the response that best describes your answer

1. How much bodily pain have you had during the past 4 weeks?

None	Very mild	Mild	Moderate	Severe	Very severe
☐	☐	☐	☐	☐	☐

2. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all	A little bit	Moderately	Quite a lot	Extremely
☐	☐	☐	☐	☐

3. In the past 4 weeks, how much of the time did pain interfere with your enjoyment of life?

Never	Rarely	Sometimes	Very often	Always
☐	☐	☐	☐	☐

4. In the past 4 weeks, how often did pain make simple tasks hard to complete?

Never	Rarely	Sometimes	Very often	Always
☐	☐	☐	☐	☐

5. In the past 4 weeks, how often were your leisure activities affected by your pain (including exercise and hobbies)?

Never	Rarely	Sometimes	Very often	Always
☐	☐	☐	☐	☐

6. In the past 4 weeks, how often did pain make you feel fed up and frustrated?

Never	Rarely	Sometimes	Very often	Always
☐	☐	☐	☐	☐

APPENDIX 3

Haemophilia Carrier Questionnaire

Part 1 – General Information about you

Name:

Date of Birth:

Age:

Type of haemophila (A or B): _____ Baseline level: _____

Severity of haemophilia in family (if known)

Mild □
Moderate □
Severe □

Family history of haemophilia-related complications:

HIV/Hepatitis □
Inhibitors □
Early death □

Other, please specify_____
_____

What is your relationship to the affected individual in the family? (daughter/mother/sister)

_____

Have you undergone genetic testing to confirm your carrier status?

Yes □
No □
Not sure □

Haven't had any children? please go on to Part 3

Part 2 – Pregnancy related information

For each ongoing pregnancy please complete:-

Pregnancy No.1_____

Year of pregnancy:

Were you aware of your carrier status prior to or during this pregnancy?
Yes □ No □

Were you offered prenatal diagnosis? Yes □ No □
Did you opt for prenatal diagnosis? Yes □ No □

Type of prenatal diagnosis:
Was this **invasive** prenatal diagnosis?

Chorionic Villus Sampling □
Amniocentesis in 2nd Trimester (week 15-28) □

Did miscarriage occur due to invasive test? Yes □ No □

If unsure, please comment_____
_____
_____

Was this **non-invasive** prenatal diagnosis?

Ultrasound to determine gender □
(please state gestational age this was performed_____)
Free fetal DNA (blood test) to determine gender □
Free fetal DNA (blood test) to determine haemophilia status (currently only available in the research and development stage) □

Was this **3rd Trimester Amniocentesis** (usually performed after weeks 34 gestation)? Yes □ No □

Complications:

Unsuccessful □
Required more than one attempt □
Premature labour □
Premature rupture of membranes □
Fetal or maternal bleeding □

If unsure, please comment_____
_____
_____

Part 2 – Pregnancy related information

Reasons for **opting** for prenatal diagnosis

To undergo termination of affected foetus	□
To be aware of haemophilia status so that a management plan could be made about delivery	□
Reasons other than haemophilia (please state)	□

_____
_____

Reasons for **not opting** for prenatal diagnosis

Fear of fetal loss from prenatal diagnosis	□
Fear of procedure and potential adverse effects	□
Did not think haemophilia was severe enough to warrant prenatal diagnosis	□
Weren't aware or given options for prenatal diagnosis	□
Accepting of possibility of having a child with haemophilia	□
Being opposed to having a termination	□
Other (please state)	□

_____
_____

Outcome of prenatal diagnosis

Fetus male	Yes □ No □
Fetus affected	Yes □ No □

Opted for termination during this pregnancy? Yes □ No □

What was the **mode of delivery**?

Normal vaginal delivery	□
Ventouse delivery	□
Forceps delivery	□
Emergency casearean section	□
Elective caesarean section	□

If you (and the doctors) were unsure of the haemophilia status of the fetus were you offered **caesarean section** as mode of delivery?

Yes □ No □

Part 2 – Pregnancy related information

For each ongoing pregnancy please complete:-

Pregnancy No.2_____

Year of pregnancy:

Were you aware of your carrier status prior to or during this pregnancy?
Yes □ No □

Were you offered prenatal diagnosis? Yes □ No □
Did you opt for prenatal diagnosis? Yes □ No □

Type of prenatal diagnosis:
Was this **invasive** prenatal diagnosis?

Chorionic Villus Sampling □
Amniocentesis in 2nd Trimester (week 15-28) □

Did miscarriage occur due to invasive test? Yes □ No □

If unsure, please comment_____
_____
_____

Was this **non-invasive** prenatal diagnosis?

Ultrasound to determine gender □
(please state gestational age this was performed_____)
Free fetal DNA (blood test) to determine gender □
Free fetal DNA (blood test) to determine haemophilia status (currently only available in the research and development stage) □

Was this **3rd Trimester Amniocentesis** (usually performed after weeks 34 gestation)? Yes □ No □

Complications:

Unsuccessful □
Required more than one attempt □
Premature labour □
Premature rupture of membranes □
Fetal or maternal bleeding □

If unsure, please comment_____
_____
_____

Part 2 – Pregnancy related information

Reasons for **opting** for prenatal diagnosis

To undergo termination of affected foetus	□
To be aware of haemophilia status so that a management plan could be made about delivery	□
Reasons other than haemophilia (please state)	□

_____

_____

Reasons for **not opting** for prenatal diagnosis

Fear of fetal loss from prenatal diagnosis	□
Fear of procedure and potential adverse effects	□
Did not think haemophilia was severe enough to warrant prenatal diagnosis	□
Weren't aware or given options for prenatal diagnosis	□
Accepting of possibility of having a child with haemophilia	□
Being opposed to having a termination	□
Other (please state)	□

_____

_____

Outcome of prenatal diagnosis

Fetus male	Yes □ No □
Fetus affected	Yes □ No □

Opted for termination during this pregnancy? Yes □ No □

What was the **mode of delivery**?

Normal vaginal delivery	□
Ventouse delivery	□
Forceps delivery	□
Emergency casearean section	□
Elective caesarean section	□

If you (and the doctors) were unsure of the haemophilia status of the fetus were you offered **caesarean section** as mode of delivery?

Yes □ No □

Part 2 – Pregnancy related information

For each ongoing pregnancy please complete:-

Pregnancy No.3_____

Year of pregnancy:

Were you aware of your carrier status prior to or during this pregnancy?
Yes □ No □

Were you offered prenatal diagnosis? Yes □ No □
Did you opt for prenatal diagnosis? Yes □ No □

Type of prenatal diagnosis:
Was this **invasive** prenatal diagnosis?

Chorionic Villus Sampling □
Amniocentesis in 2nd Trimester (week 15-28) □

Did miscarriage occur due to invasive test? Yes □ No □

If unsure, please comment_____
_____
_____

Was this **non-invasive** prenatal diagnosis?

Ultrasound to determine gender □
(please state gestational age this was performed_____)
Free fetal DNA (blood test) to determine gender □
Free fetal DNA (blood test) to determine haemophilia status (currently only available in the research and development stage) □

Was this **3rd Trimester Amniocentesis** (usually performed after weeks 34 gestation)? Yes □ No □

Complications:

Unsuccessful □
Required more than one attempt □
Premature labour □
Premature rupture of membranes □
Fetal or maternal bleeding □

If unsure, please comment_____
_____
_____

Part 2 – Pregnancy related information

Reasons for **opting** for prenatal diagnosis

To undergo termination of affected foetus	□
To be aware of haemophilia status so that a management plan could be made about delivery	□
Reasons other than haemophilia (please state)	□

_____
_____

Reasons for **not opting** for prenatal diagnosis

Fear of fetal loss from prenatal diagnosis	□
Fear of procedure and potential adverse effects	□
Did not think haemophilia was severe enough to warrant prenatal diagnosis	□
Weren't aware or given options for prenatal diagnosis	□
Accepting of possibility of having a child with haemophilia	□
Being opposed to having a termination	□
Other (please state)	□

_____
_____

Outcome of prenatal diagnosis

Fetus male	Yes □ No □
Fetus affected	Yes □ No □

Opted for termination during this pregnancy? Yes □ No □

What was the **mode of delivery**?

Normal vaginal delivery	□
Ventouse delivery	□
Forceps delivery	□
Emergency casearean section	□
Elective caesarean section	□

If you (and the doctors) were unsure of the haemophilia status of the fetus were you offered **caesarean section** as mode of delivery?

Yes □ No □

Part 3 – Future developments in prenatal diagnosis and haemophilia care

Did you ever made a conscious decision not to have children, or not to have any more children? Yes □ No □

If so, could you please state reasons, or factors influencing your decision?

Fear of passing haemophilia on to your child	□
Previous experience of haemophilia	□
The stress of going through prenatal tests	□

Other, please state

_____
_____
_____

If you currently fell pregnant, would you opt for 3rd trimester amniocentesis to diagnosis the haemophilia status of your child, considering there is a 1% risk of preterm labour?

Yes □ No □ Unsure □

If available, would you opt for a non-invasive blood test to diagnosis the haemophilia status of your child, considering there is no risk to you or the pregnancy?

Yes □ No □ Unsure □

Would having the option of diagnosing haemophilia safely, effectively and early on in pregnancy influence your decision to have children?

Yes □ No □ Unsure □

Further comments if you wish:

_____
_____
_____
_____

What is your estimation as to the risk of intracranial haemorrhage (bleeding in the brain) occuring during delivery of a male baby with haemophilia?

1/1000 □ 1% □ 5% □ 10% □

Which of these figures would you consider to be a signifcant risk?

1/1000 □ 1% □ 5% □ 10% □

At what level of risk would you opt for caesarean section?

1/1000 □ 1% □ 5% □ 10% □

Further comments if you wish:

_____
_____
_____
_____

If you are happy to be contacted by the Clinical Research Doctor, please could you provide us with your contact telephone number below? This would very useful if we need to clarify any information you have provided.

Telephone number:

MANY THANKS FOR GIVING UP YOUR TIME TO COMLETE THIS QUESTIONNAIRE. YOUR FEEDBACK IS APPRECIATED.

Please return this questionnaire in the pre-paid envelope provided to:

Miss Rezan Kadir,
The Haemophilia Centre,
The Royal Free Hospital,
Pond Street,
Hampstead,
London, NW32QG

APPENDIX 4 Condensed MCMDM-1 Bleeding questionnaire

Scoring Key

Symptom	Score -1	0	1	2	3	4
Epistaxis	--	No or trivial (less than 5)	> 5 or more than 10'	Consultation only	Packing or cauterization or antifibrinolytic	Blood transfusion or replacement therapy or desmopressin
Cutaneous	--	No or trivial (< 1cm)	> 1 cm and no trauma	Consultation only	--	--
Bleeding from minor wounds	--	No or trivial (less than 5)	> 5 or more than 5'	Consultation only	Surgical hemostasis	Blood transfusion or replacement therapy or desmopressin
Oral cavity	--	No	Referred at least one	Consultation only	Surgical hemostasis or antifibrinolytic	Blood transfusion or replacement therapy or desmopressin
Gastrointestinal bleeding	--	No	Associated with ulcer, portal hypertension, hemorrhoids, angiodysplasia	Spontaneous	Surgical hemostasis, blood transfusion, replacement therapy, desmopressin, antifibrinolytic	--
Tooth extraction	No bleeding in at least 2 extractions	None done or no bleeding in 1 extraction	Reported, no consultation	Consultation only	Resuturing or packing	Blood transfusion or replacement therapy or desmopressin
Surgery	No bleeding in at least 2 surgeries	None done or no bleeding in 1 surgery	Reported, no consultation	Consultation only	Surgical hemostasis or antifibrinolytic	Blood transfusion or replacement therapy or desmopressin
Menorrhagia	--	No	Consultation only	Antifibrinolytics, pill use	Dilation & curettage, iron therapy, ablation	Blood transfusion or replacement therapy or desmopressin or hysterectomy
Postpartum hemorrhage	No bleeding in at least 2 deliveries	None done or no bleeding in 1 surgery	Consultation only	Dilation & curettage, iron therapy, antifibrinolytics	Blood transfusion or replacement therapy or desmopressin	Hysterectomy
Muscle hematomas	--	Never	Post trauma, no therapy	Spontaneous, no therapy	Spontaneous or traumatic, requiring desmopressin or replacement therapy	Spontaneous or traumatic, requiring surgical intervention or blood transfusion
Hemarthrosis	--	Never	Post trauma, no therapy	Spontaneous, no therapy	Spontaneous or traumatic, requiring desmopressin or replacement therapy	Spontaneous or traumatic, requiring surgical intervention or blood transfusion
Central nervous system bleeding	--	Never	--	--	Subdural, any intervention	Intracerebral, any intervention

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