CARDIFF UNIVERSITY

Master of Philosophy Thesis

Thrombin Generation in Obstetric Haemorrhage

Author:

Julia FREYER MARTINS PEREIRA

Supervisors:

Prof Peter William COLLINS

Prof Judith Elizabeth HALL

Dr Peter Vincent JENKINS

A thesis submitted in fulfilment of the requirements for the degree of Master of Philosophy in the

Division of Population Medicine

05 November 2023

Word count: 22.745

“As we acquire more knowledge, things do not become more comprehensible, but more mysterious.”

Albert Schweitzer

CARDIFF UNIVERSITY

Abstract

Faculty: Division of Population Medicine

Master of Philosophy

Thrombin Generation in Obstetric Haemorrhage

by Julia FREYER MARTINS PEREIRA

The haemostatic system undergoes significant changes during pregnancy, particularly at term, leading to alterations in coagulation factors and anticoagulants. These changes result in an increase in procoagulant factors, such as, fibrinogen, factor VIII, and von Willebrand factor, along with reduced levels of anticoagulants like protein S. Consequently, a procoagulable state is established.

Postpartum haemorrhage (PPH) is the leading cause of maternal mortality worldwide and may be caused or exacerbated by haemostatic impairment. Current research efforts have primarily focussed on examining coagulation factors and traditional laboratory investigations to monitor PPH and guide replacement of coagulation factors with fresh frozen plasma (FFP). Thrombin generation can be used to measure the integrated effects of procoagulant factors and so give an overview of haemostatic competence. There has been limited research into changes in thrombin generation during PPH and its potential to identify women who might benefit from FFP infusion.

This thesis investigated thrombin generation in obstetric patients experiencing PPH and compared the results with non-bleeding pregnant women and non-pregnant participants. The main findings are that thrombin generation confirmed the prothrombotic state at term and remained raised or normal during almost all cases of PPH. This suggests that FFP would not improve haemostasis in most cases of PPH. By examining thrombin generation, a more comprehensive understanding of the haemostatic alterations in PPH can be obtained with the aim to improve individualised treatment.

Acknowledgements

The work presented in this document would not have been possible without the support and guidance of various people. Most prominent amongst these individuals, are my supervisors, Professor Peter Collins, Professor Judith Hall, and Dr Vincent Jenkins. I am eternally grateful for their continuous support, direction, and considerable insight in all aspects of this thesis.

Furthermore, I would like to express my deepest thanks to Dr Lucy de Lloyd, Dr Sarah Bell and everyone in the Obstetric Plus research team at the University Hospital of Wales for enabling me to undergo this Master of Philosophy project. Their generous support, through a partial scholarship with the OBS+ Research Fund, has been truly invaluable as well as their help throughout the process. I would also like to thank the patients for consenting for their samples to be used in this project to further improve our understanding of postpartum haemorrhage. Many thanks also to the routine laboratory staff for adding to their already busy workload by processing the initial receipt of samples, the storage, and collection.

Finally, I would like to thank my family and friends but in particularly my husband, who has always been there for me throughout the entire process.

Table of Contents

List of figures

List of tables

List of abbreviations

aPTT	Activated partial thromboplastin time
APH	Antepartum haemorrhage
AT III	Antithrombin III
CAT	Calibrated automated thrombography
CS	Caesarean section
DIC	Disseminated intravascular coagulation
ETP	Endogenous thrombin potential
F	Factor
FI	Factor I (fibrinogen)
FII	Factor II
FV	Factor V
FVII	Factor VII
FVIII	Factor VIII
FIX	Factor IX
FX	Factor X
FXI	Factor XI
FXII	Factor XII
FXIII	Factor XIII
FDP	Fibrinogen degradation products
FFP	Fresh frozen plasma
GPIIbIIIa	Glycoprotein IIb/IIIa
HELLP	haemolysis, elevated liver enzymes, low platelets syndrome
IQR	Interquartile range
K_2EDTA	Dipotassium ethylenediaminetetraacetic acid
MBL	Measured blood loss
NA	Not applicable
PAI-1	Plasminogen activator inhibitor 1
PAI-2	Plasminogen activator inhibitor 2
PPH	Postpartum haemorrhage
PAR	Protease-activated receptor

PPP	Platelet poor plasma
PRBC	Packed red blood cells
PT	Prothrombin time
SD	Standard deviation
TAFI	Thrombin activatable fibrinolysis inhibitor
TF	Tissue factor
TG	Thrombin generation
TGA	Thrombin generation assay
TM	Thrombomodulin
TFPI	Tissue factor pathway inhibitor
TXA	Tranexamic acid
vWF	von Willebrand factor
WHO	World health organisation

Dedicated to my parents, Annett and Gunter Freyer, who encouraged me to be the best that I can be and for enabling me this journey from an early stage.

Chapter 1

1 Introduction

1.1 Pregnancy

Normal pregnancy induces significant physiological changes resulting from hormonal, metabolic, and mechanical factors [1]. These adaptations are necessary to meet metabolic demands of the growing foetus and prepare the female body for pregnancy and birth. Respiratory adaptations, e.g., increased minute ventilation through higher respiratory rate and tidal volume, aid oxygen delivery, while cardiac output rises to support enhanced uterine blood flow [2]. Haematological changes involve an increase in both red blood cell and plasma volume [2] and haemostatic changes will be the focus of this thesis.

1.2 Haemostasis

1.2.1 Introduction

Haemostasis is a multifaceted, dynamic, and self-regulating process designed to stop bleeding at the site of vascular injury. It derives from the Greek words *haeme* meaning blood and *stasis* meaning causing to stop [3].

In a healthy individual, haemostasis maintains a delicate equilibrium between coagulation, anticoagulation, and fibrinolysis [4]. Traditionally, it is described as being comprised of primary and secondary haemostasis, with primary haemostasis involving the formation of a platelet plug through platelet adhesion and aggregation and secondary haemostasis resulting in fibrin formation, which stabilises the platelet plug [5]. Historically, it was divided into intrinsic and extrinsic pathways that converge into a common pathway. However, since this disregards the link between primary and secondary haemostasis, it fails to accurately describe coagulation in vivo by overemphasizing the extrinsic pathway [6] [7] [8] [9].

The cell-based model (see Figure 1) provides a more comprehensive explanation of haemostasis, describing four interconnected steps: initiation, amplification, propagation, and termination [10]. The ultimate goal of haemostasis is to generate thrombin, a serine protease, that converts fibrinogen to fibrin, leading to clot formation [11]. Thrombin generation, however, is not linear throughout. Initiation takes place when blood and freely circulating factor VII is exposed to tissue factor (a transmembrane protein) bearing cells, such as fibroblasts and smooth muscle cells, which are localised outside the vasculature [12]. Factor VII binds to TF initiating a positive feedback loop, activating additional FVII and producing more TF-FVIIa complexes. These complexes activate small amounts of FX and FIX. FXa can subsequently activate factor V and, as a prothrombinase complex (FXa/FVa), activate factor II to generate a small amount of factor IIa (thrombin) (3-5% of total thrombin) [12] [13] [14].

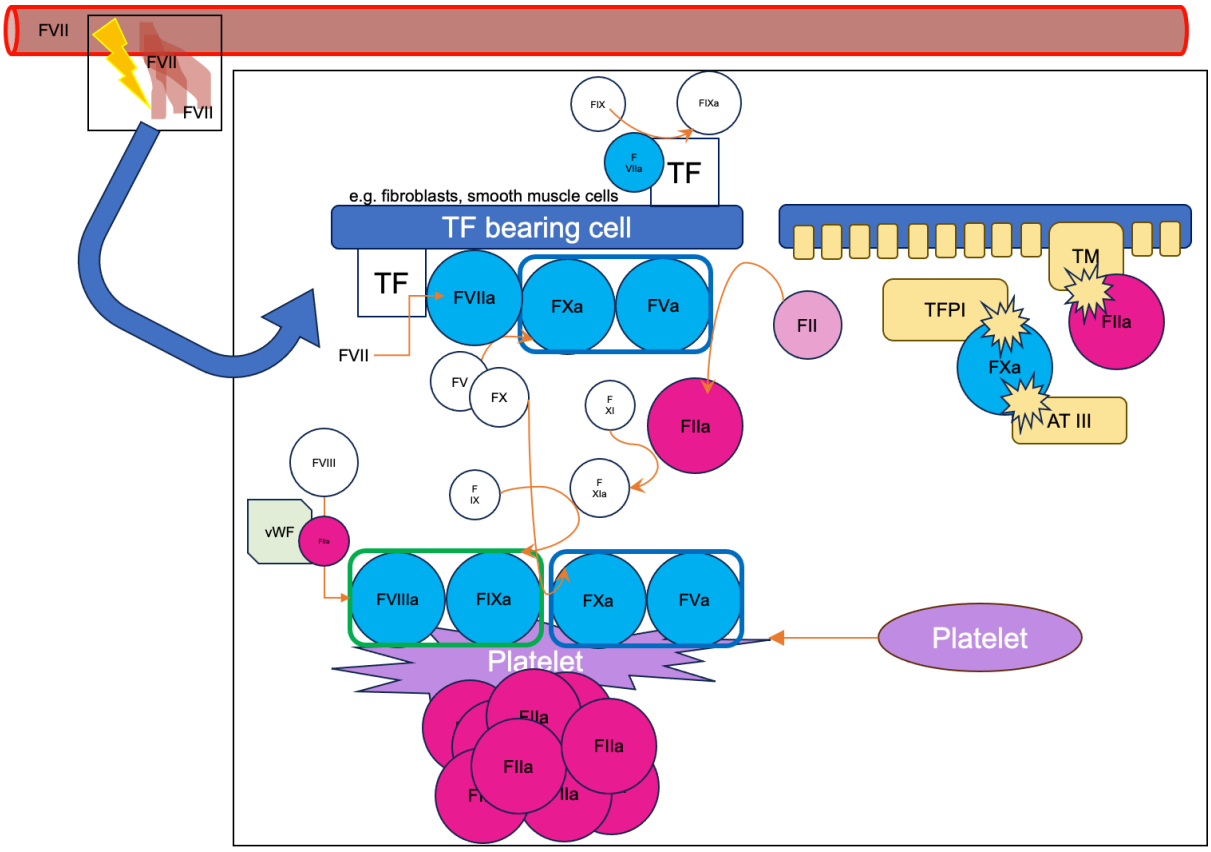

Figure 1. Cell-based model of haemostasis.

Inspired by Monroe & Hoffmann and Metze et al. [15] [16]. *FII (factor II, prothrombin), FIIa (activated factor II, thrombin), AT III (antithrombin III), FVa (activated factor V), FVIIa (activated factor VII), FVIIIa (activated factor VIII), FIXa (activated factor FIX), FXa (activated factor FX), FXIa (activated factor FXI), TF (tissue factor), TFPI (tissue factor pathway inhibitor), TM (thrombomodulin), vWF (von-Willebrand factor).*

During the second phase, amplification, the small amount of thrombin of the initiation phase acts as a potent platelet activator. It diffuses away from the TF bearing cell and activates platelets causing a shape change, activation of glycoprotein IIbIIIa (GPIIb/IIIa) and rapid externalisation of phosphatidylserine in the membrane [12] [13]. The externalised phosphatidylserine supports further coagulation reactions on the platelet surface. Furthermore, thrombin leads to the activation of the cofactors FVIII and FV to FVIIIa and FVa. Thrombin also activates FXI, which then cleaves FIX to FIXa. FVIIIa combines with factor IXa to form the intrinsic tenase complex, which rapidly converts FX to its active form FXa. FXa in turn combines with FVa to lead to accelerated formation of more prothrombinase complexes [12] [13].

During the propagation phase, the prothrombinase complex activity is exponentially enhanced by the tenase complex, ultimately resulting in a burst of rapid thrombin generation. Thrombin cleaves fibrinopeptide A and B from fibrinogen (factor I) to form fibrin. Once enough fibrin is produced, it polymerises to form an insoluble fibrin matrix which stabilises the platelet plug and forms the clot [13]. However, thrombin does not only contribute to producing fibrin but also promotes the stabilization of the platelet plug within a fibrin meshwork by activating FXIII, which forms cross-linked fibrin [13].

During the termination phase, to control coagulation and prevent thrombotic occlusion of intact blood vessels, natural anticoagulants (including tissue factor pathway inhibitor, protein C, protein S, antithrombin III, heparan sulfate proteoglycans, adenosine diphosphatase activity) are mobilized, along with the activation of the fibrinolytic process [13].

The cell-based model assigns importance to FXIa as a potent enhancer on the platelet surface. The primary function of FXIa is to augment the quantity of FIXa on the surface of platelets. This increased presence of FIXa promotes the production of FXa on the platelet surface, resulting in an amplified generation of thrombin. Therefore, while FXI itself is not indispensable for thrombin generation on the platelet surface, it acts as an auxiliary mechanism, enhancing the process [15].

Abnormalities of any haemostatic step can lead to imbalances and thus coagulopathy, which is broadly defined as any single or multiple coagulation factor or platelet deficiency or dysfunction [4] [17]. It is not known whether coagulopathies associated with trauma differ from those associated with postpartum haemorrhage (PPH). Extensive research has been conducted into trauma-induced coagulopathy, aiming to explain the underlying haematological abnormalities. Two hypotheses have emerged: the disseminated intravascular coagulation (DIC) -fibrinolysis hypothesis, and the activated protein C hypothesis [4]. The DIC-fibrinolysis hypothesis suggests that bleeding is due to hypoperfusion, shock, and endothelial injury that leads to "increased thrombin-generating potential, lower antithrombin levels, consumption of clotting factors, decreased fibrinogen, increased levels of fibrinogen degradation products (FDP), and a higher FDP/D-dimer ratio" [4]. While the activated protein C hypothesis agrees that bleeding is secondary to hypoperfusion, shock, and endothelial injury, it further proposes the activation of anticoagulation via activated protein C to be an important cause [4]. Activated protein C inhibits factor VIIIa, Va and thus the tenase and prothrombinase complexes, as well as stimulates hyperfibrinolysis by inhibiting plasminogen activator inhibitor (PAI)-1, resulting in dysfibrinogenaemia and increased D-dimer production [4] [18]. Ultimately, trauma results in the loss and consumption of coagulation factors directly whilst releasing anticoagulation factors [18]. It is not known whether coagulopathies associated with trauma differ from those associated with PPH.

Limited peer-reviewed studies on coagulation profiles in PPH suggest that, except for fibrinogen, most women's coagulation profiles remain normal until large bleeds occur [19] [20]. Common causes of PPH, such as surgical trauma and atony are associated with limited coagulopathy association unless intervention is delayed or ineffective, leading to dilutional coagulopathy [20] [21]. Placental abruption and amniotic fluid emboli may cause rapid consumptive coagulopathy despite minimal initial blood loss [21]. In a recent large-scale observational study, coagulation abnormalities were uncommon, but a small subgroup displayed an acute obstetric coagulopathy characterized by severe hyper-fibrinolysis and hypodysfibrinogenemia [22]. PPH coagulopathy will be further discussed in section 1.3.5.

1.2.2 Laboratory investigations

1.2.2.1 Platelet count

Platelet count is a measure of platelet number in the blood performed on an automated haematology analyser. Normal platelet count ranges from 150–400×10^{9}/L [23] and can vary according to age, sex, and ethnicity. Females tend to have a higher mean platelet count than males and age causes a drop in platelet count in both [23]. This test solely quantifies the platelet number and does not assess the functional quality of platelets, thereby limiting its clinical use in haemorrhages.

1.2.2.2 Prothrombin time

Prothrombin time (PT), reported in seconds, evaluates the integrity of the classical extrinsic and final common pathway of coagulation, specifically factors VII, X, V, II and fibrinogen [3]. PT measures the clotting time of citrated platelet-poor plasma (PPP) after the addition of calcium and tissue thromboplastin (affinity-purified TF and phospholipids) which activate coagulation through the TF/FVIIa complex [17] [24] [25]. PT is "most sensitive to factor VII deficiencies, moderately sensitive to factor V and X deficiencies, sensitive to severe fibrinogen and prothrombin deficiencies" [17]. The high concentration of TF bypasses the propagation phase because the action of TFPI is overwhelmed and, thus, PT is not sensitive to deficiencies in FVIII or FIX, and only reflects partial thrombin formation, making it an imperfect measure of total thrombin generation [17] [26]. Given that pregnancy is a prothrombotic state (see section 1.2.3.1), PT is shortened in normal pregnancy compared to non-pregnant people [3].

1.2.2.3 Activated partial thromboplastin time

Activated partial thromboplastin (aPTT) evaluates the integrity of the intrinsic and final common pathway of coagulation [27]. It measures high-molecular-weight kininogen, prekallikrein, factors I, II, V, and VIII-FXII. The test assesses the clotting time of PPP and is initiated by adding an intrinsic pathway activator (e.g., celite, kaolin) and a phospholipid (e.g. cephalin), activating FXII, FXI and FIX, while the rest of the pathway remains inactivate until calcium is introduced [24] [28]. Tissue factor is excluded in aPTT, hence the term partial thromboplastin [24]. The normal aPTT range tends to be

between 20-35 seconds [28] [29] [30]. A prolonged aPTT is seen in all congenital and acquired procoagulant deficiencies with exceptions of factor VII and XIII deficiencies [17]. However, in normal pregnancy, it is shortened compared to the non-pregnant normal range mainly due to increased levels of FVIII [3].

1.2.2.4 Fibrinogen assays

The most common method used to measure functional fibrinogen is the Clauss Technique, where a diluted plasma sample is clotted with excess thrombin solution [100 U/mL], thus the clotting time is independent of thrombin concentration [31] [32]. During the clot formation, a spectrophotometer reads the absorbance changes in the sample [27], thus representing an inversely proportional relationship. The higher the fibrinogen concentration, the shorter the time to clot formation in the sample [27]. Normal fibrinogen levels in non-pregnant people range between 1.8 to 4 g/L [31] and the normal range in our laboratory is 2 to 4 g/L. Elevated fibrinogen levels can be seen with advancing age and the female sex, as well as, pregnancy, acute exercise, smoking and diseases, e.g. inflammatory conditions and infections [32]. On the other hand, a reduced fibrinogen level is seen in inherited conditions such as hypofibrinogenaemia and dysfibrinogenaemia, viral hepatitis, disseminated intravascular coagulation, and decompensated liver failure [32].

1.2.2.5 Thrombin generation assays

See section 1.2.4.2.

1.2.3 Normal haemostatic changes in pregnancy

1.2.3.1 Coagulation

During pregnancy, multiple physiological adaptations occur in the haematological system to prevent excess bleeding during placental implantation, maintenance, and delivery of baby and placenta [33]. These include a 40% increase in plasma volume and a 25% increase in red blood cell volume, leading to dilutional anaemia with a 15% decrease in haemoglobin [2].

In normal pregnancy, physiological alterations in the coagulation system occur, leading to elevation in procoagulant factors with a reduction in natural anticoagulants and fibrinolytic activity [3] [8] (see Table 1). Primarily, pregnancy results in a pro-thrombotic state to reduce the risk of haemorrhage at the time of delivery, but subsequently predisposes pregnant women to thrombosis and potential vascular complications [8] [34]. The most common coagulation abnormality noted in pregnancy is thrombocytopenia, which is defined as a platelet count of $<150x10^9$/L and thought to be due to increased platelet destruction as well as haemodilution [9] [10]. Destruction is initially compensated by an increase in platelet production, with a maximal platelet decrease is seen in the third trimester [3] [35]. A platelet count $<70 \times 10^9$/L at term may suggest the presence of HELLP (haemolysis, elevated liver enzymes, low platelets) syndrome, DIC, immune thrombocytopaenia or other rare pregnancy-related conditions [3]. But in uncomplicated pregnancy, the platelet count seldom falls to a level that would impact bleeding [10].

Table 1. Haemostatic changes in pregnancy.

	Pro-coagulation	**Anti-coagulation**
Increased during pregnancy	Factor I (fibrinogen) Factor VII Factor VIII Factor IX Factor X Factor XII von Willebrand Factor Plasminogen activator inhibitor 1 Thrombin-antithrombin complex Prothrombin fragment 1 + 2 TAFI	D-dimer Fibrinopeptide A
Variably, increased/decreased or no overall change	Factor V Factor XI Factor XIII	Protein C Antithrombin
Decreased during pregnancy	Platelet count	Protein S Tissue plasminogen activator

Inspired by Solomon et al. 2012. [36] *TAFI (Thrombin Activatable Fibrinolysis Inhibitor).*

The alteration of the coagulation system entails significant changes in clotting factor levels. Notably, factors FVIII, FIX, FX, FXII, and vWF exhibit marked increases as pregnancy progresses, typically peaking shortly before delivery [3] [37] [38] [39]. Factor XI levels tend to be unchanged, while FV initially increases followed by a decrease and stabilisation during the later stages of pregnancy [3] [33]. Factor XIII

may display a slight increase during the first trimester, but progressively decreases to about 50% of non-pregnant levels throughout pregnancy [3] [37]. Studies regarding coagulation factors II and VII still yield conflicting results. Some suggest no change or only a slight decrease in FII, whilst others argue for significantly higher levels [33] [37]. Thornton et al. report an initial increase in FII followed by a decrease, ultimately resulting in no overall change by term [3]. In contrast, factor VII has been observed to increase during pregnancy [3] [34]. Overall, these changes, in conjunction with the well-documented elevation in plasma fibrinogen levels, contribute to the establishment of a hypercoagulable state throughout pregnancy [1] [38] [40].

1.2.3.2 Anticoagulation

In addition to the changes in the procoagulation system, an overall decrease in natural anticoagulants have been documented in pregnancy [34]. Protein C and protein S, vitamin-K dependent proteins, inhibit coagulation by inactivating factors Va and VIIIa, resulting in negative feedback on coagulation [11] [41]. Protein S activity decreases with increasing gestation [3] [37] [38]. Although protein C levels initially peak in gestational weeks 20-30, they appear to be within normal non-pregnant values at the end of pregnancy [3] [34]. Some suggest an immediate increase in protein C levels after delivery that continues up to 3 days postpartum [35]. Furthermore, protein S also acts as a co-factor for TFPI, which further inhibits FVIIa and FXa [11] [41] and the cell surface-expressed glycoprotein TM binds to thrombin, leading to the further activation of protein C [11]. TM increases during pregnancy and is even higher in women suffering from pre-eclampsia [34] [42]. Another anticoagulant is antithrombin, a plasma glycoprotein and serine protease inhibitor of thrombin, factors IXa, Xa, XIa, XIIa, plasmin, kallikrein and complement C1, in pregnancy. There is evidence that AT levels remain within the normal range during pregnancy, whereas other studies have shown a decrease throughout pregnancy and particularly within the third trimester or after a caesarean section [35].

1.2.3.3 Fibrinolysis

Along with the activation of the coagulation system and the suppression of natural anticoagulants, the fibrinolytic activity is also reduced during pregnancy. In healthy humans, the balance between coagulation and fibrinolysis enables haemostatic plug formation at an injury site while ensuring the clot will not reach a vessel occluding size.

Fibrinolysis is an enzymatic process preventing accumulation of intravascular clots by encouraging fibrin breakdown [43] [44]. The primary fibrinolytic protein is plasmin, a protease enzyme, which cleaves fibrin forming fibrin degradation products, including D-dimer [43] [44]. Plasmin's precursor plasminogen is activated by either tissue plasminogen activator or urokinase plasminogen activator [44]. In pregnancy, there is a decrease in tissue plasminogen activator activity, which is attributed to the gradual increase in uterine produced PAI 1 and 2 [35]. Furthermore the "concentration of PAI-2 varies with birth weights, indicating a dependency not only upon the quantity and quality of the placental tissues but also upon fetal growth" [35]. In addition, levels of TAFI are increased in the third trimester, which contribute to reduced fibrinolytic activity [3]. TAFI reduces the production and function of plasmin by decreasing available plasminogen binding sites [43].

1.2.3.4 Laboratory results

Pregnant women routinely have blood tests taken during antenatal care. In the United Kingdom, this involves a full blood count at booking with the midwife and at 28 weeks with expected normal haemoglobin values of 11g/dL and 10.5g/dL, respectively [45]. If haemoglobin levels are outside the norm, further investigations are required, and first line treatment is usually iron supplementation [45]. With a platelet count of 50 x 109/L (gestational thrombocytopenia) considered sufficient for caesarean section [3], it suggests that a slightly lower than normal platelet count during pregnancy is benign, and it does not significantly impact haemostasis or increases haemorrhage risk [3] [46].

Coagulation tests in the third trimester are not routinely performed. However, various research studies have suggested normal reference ranges. Abbassi et al. has shown a fibrinogen range in the third trimester of 3.73-6.19 g/L, which has been confirmed by newer research studies from Slovakia (fibrinogen 4.967 ± 1.272 g/L in the third trimester), as well as, United Kingdom finding term fibrinogen in the range between 4-6 g/L in comparison to 2-4 g/L in healthy non-pregnant women [20] [39] [47]. Regarding aPTT, research studies agree that it is shortened at the end of pregnancy due to the elevated FVIII [36] [38] [39]. Normal reference ranges lie anything between 24.7-35.0 seconds, 31.92 ± 2.90 seconds, and 30.43 ± 3.201 seconds [8] [47]. Prothrombin time references are a little more ambiguous. Abbassi et al. showed a shortened PT (9.6-12.9 seconds) in the third trimester, whereas Uchikova reported that PT and TT were statistically significantly longer in their pregnant participants [8]. But their results were confirmed by, albeit in a very small cohort, by Srimala et al. (14.32 ± 1.91) [48].

For discussion of normal ranges of thrombin generation results, see section 1.2.4.2.

1.2.4 Thrombin generation

The combined interaction of procoagulant factors during secondary haemostasis leads to the production of thrombin making it the end-product of coagulation [49]. The word thrombin is derived from the Greek word *thrombos* meaning blood clot, thus representing its vital enzymatic role in the fibrinogen to fibrin conversion [50].

1.2.4.1 Structure and function

Thrombin, a multifunctional serine protease of the chymotrypsin family, exhibits unique properties as a procoagulant, anticoagulant, and potent platelet activator both in vivo and in vitro settings [50] [51]. It is derived from its precursor prothrombin, a protein synthesized by the liver as an inactive zymogen and its conversion occurs in the presence of Ca2+ through the prothrombinase complex on a negatively charged phospholipid membrane [50] [52]. Thrombin is composed of an A chain (36 residues) and a B chain (259 amino acids), with the B chain playing a vital role in catalytic activity, substrate recognition, and allosteric regulation [51] [52] [53].

Thrombin possesses distinct binding sites, including the catalytic centre at its core and anion-binding exosite I, which interacts with thrombin's co-factors and substrates such as fibrinogen, fibrinopeptides, and fibrin [51] [53] [54] [55]. TM and protease-activated receptor 1 (PAR) are additional factors that bind to exosite I, with TM promoting thrombin's anticoagulant function [51]. The anion-binding exosite II interacts with the platelet glycoprotein receptor Gplbα [53] [54]. Thrombin can exist in slow (Na^+ free) and fast (Na^+ bound) forms [53] [54], with Na^+ facilitating enhanced binding and catalysis of procoagulant substrates (i.e., fibrinogen, FV, FVIII, FXI, PAR-1) [51] [55]. The slow form of thrombin is known to cleave the anticoagulant protein C, while the equilibrium between slow and fast thrombin is maintained at a ratio of 2:3 (slow:fast) *in vivo* [53] [54].

Thrombin's multiple functions are a result of its complex structural composition. It exhibits procoagulant functions such as converting fibrinogen to fibrin and activating factors V, VIII, XI, and XIII [50] [51]. On the other hand, thrombin has prothrombotic effects by cleaving PAR and facilitating platelet activation [50] [51]. Additionally, it can function as an anticoagulant through the formation of the thrombin-TM complex, which activates the protein C pathway and inhibits coagulation [50] [51].

1.2.4.2 Thrombin generation assays

Traditional laboratory investigation tools, such as the PT and aPTT, only investigate the initiation phase of thrombin generation, where only a very small proportion of the total amount of thrombin is produced. Thrombin generation (TG) assays measure the total amount of thrombin generated by plasma after coagulation is activated. The first thrombin generation assay was developed in 1953. In 1986, the first calibrated automated thrombography (CAT) was introduced [12]. At the time, the assays used chromogenic reagents. Newer CAT assay methods rely on low-affinity fluorogenic substrate (Z-Gly-Gly-Arg-AMC), thus enabling continuous monitoring of TG in PPP and platelet-rich-plasma [56] [57]. "Since fluorescence is not disturbed by modulations in turbidity associated with clot formation, the test can be performed in whole (not defibrinated) platelet-poor or platelet-rich plasma, close to the *in vivo* situation" [56]. PPP is often preferred due to its reliable and consistent composition of exact

concentrations of tissue factor and phospholipids in each sample, enabling standardisation [58].

Each plasma sample is run in two wells: a measurement and a calibration well. Both contain 80 µl of the patient's platelet poor plasma. In the measurement well, the plasma is combined with TF (final concentration 5 pM) and an excess of synthetic phospholipid vesicles (final concentration 4 µmol) to activate coagulation, whereas the calibration well is combined with a known quantity of a thrombin calibrator, which does not initiate coagulation [56]. If the assay is required to be sensitive to low concentrations of FVIII, for example for monitoring people with haemophilia, then low concentrations of TF (1 pM) are required to activate the thrombin generation assay, and in this situation, corn trypsin inhibitor is required to prevent contact activation. When FVIII levels are known to be high, as is the case for this study, standard TF concentrations (5 pM) can be used, and corn trypsin inhibitor is not required. The thrombin calibrator is made up of a thrombin-like enzyme linked to an α-2-macroglobulin that is not inhibited by plasma protease inhibitors or plasma colour [56] [57] [58]. Subsequently, each well has $CaCl_2$ and FluCal added [26]. FluCal is a combination of a Fluo-Buffer and Fluo-Substrate. Fluo-Buffer is made up of Hepes (pH 7.35) and $CaCl_2$. Fluo-Substrate consists of the fluorogenic substrate in a dimethyl sulfoxide solution. In the measurement well, thrombin splits the fluorogenic substrate releasing the fluorescent 7-amino-4-methylcoumarin.

The 7-amino-4-methylcoumarin molecule has distinctive optical properties, demonstrating signal generation upon excitation with 390 nm light and emitting measurable fluorescence at approximately 460 nm. [58] The fluorescence intensity recorded by a fluorometer is directly proportional to the concentration of thrombin generated over time [59] [60]. In the calibration well, the thrombin calibrator converts the fluorogenic substrate at a known constant rate [56]. After the measurement is completed, the internal software calculates the parameters of the thrombogram: the lag time (min), the velocity index (nM/min), the peak thrombin (nM), the time to peak (min), the tail and the endogenous thrombin potential (ETP), i.e., the area under the curve. Several studies have shown low intra- and inter-assay variabilities (<10%) for all parameters, suggesting a high level of reproducibility, in both PPP and platelet-rich-plasma [61] [62]. One study showed, when the same individuals were tested

repeatedly over a one-year period, the assay consistently provided reasonably consistent results with a variation coefficient ranging from 10-15% [56].

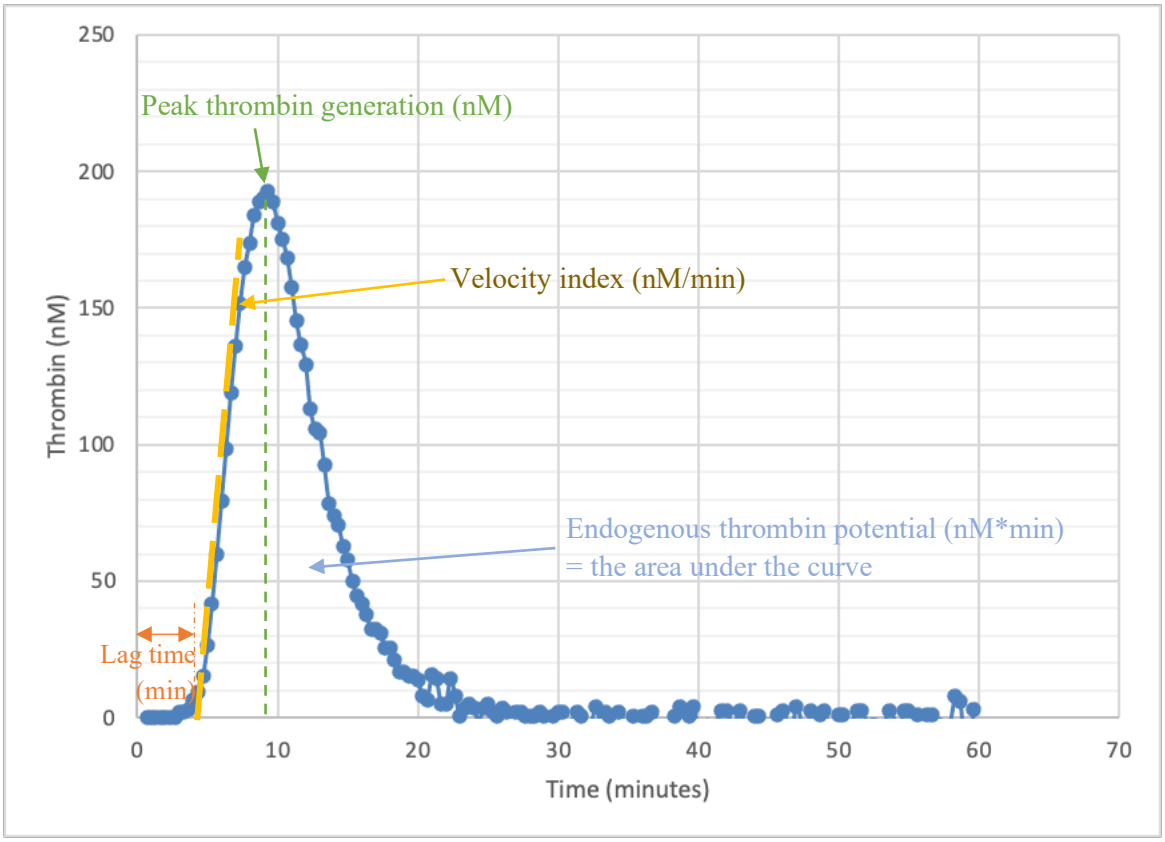

Figure 2. Typical thrombin generation curve including thrombin generation parameters (lag time, peak thrombin generation, endogenous thrombin potential, and velocity index).
Source: Generated using data from the MPhil data set.

Figure 2 shows a typical thrombin generation trace. The lag time corresponds to the initiation phase of coagulation after the addition of the trigger and is the time required for thrombin concentration to reach 1/6th of the peak concentration [49] [56] [59]. Thus, it is mainly determined by factors VII and IX, as well as, free TF, TFPI, and fibrinogen [5]. At the end of the lag time, plasma clots are formed, and lag time may be similar to the plasma clotting time seen in PT and aPTT [56] [59] [61]. This is followed by the amplification and propagation phase, which shows a steep increase of thrombin generation to the peak value, representing the maximum concentration of thrombin generated [49] [59]. The rate of increase of thrombin is depicted as the velocity index of thrombin generation and gives the slope between the start of thrombin generation and the time to peak thrombin [63]. The amplification phase relies on the quantity and quality of platelets; thus, the velocity and peak values tend to be higher with a higher platelet count [5]. However, this is not relevant to the assays described in this thesis,

which were performed in PPP with an excess of external phospholipid was supplied. The area under the curve corresponds to the total amount of thrombin generated in the sample, i.e., represents the total enzymatic activity of thrombin [56] [59]. Some suggest that ETP is the most useful variable for assessing bleeding and thrombosis risk [56] [57]. However, others argue that "peak height is sometimes a more sensitive indicator of the plasma thrombin-generating capacity, as it is less readily saturated than ETP at increasing plasma concentrations of coagulation factors or trigger" [56]. Hypocoagulability is represented by prolonged lag time, low peak thrombin, velocity index, and/or ETP, whereas the opposite is true for hypercoagulability [56] [59].

1.2.4.3 Thrombin generation and pregnancy

In recent years, there has been a surge of scientific investigations focused on elucidating the intricacies of thrombin generation. One study investigated thrombin generation in healthy males and non-pregnant females using a home-made trigger made up of 13.6 pm TF [49]. The male group had a mean ETP value of 1480 ± 239 nM*min, a mean lag time of 2.72 ± 0.39 minutes, and a mean peak value of 328 ± 38.0 nM. The female group had similar mean ETP values at 1490 ± 212 nM*min, a mean lag time of 2.62 ± 0.36 minutes, and a mean peak value of 342 ± 42.1 nM [49]. However, a more recent large-scale study showed that females exhibited a shorter lag time ($p < 0.0001$) and higher ETP and peak height at both 1 pM TF and 5 pM TF ($p < 0.0001$ for both) compared to males, which would suggest a more procoagulant profile in females [64]. Another study using commercialised reagents showed similar median lag times of 3.0 min and median peak heights of 359 nM in healthy non-pregnant females, however, they reported median ETP values of 2149 nM*min [65]. These conflicting findings highlight the need for further research to assess the inconsistency and variability in results and thus to resolve those discrepancies.

The variability in thrombin generation results may also be attributed to differences in individual fitness levels. One study examining thrombin parameters in trained and sedentary healthy individuals found mean lag times for thrombin generation were notably shorter in the trained group (2.33 minutes) compared to the sedentary group (2.72 minutes) [66]. Moreover, the mean ETP values were lower in the trained individuals (1664 nM*min) as opposed to the sedentary counterparts (1821 nM*min)

[66]. The peak thrombin values were 431 nM and 450 nM, for the trained and sedentary groups, respectively [66]. Additionally, the velocity indices were marginally higher in the trained subjects (251 nM) compared to the sedentary individuals (244 nM) [66]. Their statistical analysis revealed a significant difference in ETP between the two groups (p = 0.007) [66]. These findings suggest that regular physical fitness may exert an influence on thrombin generation [66].

Additionally, age seems to play a role in coagulation factors and inhibitors. Therefore, older individuals tend to have higher ETP values [49] [67]. However, a study demonstrated that in females, age was associated with lower ETP at 1 pM TF (p = 0.015) and lower peak height at 5 pM TF [64]. Females using oral contraceptives or hormone replacement therapy exhibited a shorter lag time, higher ETP, and peak height independent of TF volume. The observed sex differences in thrombin generation can be partly attributed to the influence of female endogenous sex hormones on the coagulation cascade. Females, regardless of hormonal treatment, exhibited higher levels of fibrinogen and lower levels of protein S, antithrombin, and protein C compared to males [64]. Another study indicated variations in thrombin generation based on the menstrual cycle, with a median lag time of 2.59 ± 0.4 min in the follicular phase versus 2.67 ± 0.5 min in the luteal phase, a median ETP value of 1524.5 ± 283.2 nM*min in the follicular phase versus 1609.2 ± 342.8 nM*min in the luteal phase, and a peak value of 300.1 ± 50 nM in the follicular phase versus 308.1 ± 54.4 nM in the luteal phase. These findings suggest higher thrombin generation during the luteal phase of the menstrual cycle. However, the study also investigated coagulation factors and found that the actual coagulation cascade was marginally faster during the follicular phase, suggesting a potential role of progesterone in the coagulation system.

Research on thrombin generation changes during pregnancy is limited and often contradictory. Some studies suggest that thrombin generation remains stable throughout pregnancy. For example, Hron et al. observed stable ETP in healthy pregnant women during the first and second trimesters, followed by a decrease in the third trimester [38]. Eichinger et al. reported unchanged ETP in healthy women, both with and without factor V Leiden, throughout their uncomplicated pregnancies [68]. However, more recent studies, such as Rosenkranz et al., report that there is an

increase in thrombin generation from non-pregnancy to pregnancy, reflecting the prothrombotic nature of pregnancy [65] [69] [70]. The variability in findings compared to Eichinger et al. could be attributed to methodological differences. Eichinger et al. used a chromogenic substrate, whereas Rosenkranz utilized a fluorogenic substrate. Unlike optical density measurements, the fluorescent signal remains unaffected by turbidity and clot formation. Supporting the notion of increased thrombin potential during pregnancy, Macey et al. found that healthy pregnant females had median lag times of 2.8 minutes, median ETP values of 2525 nM*min, and median peak heights of 452 nM [65]. Thus both, Macey et al. and Rosenkranz et al. showed an increase in ETP and peak in pregnancy but not lag time [65] [70]. As shown later, the results from this study support that thrombin generation is significantly enhanced at term when compared to non-pregnant healthy women.

To my knowledge, only one study has investigated an individual woman's capacity to generate thrombin before, during, and after pregnancy [1]. This study showed that an individual woman's endogenous thrombin potential increased from pre-pregnancy (1162 ± 446 nM*min) to early pregnancy at 11-15 weeks (2157 ± 466 nM*min) to late pregnancy at 30-34 weeks (2140 ± 543 nM*min) [1]. However, the difference between early and late pregnancy values was not statistically significant [1]. Peak values also increased from 81 nM ± 41 nM pre-pregnancy to 219 nM ± 117 nM in early pregnancy and 336 nM ± 178 nM in late pregnancy [1]. A similar trend was observed in women undergoing natural cycle *in vitro* fertilization [71]. Significant increases in ETP, peak height, and velocity index were observed from pre-pregnancy to day 43 of gestation (p = 0.024–0.0004), which persisted until day 59 [71]. Accounting for inter-individual variation, there were significant increases in these parameters from pre-pregnancy to day 32 (p = 0.0351–<0.0001), with the increases remaining until day 59 [71].

However, research into thrombin generation during postpartum haemorrhage is sparse. Dallaku et al. investigated women with primary PPH that were recruited into the WOMAN trial to establish whether tranexamic acid (TXA) increases the prothrombotic effect during PPH [72]. They found mean ETPs (SDs) of 1491 nM*min (378.7 nM*min) in the placebo and 1537 nM*min (375.9 nM*min) in the group that was given TXA, thus demonstrating no evidence of TXA affecting thrombin generation. However, when compared to above mentioned research showing higher ETP values

during pregnancy, it suggests a potential decrease in thrombin generation during a postpartum haemorrhage event [72].

A more recent study conducted in the United States examined thrombin generation in women experiencing PPH, defined as a blood loss of 1 L at the time of delivery and/or within 24 hours after delivery [73]. Blood samples were collected at the time of diagnosis, as well as 2 and 4 hours thereafter [73]. The majority of PPH cases (95%) occurred at the time of delivery, with 74% attributed to uterine atony [73]. Compared to patients without PPH, those with PPH exhibited a significant decrease in peak thrombin concentration in whole blood (median values of 150.2 nM vs. 46.9 nM; p = 0.01) [73]. However, this significant difference was not observed when analysing PPP alone, as the peak thrombin values showed no significant difference (median values of 276.9 nM vs. 291.6 nM; p = 0.66) [73]. Patients with PPH also experienced a longer time to reach peak thrombin formation compared to patients without PPH (median values of 3.0 minutes vs. 0.2 minutes in plasma, respectively; p = 0.44) [73]. Notably, this prolongation in time to thrombin formation was shown to recover within 4 hours [73].

1.3 Postpartum haemorrhage

1.3.1 Definition

Postpartum haemorrhage is a common clinical emergency globally [74] [75] [76] [77] [78]. It is the most common cause of maternal mortality worldwide [79] [80], contributing to 19% of maternal deaths with a significant variation between high-resource countries (8.0%) and low-resource countries (Northern Africa – 32.0%) [74] and also a leading cause of maternal morbidity [72]. Consequences of maternal morbidity range from multiple blood transfusion to peripartum hysterectomy and to multi-organ failure [81]. Analysing data from 186 out of 195 countries, the Global Burden of Disease 2015 Maternal Mortality Collaborators evaluated the global number of maternal deaths in 2015. They estimated that out of 275,288 deaths, up to 34% were caused by haemorrhage, with the highest incidence observed in the 15-29 age group [82]. Furthermore, the maternal mortality ratio varied significantly, ranging from 15 in 100,000 live births in high vs to 443 in 100,000 live births in low socio-

demographic index countries, where haemorrhage serves as the primary cause of maternal death [82].

The challenge when attempting to calculate prevalence and comparing data from various countries is the varying definition of postpartum haemorrhage and the methods used to assess blood loss, including estimation versus quantification. For example, in Flanders, some studies define PPH by proxy, i.e., equalling it to "women receiving a blood transfusion within 24 hr of birth" [83]. This definition lacks clarity and does not consider factors like antenatal anaemia. In Norway, Rossen et al. defined severe PPH as an estimated blood loss of more than 1,000 ml [84]. Similarly, in Australia, Fyfe et al. defined major primary postpartum haemorrhage as "blood loss equal to or greater than 1,000 mls within 24hrs of delivery" [85], despite, the Australian Modification of ICD defines PPH as "haemorrhage of 500 ml or more following vaginal delivery or 750 ml or more following a caesarean delivery" [83]. Similar definitions are seen in Canada, where PPH is defined as blood loss exceeding 500 ml after a vaginal delivery and 1,000 ml after a caesarean delivery, or if the physician noted PPH in the medical chart [83]. In Albania, they also rely on visual estimation of blood loss, and PPH is defined as blood loss of more than “500 mL after vaginal birth or 1,000 mL after caesarean birth [or] [...] blood loss sufficient to cause hemodynamic instability" [72].

These varying definitions and assessment methods make it challenging to obtain consistent and comparable data on PPH prevalence across different countries. This research paper will use the WHO guidelines’ definition for PPH, which can be divided into primary and secondary PPH. Primary PPH is defined as the blood loss of ≥500 ml from the genital tract within 24 hours of the birth of the baby and may start pre-placenta delivery [76] [80] [86]. It can be further grouped into minor (500-100 ml), moderate major (1000-2000 ml) and severe major PPH (more than 2000 ml) [85]. Secondary PPH describes any abnormal bleeding from the genital tract between 24 hours and 12 weeks postnatally [79]. As can be noted, some of these definitions above are not in conformity with WHO guidelines. In a systematic review, it was identified that almost 77% reports in the WHO database 1997-2002 included incorrect definitions of PPH [87]. Furthermore, the problem with all of these definitions is that they rely on visual estimation of blood loss, which has been shown to underestimate the actual blood loss

volume and underestimating blood loss ultimately will result in lower PPH rates [88] [89]. Carroli et al. showed in their systematic review of 120 datasets (including a total of 3,815,034 women) that the prevalence of PPH, defined as blood loss ≥ 500 mL, was higher when measured objectively (10.55%) vs. subjectively (7.23%). This difference gets larger the bigger the blood loss as shown by the prevalence of severe PPH, defined as blood loss ≥1000mL, measured objectively (3.04%) vs subjectively (1.68%) [87]. The recent E-MOTIVE study examined factors influencing the current detection and management of PPH in sub-Saharan Africa [90]. One common barrier identified was the lack of tools and equipment to accurately measure blood loss (MBL). Healthcare professionals acknowledged the challenge of quantifying the amount of blood loss without utilizing proper measurement techniques [90].

1.3.2 Aetiology

The causes of PPH are varied. The four most common causes for PPH can been summarised in the mnemonic 'The Four T's': Tone (uterine atony), Trauma (laceration, haematoma, inversion, uterine rupture), Tissue (retained tissue, e.g., placenta, placental fragments, or blood clots, or invasive placenta), Thrombin (coagulopathy) [86]. These causes can coexist with each other and may be associated with coagulopathy, particularly when there is delayed intervention or inadequate response, with dilutional coagulopathy developing due to massive blood loss and fluid resuscitation [19]. Additionally, hypodysfibrinogenaemia, characterised by structural or functional abnormalities in fibrinogen, and hyperfibrinolysis, which involves the excessive breakdown of fibrin clots, can be associated with PPH. Certain conditions, such as placental abruption (the separation of the placenta from the uterus) and amniotic fluid embolism (the entry of amniotic fluid into the maternal circulation), can cause rapid and consumptive coagulopathy despite minimal initial blood loss.

Uterine atony is the leading cause of primary PPH and consequently the leading cause of maternal mortality worldwide [72] [79] [80] [91] [92] [93]. It is characterized by the inadequate contraction of the uterus. The uterus is composed of interlacing muscle fibres that form a distinct network structure [91], comprising the endometrium, myometrium, and perimetrium. To initiate muscular contraction, calcium influx is necessary, which occurs through diffusion across membranes via voltage-gated

calcium channels and release from the sarcoplasmic reticulum, with L-type calcium channels serving as the main entry point in the uterus [94]. Uterine relaxation is essentially the reversal of that pathway [94]. Blood vessels which eventually supply the placenta pass through the muscular layer of the uterus [95]. Post-delivery, uterine contractions are necessary for placental separation as well as haemostasis by restricting those uterine blood vessels [91] [96]. "This blood-saving mechanism is known as the ’physiological sutures’ or ’living ligatures’." [91].

Uterine atony is managed with bimanual massaging/compression of the uterus as well as uterotonics. Uterotonics such as oxytocin, carboprost, misoprostol, or methylergonovine are utilized based on individual patient factors and contraindications [86]. Trauma can result through direct injury, for example, caesarean section incision, or indirectly through delivery trauma, like lacerations. Optimal management includes early bleed identification and suturing [86]. Regarding retained tissue, any fragments left in the uterus can cause impairment of uterine contraction and subsequent poor haemostasis. It has been suggested that the longer fragments are left in uterus, the higher the risk of PPH. Some suggested that the risk doubles after 10 minutes [86]. Optimal management includes manual removal of the fragments. Another cause for retained tissue is invasive placenta, which describes conditions where the placenta attaches either too strongly or invades too deeply the uterine wall, for example, placenta accreta, increta, or percreta. Any of these can cause life-threatening PPH and should ideally be identified pre-delivery [86]. As discussed previously, there are various changes in the coagulation system during pregnancy. During PPH, coagulation can become compromised contributing to defective haemostasis.

1.3.3 Risk factors

There are numerous methods that attempt to differentiate and group risk factors for PPH. Some studies distinguish risk factors into patient- and pregnancy-related characteristics, for example, maternal age, parity, ethnicity, and obstetric practices, such as mode of delivery, use of syntocinon for augmentation, and episiotomy [83]. However, a more recent multidisciplinary consensus statement proposes categorizing risk factors according to time of occurrence, i.e., pre-pregnancy, during pregnancy, during labour, or after delivery [97].

Extensive research into risk factors of PPH has identified various elements. A Norwegian study analysed data from the Medical Birth Registry of Norway, including 307,415 women between 1999-2004, and found that induction of labour and prelabour were associated with a higher risk of PPH (odds ratio [OR] 1.71 and 2.05, respectively) [98]. Emergency caesarean section (CS) carried a 55% higher risk, whereas vaginal deliveries had a 52% lower risk compared to prelabour CS [98]. The highest risk was observed for emergency CS after induction in mothers with a previous CS (OR 6.57), compared to spontaneous vaginal delivery in mothers with no previous CS [98]. An American study using logistic regression modelling on a dataset of 76,641 hospital admissions, including 25,654 PPH cases, between 1995-2004 found independent risk factors for PPH from uterine atony resulting in transfusion to be age <20 or ≥40 years, caesarean delivery, hypertensive diseases of pregnancy, polyhydramnios, chorioamnionitis, multiple gestation, retained placenta, and antepartum haemorrhage (APH) [99]. Excluding maternal age and caesarean delivery, at least one of these risk factors was present in 38.8% of the patients [99]. A randomized control trial of 106 French hospitals between 2004-2006 identified the factors that were independently associated with greater severity of PPH: primiparity, previous PPH or CS, cervical softening, prolonged labour, episiotomy, delay in initial care for PPH, administration of oxytocin more than 10 minutes after PPH diagnosis, manual examination of the uterine cavity more than 20 minutes after, call for additional assistance more than 10 minutes after, and delivery in a public non-university hospital [100]. This study focused specifically on PPH cases due to atony after vaginal delivery, which might introduce bias and account for the slight differences in identified PPH risk factors.

Nationally collected data from Scottish maternity hospitals between 1980 and 2005 showed an association between advanced maternal age and an increased risk of caesarean delivery [101]. Advanced maternal age was also positively correlated to prolonged labour, higher likelihood of operative vaginal delivery, and impaired uterine function [101]. Additionally, there was an increased occurrence of multiphasic spontaneous myometrial contractions with age [101]. Considering age as an independent risk factor and the association with increased comorbidities, it is not surprising the maternal age has been shown to increase the risk of suffering from PPH [82] [99] [102] [103]. That tendency could cause problems as Zeitlin et al. [104] showed that maternal age, especially over 35-year-olds, increased in all countries, with the

exception of Finland. Furthermore, they demonstrate an increase in multiparty, possibly due to the increase in maternal age and improvement of assisted reproduction techniques [104]. A New Zealand study analysed 30,231 term nulliparous singleton deliveries to investigate the association between body mass index (BMI) and major postpartum PPH [85]. Obesity was defined as a BMI ≥ 30.0. They found a twofold increased risk of major PPH in nulliparous obese women compared to those with a normal BMI (OR 2.37) [85]. This risk was higher for vaginal delivery (OR 2.11) compared to emergency/planned caesarean section (OR 1.73) in nulliparous obese women. Independent risk factors for major PPH after vaginal delivery included episiotomy (OR 1.94), pre-eclampsia (OR 1.97), retained placenta (OR 4.88), and 3rd/4th-degree tear (OR 5.09) [85]. In comparison to caesarean delivery, antepartum haemorrhage (OR 1.65), chronic hypertension (OR 1.90), and placenta praevia (OR 3.08) were identified as risk factors [85]. Furthermore, a study identified that uterine bleeding beyond 28 weeks of gestation and the presence of placenta praevia were the most significant predictors of placental abruption [105].

Pre-eclampsia is known to increase the risk of PPH [93] [106]. While the precise pathogenesis and pathophysiology are not fully described, a dysfunction in the coagulation-fibrinolysis system appears to contribute to a hypercoagulable state [107] [108]. Placental dysfunction caused by oxidative stress, immune abnormalities, genetic factors, etc., results in the release of antiangiogenic factors [109]. This causes endothelial and immune dysfunction along with coagulation abnormalities. The hypercoagulable state leads to micro-emboli formation in various organs and the placenta, resulting in organ dysfunction [108] [110] [111].

It becomes evident that there is no single factor solely responsible for the development of PPH [97] (see Table 2). A global multidisciplinary consensus stated the following risk factors for PPH development: "multiple pregnancies (OR 2.3-4.7); a history of PPH (OR 3.3); pregnancy-induced hypertension (OR 1.9-2.5); chorioamnionitis (OR 2.5); episiotomy (OR 1.4 to 2.2); pre-labour caesarean section (OR 1.3-2.3); caesarean section during labour (OR 1.7-3.6); macrosomia (OR 1.7 to 3.5); [and] operative vaginal delivery (OR 2.3)" [97].

However, it is not unprecedented for PPH to occur without any identifiable clinical risk factor for haemorrhage present [79] [86] [99]. Evensen et al. cited that 20% of PPH transpire in pregnancies without risk factors [86]. To reduce risk, WHO guidelines suggest active management of the third stage of labour, including, "(i) administration of a uterotonic soon after the birth of the baby; (ii) clamping of the cord following the observation of uterine contraction (at around 3 minutes); and (iii) delivery of the placenta by controlled cord traction, followed by uterine massage." [79].

Table 2. Summary of postpartum haemorrhage risk factors.

Pre-Pregnancy	During Pregnancy	During Labour	After delivery
Parity	Pre-eclampsia	Prolonged labour	3rd/4th degree tear
Ethnicity	APH	Induction	Episiotomy
BMI	Polyhydramnios	Eclampsia	Retained placenta
Age		Chorioamnionitis	Caesarean section
Previous PPH			
Previous CS			

Inspired by [83] [85] [93] [97] [98] [100] [101] [106].

1.3.4 Epidemiology

Recent reports demonstrate an increase in frequency of PPH globally, despite varying significantly from hospital to hospital and among countries, for example, high vs low resource countries [112] [113] [114]. Some of this trend might be explained by risk factors for PPH although others have not shown any association [85] [115] [116] [117].

One hypothesis argues that an increase in atony results in an increase in PPH. Two American studies show an increase in PPH by 26% between 1994-2006 and by 27.5% from 1995 to 2004 and argue this is due to the increase in incidence of uterine atony [99] [115]. Callaghan et al. also demonstrated an increase of uterine atony cases from 1.6% to 2.4%, whereas Bateman et al. demonstrated a stable rate of PPH from other aetiologies, e.g., retained placenta or coagulopathy [99] [115]. Lutomski et al. support the hypothesis of an increase in atony resulting in an increase of PPH incidence across various modes of delivery, including, vaginal, instrumental, emergency, and elective caesarean deliveries [118].

However, some suggest that the increase in severe PPH is due to mode of delivery. A Norwegian study showed the risk to develop severe PPH was doubled after a CS vs a vaginal delivery (5.9% vs 2.8%) [84]. Twins' delivery posed the highest risk for PPH in both delivery methods, while vaginal deliveries had an increased risk with retained placenta and induction [84]. This is supported by an Australian study showing unassisted vaginal births have the lowest PPH risk, whereas unplanned caesarean sections were associated with the highest incidence of PPH and women with forceps deliveries had the highest incidence of severe PPH [112].

The rising maternal age at childbirth may contribute further to an increase in PPH incidences [83]. While some suggest no correlation between age and PPH [103], it is evident that maternal age is increasing globally [83]. Advanced maternal age could be associated with a higher utilization of assisted reproductive techniques, potentially contributing to PPH rates [83]. An international collaboration investigating PPH incidences in Australia, UK, Canada, and USA aimed to identify contributing factors [83]. They suggested practice changes, including the rise in CS and labour inductions, as possible reasons [83]. Additionally, improved record-keeping and changes in how data are recorded may contribute to the observed trend [83].

Reviewing a wide range of international research, the observed increased trends appear to be most likely due to multifaceted factors ranging from patient-focused factors (e.g., obesity, comorbidities, age) to delivery-focused factors (e.g., rise in CS).

1.3.5 Coagulopathy

Throughout pregnancy, an increase in coagulation factors produces a prothrombotic state [10]. This is greatest at time of delivery with placental expulsion releasing thromboplastic substances [3]. However, in a minority of PPH cases, clot formation is insufficient to stop the bleeding. Over the years, various hypotheses attempted to explain the complexity of coagulopathy in PPH, with different bleeding causes potentially influencing the extent of coagulopathy, ranging from dilutional coagulopathy to localized consumption, DIC, and increased fibrinolysis [19] [119] [120]. Bleeding can be further affected by complications of pregnancy, such as preeclampsia, sepsis, and impaired liver function [20].

Hypofibrinogenemia is commonly observed in PPH and predicts the progression form moderate to severe cases [121] [122] [123], whereas haemoglobin levels are frequently adequately maintained irrespective of blood loss [122]. The drop in fibrinogen levels can result from blood loss, and therefore loss of coagulation factors directly, or "consumption of factors associated with coagulation activation" [121]. De Lloyd et al. examined all 18 501 deliveries at a Welsh hospital over 3 years and identified 456 PPHs with an estimated blood loss ≥ 1500 ml and found that blood loss and drop in fibrinogen levels was best correlated (r 0.48, *p* value < 0.01) irrespective of mode of delivery [122]. Cortet et al. reported similar findings looking at 738 women with PPH after a vaginal delivery across France [121]. At time of diagnosis, the fibrinogen concentration in the group without worsening PPH was 4.2 g/L in comparison to fibrinogen levels of 3.4 g/L in severe PPH. They argue that a low fibrinogen level at the time of PPH diagnosis is independently associated with an increased risk of severe PPH, regardless of other laboratory indicators [121].

Furthermore, others have reported that large volumes of fluid administration do not only affect fibrinogen levels, but also haemoglobin, platelet count, as well as conventional coagulation studies (haematocrit, PT, and aPTT), although in the majority of women, coagulation studies like PT remain within the normal range despite large volume loss and subsequently do not develop significant coagulopathy [19] [124] [125]. However, PT and aPTT can be affected when PPH loss exceeds 4000 ml [20].

1.3.6 Treatment

A 4-stage approach has been suggested to guide PPH resuscitation treatment. This may involve infusing warmed isotonic fluids when clinically required and making blood transfusion decisions based on clinical and haematological assessments [126]. During bleeding, coagulation factor concentrations should be maintained within normal ranges. This involves targeting specific values, including normal PT/aPTT, fibrinogen levels above 2 g/L, EXTEM clot time below 75 seconds, and Fibtem A5 above 11 mm [126].

Fresh frozen plasma (FFP) is a blood product that plays a role in the management of PPH [127] [128]. Derived from whole blood donations, FFP is frozen at a temperature of ≤-18°C within 8 hours of collection. It contains all clotting factors at physiological concentrations.

In the context of PPH management, FFP is administered to replace depleted procoagulant clotting factors and fibrinogen [129]. However, the optimal fibrinogen replacement target in PPH remains uncertain and there are concerns are that the infusion of FFP during PPH may lead to dilution, potentially reducing fibrinogen levels and impacting its effectiveness in improving haemostasis [127] [128]. In addition, the role of FFP to replace procoagulant clotting factors is unclear because the incidence of depletion of these factors is unknown. Consequently, recent studies suggest that FFP infusion should be tailored to individual patient needs, guided by laboratory tests and viscoelastometric testing [127] [128]. In cases where haemostatic tests are unavailable, guidelines recommend that early consideration of FFP infusion is warranted for conditions with a suspected coagulopathy, such as placental abruption or amniotic fluid embolism, or when PPH detection has been delayed [126] [127] [128].

1.3.7 Problem with traditional coagulation tests

The process of coagulation is dynamic and complex. Various coagulation factors are required to be activated or inactivated to form complexes that generate thrombin and produce a fibrin clot. Traditional coagulation tests, such as PT and aPTT, solely focus on procoagulant factors with varying sensitivity for coagulation factor deficiency.

For example, PT reagents are sensitive to FVII deficiencies in the extrinsic pathway but less sensitive to deficiencies in the final common pathway [24]. Furthermore, both tests can be affected by various factors, for example, timing and procedure of sample collection (e.g., storage of ≥24 hours leads to prolongation, under-filling of collection tubes), anaemia, high haematocrit count, plasma discolouration (e.g. due to haemolysis or hyperbilirubinaemia) and lupus anticoagulant [28] [44]. Furthermore, considering that patients affected by plasma defects or on low molecular weight heparin may suffer significant haemorrhaging but may have a normal aPTTs, suggests aPTT is not satisfactory for predicting haemorrhagic risk [25]. In a recent obstetric

major haemorrhage study, aPTT was less sensitive with increasing blood volumes and PT values did not correlate with blood loss [122]. In cases of major haemorrhages, including PPH, PT and aPTT may remain normal due to their limited evaluation of the initiation phase of coagulation and their susceptibility to fluid dilution [57] [129].

Therefore, their ability to assess clinical coagulopathy and to provide a complete summary of haemostasis is limited. This is especially the case in pregnancy, where there are pronounced changes not only in coagulation factor levels, but also in natural anticoagulants as well as the fibrinolytic system. Thus, for this research project, global haemostatic assays will be used to assess total thrombin generated and thus, quantifying the combined effect of various coagulation factors [12]. The results can be used to investigate whether the women recruited had developed deficiencies of coagulation factors that were sufficient to impair thrombin generation and hence would potentially benefit from replacement therapy with FFP.

Chapter 2

2 Methodology

2.1 Objectives

2.1.1 Study objectives

The aim of this project is to measure thrombin generation assays in women suffering from PPH to investigate whether FFP transfusion has the potential to improve haemostasis in this cohort.

2.1.2 Endpoints

2.1.2.1 Primary endpoint

The primary endpoint is to investigate what proportion of women, suffering from PPH, have thrombin generation below the normal range found in healthy non-pregnant female controls for velocity index, peak thrombin, and ETP, or above the normal range for lag time. The hypothesis is that, if thrombin generation fulfils these criteria, then infusion of FFP could have the potential to improve haemostasis.

2.1.2.2 Secondary endpoints

- Do women suffering from PPH have different thrombin generation parameters compared to pregnant women at term who are not bleeding (non-bleeding pregnant controls)?
- Do women suffering from PPH have different thrombin generation parameters compared to pooled normal plasma (used as a model for FFP)?
- How do PPHs with different primary cause differ in terms of thrombin generation?
- How does postpartum haemorrhage size affect thrombin generation?

2.2 Study design

This Master of Philosophy research project is part of the Obstetric Bleeding Study Plus (OBS+: “Characterising the Coagulopathy of Obstetric Haemorrhage”) – a single centre observational cohort descriptive study (ethical approval REC16/WA/0282) – investigating female patients, over the age of 16 years, experiencing significant obstetric haemorrhage on the consultant-led delivery suite at Cardiff and Vale University Health Board. The blood loss in all women was quantitatively assessed using validated gravimetric and volumetric techniques, ensuring objective measurements rather than estimations. Samples meeting the inclusion criteria for OBS+ (see below) were collected between May 2017 and May 2020. This thesis will investigate a cohort of women from OBS+ with the most severe postpartum bleeding.

2.2.1 Inclusion criteria

Women were included into the OBS+ study if they fulfilled one or both criteria:

- Women who activate the institution’s major obstetric haemorrhage protocol (> 1000 ml MBL with ongoing PPH, or earlier if clinical concern of bleeding or coagulopathy), on the consultant-led Delivery Suite at Cardiff and Vale University Health Board.
- Women with suspected placental abruption or amniotic fluid embolus.

In the sub-analysis for this thesis, I have selected a subgroup of the women recruited to OBS+ to reflect the most severe bleeds and hence those most likely to have impaired thrombin generation and hence women who could potentially benefit from FFP. The subgroup includes patients suffering from severe PPH (>1500 ml), all placental abruptions, and those requiring blood product transfusion or women having multiple sampling episodes (suggesting prolonged bleeding or ongoing clinical concern). All of these were selected for further extended testing with thrombin generation and coagulation factor levels. To group the women, the primary cause of postpartum haemorrhage was determined. Although many women experienced multiple factors contributing to the bleeding, the primary cause was determined in collaboration with the treating clinicians and as such, the following categories were identified: uterine atony, surgical/trauma (encompassing surgical bleeds and genital

tract trauma), placental abruption, placenta accreta/praevia, and retained products of conception (involving retained placenta or membranes).

2.2.2 Consent

Following the cessation of bleeding, consent was sought retrospectively in accordance with the ethical approval (REC16/WA/0282). Consent covered agreement to collect and analyse data relating to the PPH and performing non-routine testing, such as thrombin generation, on stored samples for research purposes. Patients also received a leaflet containing all information, including, contact details to be able to withdraw from the study at any point.

2.2.3 Control groups

In addition to study participants, a control cohort of 35 woman with uncomplicated term pregnancies booked for elective caesarean section were approached and had the same blood samples taken as the study participants for comparison. These will be termed non-bleeding pregnant controls (PCs). In addition, 20 healthy non-pregnant female controls (NPCs) were also used to create a local normal range for each thrombin generation parameter and to compare with women experiencing postpartum haemorrhage. Furthermore, normal pooled plasma samples were analysed as reference samples. Normal pooled plasma (NPP) contains citrated plasma from screened normal non-pregnant human donors with an approximate equal ratio of male to female. The thrombin generation in NPP will be interpreted as a surrogate for the thrombin generation in fresh frozen plasma. For this purpose, we used in-house NPP results:

Table 3. Reference ranges for normal pooled plasma.

	Median	Mean	Minimum	Maximum
Lag time (min)	3.89	4.12	2.67	5.78
Peak (nM)	220.96	218.49	137.89	368.62
ETP (nM*min)	1426.36	1422.67	894.49	2195.51
Velocity index (nM/min)	68.15	67.15	40.08	169.06

Source: Reference ranges generated using in-house NPP data.

2.2.4 Recruitment flow diagram

The Consort flow diagram illustrates the recruitment process for the MPhil cohort.

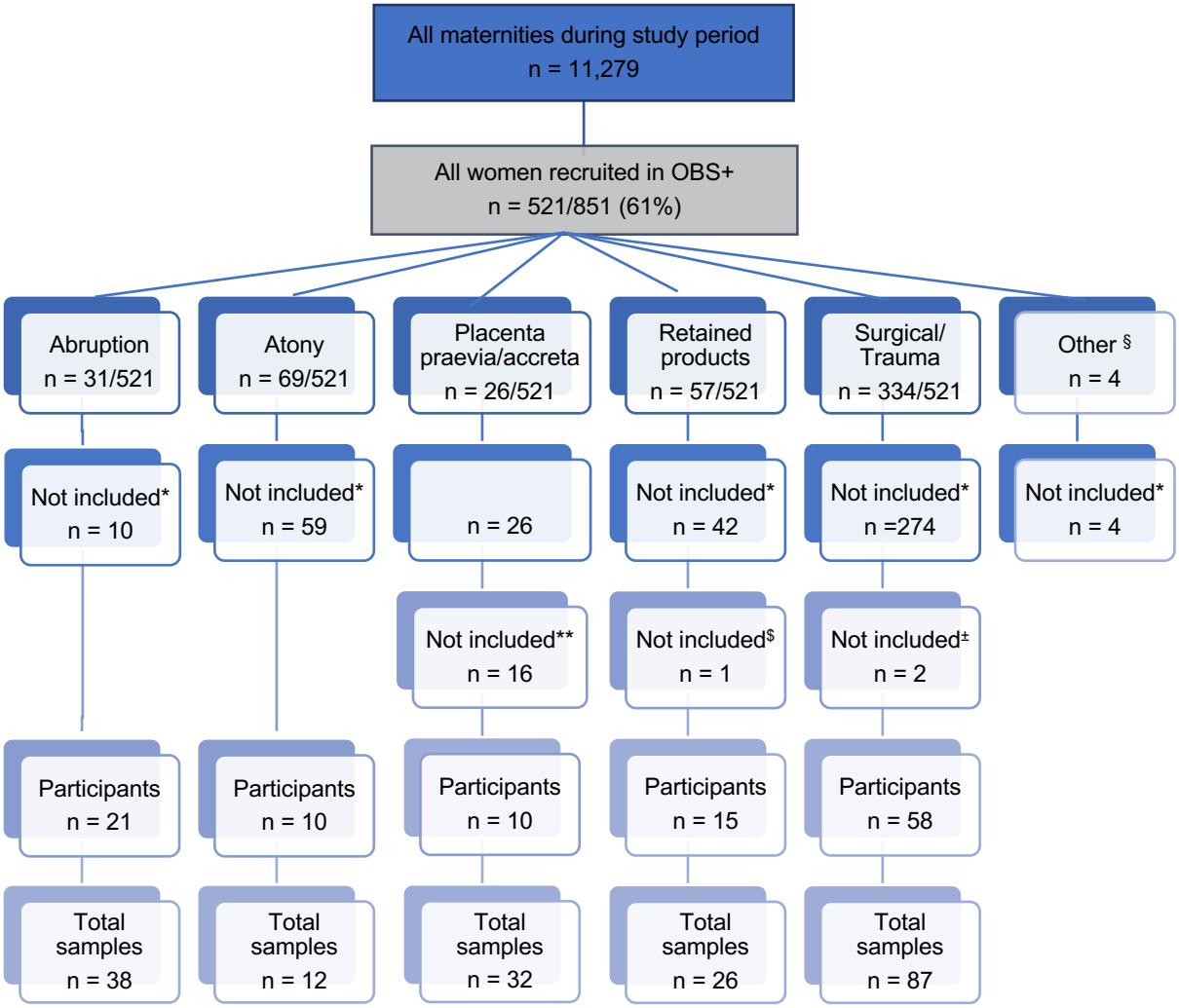

Figure 3. Consort recruitment flow diagram.

§ Four women had septicaemia identified as their primary cause of bleed.

** Women were not included as they were not meeting the inclusion criteria outlined in 2.2.1.*

*** All women with placental abruption should have been included but seven women did not have the correct study samples sent and therefore excluded. One sample was clotted, and no extended specialist testing was possible. Eight samples were insufficient in volume for specialist testing.*

$ One participant's sample was clotted, and no extended specialist testing was possible.

± Two patients did not have the correct study samples sent and seven patients did not have sufficient sampling volume.

During the period from May 6, 2017, to May 30, 2019, there were 11,279 maternities recorded in Cardiff and Vale University Health Board. Among them, 851 women experienced a PPH with a blood loss of ≥1000 ml (Figure 3). Out of the 851 cases, 357 women were not included in the study due to various reasons: 154 were discharged before consent could be obtained, 23 were unable to provide consent due to mental health or communication issues, 12 declined to participate, and the reasons for the remaining exclusions were not recorded. In addition, 27 women with a PPH of <1000 ml were recruited based on clinical concerns related to coagulopathy, such as placental abruption. Ultimately, a total of 521 women were successfully recruited into the OBS+ study (Figure 3).

Among the initial 521 women included in the study, the participants were stratified based on their primary cause of bleeding. There were 114 women who met the inclusion criteria for additional testing with thrombin generation assay specified by this thesis in section 2.2.1. The inclusion of all women with placenta praevia/accreta was intended, but seven women did not have the correct study samples sent, one sample was clotted, and eight had insufficient volume for conducting the thrombin generation assay. One woman in the group with retained products was excluded as her sample also clotted, preventing further testing. Additionally, two women in the surgical/trauma category were excluded due to either incorrect study samples being sent or insufficient volume for further testing.

In addition, as explained in section 2.3, some study participants required multiple bleeding study samples due to ongoing bleeding or clinical concerns. Consequently, the total number of analysed samples exceeds the initial number of participants in the study. Nevertheless, a total of 17 samples had to be further excluded from the analysis due to repeated error messages encountered during the thrombin generation assays. In total the study reports thrombin generation results on 197 samples.

2.2.5 Amniotic fluid embolus

An additional patient will be added to the analysis. This patient was not part of OBS+ because she presented after study closure. A separate consent has been gained to describe this patient as a case report and the case is published [130]. This patient has

a confirmed diagnosis of amniotic fluid embolus and by including this patient, it will enable a comparison of different type of coagulopathies depending on primary cause of bleeds.

2.3 Sample and data collection

Women who met the OBS+ inclusion criteria had standard laboratory blood tests (full blood count, coagulation screen, group and save) taken by clinicians as indicated by the clinical picture at the time of PPH. In total, one 4 ml BD Vacutainer K_2EDTA (dipotassium ethylenediaminetetraacetic acid) tube and one 4 ml BD Vacutainer 3.2% buffered sodium citrate tube was sent for full blood count, including haemoglobin and platelets, and a coagulation screen, including Clauss fibrinogen, prothrombin time, and activated partial thromboplastin time, to the haematology laboratory and processed according to standard laboratory processes. If bleeding continued, additional samples could be taken and analysed as per routine practice: repeat blood sampling circa every 30 minutes while the bleeding continues, or after every additional 500 ml blood loss, or if there was clinical concern, or after infusion of haemostatic components.

At the time of each blood test, two additional sodium citrate tubes were sent for further non-routine testing and analysis. These samples were stored and consent to perform further testing was sought after the bleeding had been controlled. In case of non-consent, the stored samples were destroyed, and patients were not included into the study. The additional sodium citrate samples underwent centrifugation at a speed of 1500 rpm for 15 minutes. Subsequently, the plasma was separated and subjected to further centrifugation to generate PPP. The PPP was separated into a minimum of five individual aliquots, each appropriately labelled with a unique laboratory specimen number, and stored at -80 degree C until further analysis for thrombin generation was required. Once required, the samples were thawed in a water bath. Specialist testing included individual coagulation factor testing (reported in detail here [22]) and thrombin generation. Every sample undergoing thrombin generation testing creates the following parameters: lag time (min), peak thrombin generation (nM), endogenous thrombin potential (nM*min), and velocity index (nM/min).

Pregnant controls had the same blood samples taken at the time of their elective caesarean section for comparison.

Patient demographics and pregnancy details were collected prospectively.

2.4 Calibrated automated thrombogram

2.4.1 Preparation

The Calibrated automated thrombogram (CAT) used was the Diagnostica Stago's ©Thrombinoscope relying on fluorogenic analysis method. Here, fluorescence readings are automatically converted into thrombin generation curves by dedicated software.

The platelet-poor-plasma patient samples were prepared by the laboratory as discussed in previous section. The PPP reagent (mixture of phospholipids and tissue factor), required to initiate thrombin generation, was mixed, prepared in batches and frozen for later use. The final concentration of TF was 5 pM and phospholipids 4 µmol. Stago©FluCal (mixture of fluorogenic substrate (Fluo-Substrate) and Fluo-Buffer (with Calcium Chloride)) was freshly prepared prior to every cycle. The Fluo-Buffer was warmed up in a water bath (37 degrees Celsius) for at least 5 minutes. 40 µl of Fluo-Substrate was then added to one vial of Fluo-Buffer and vortexed. Each FluCal vial was used within one hour of preparation as suggested by the manufacturer.

The T Cal solution (mixture of citrate buffered saline, bovine proteins, and saccharides) was prepared by adding 1 mL deionized water prior to running the thrombinoscope. The CAT wash buffer was prepared by adding 1 ml of 1 M calcium chloride to 9 ml sterile water, vortex and making 10 ml 0.1 M wash buffer.

2.4.2 Cycle process

Prior to and after running a CAT cycle, the machine was cleaned with propan-2-ol and sterile water.

The thrombin generation assay was performed using a Greiner Bio-one crystal-clear, u-bottom shaped, 96 well microplate. Each microplate was divided into up to 3 measurement and up to 2 calibration wells per patient sample depending on aliquot quantity. In the measurement well, by hand, 80 μl of patient plasma was combined with 20 μl PPP reagent, whereas the calibration well was combined with 20 μl T Cal. This did not activate coagulation yet. The microplate was placed into the thrombinoscope and briefly incubated for 10 minutes.

Following the incubation, thrombin generation is initiated by automatically dispensing the prepared $CaCl_2$ and the FluCal solutions (Z-Gly-Gly-Arg-AMC) into each well. The thrombinoscope registers this as zero followed by 10 seconds of shaking, i.e., mixing, the samples and initiating the reading. Measurements are taken for 60 minutes. A dedicated software program compares the readings from the measurement and calibration wells, calculates the thrombin generation and displays the thrombin concentration in real time.

2.5 Statistical analysis

First, a descriptive comparison will be made between the OBS+ cohort, pregnant non-bleeding control group, and the MPhil PPH cohort (referred to subsequently as “PPH cohort”), which will be further subdivided based on the primary cause of bleeding. Age, blood loss at study entry, and total blood loss will be presented as median, 25^{th}, and 75^{th} percentiles, while the mode of delivery will also be analysed for group comparisons.

Next, thrombin generation parameters including lag time, endogenous thrombin potential, peak thrombin value, and velocity index will be evaluated across various cohorts, including the entire PPH cohort, subdivided PPH groups based on the primary cause of bleeding, pregnant non-bleeding control group, and non-pregnant female control group. Initially, each group's parameter data will be described in terms of minimum (min), maximum (max), mean, median, standard deviation (SD), and interquartile range (IQR). Subsequently, statistical comparisons will be conducted among the groups, focusing first on the first blood sample taken during PPH, followed by the worst (lowest for ETP, peak and velocity index and highest for lag time) blood

sample within the PPH group based on individual primary causes of bleeding, and finally, considering all blood samples taken during PPH.

To account for the small sample sizes, prior to running comparisons, preliminary assessments will be conducted to evaluate the normality of data distribution using histograms and Shapiro-Wilk tests. A significance level of $p < 0.05$ will be used to determine statistical significance. If the data deviates from normality, a Kruskal-Wallis test will be employed to compare the non-pregnant control group with the non-bleeding pregnant control group and the PPH cohort. Alternatively, if the data is determined to be parametric, a one-way ANOVA test will be performed. If a significant difference is observed, a multiple pairwise-comparison tests, such as Tukey's Honest Significant Differences, will be employed. Once again, a significance level of $p < 0.05$ will be considered statistically significant.

In the case of a significant difference indicated by the Kruskal-Wallis test, a post-hoc Wilcoxon rank sum test will be used to compare pairs of groups. This comparison will be conducted using either a conservative Bonferroni-adjusted method or the Benjamin & Hochberg adjusted method. If the multiple pairwise-comparison test reveals a statistically significant difference, a paired sample t-test will be used to confirm the differences observed between the groups. Once again, a significance level of $p < 0.05$ will be considered statistically significant.

In addition to comparing PPH subgroups, this project aims to investigate the relationship between changes of thrombin generation parameters and measured blood loss (ml) in the context of postpartum haemorrhage subgrouping. Linear regression analyses will be employed to assess the potential associations between thrombin generation parameters and the amount of measured blood loss experienced by the patients. Linear regression analysis will be used to determine the strength and direction of the relationship between the dependent variable, thrombin generation parameters, and the independent variable, measured blood loss, among the PPH subgroups. In this context, the model fit will be investigated using R^2 and adjusted R^2, i.e., what proportion of the total variability in thrombin generation parameters can be explained by measured blood loss. The interpretation for R^2 values will be made according to the following: R^2 values ranging from 0 to 0.19 will indicate a very weak

or negligible relationship. A range of 0.20 to 0.39 will suggest a weak relationship, while values from 0.40 to 0.59 will indicate a moderate relationship. R^2 values falling between 0.60 and 0.79 will be indicative of a strong relationship, and values between 0.80 and 1.0 will signify a very strong relationship between the variables. The F statistic and its associated p value will help determine the overall significance of the linear regression model. Once again, the significance level will be set at $p < 0.05$ to determine statistical significance.

Statistical analyses, including, graphs, and tables, will be performed using RStudio Version 1.4.1564, with a variety of base, tidyverse, and ggplot functions for data manipulation and visualization.

2.6 Ethical approval

The presented data are anonymised and cannot be related to individual women. Ethical approval was given prior to conducting the study (REC16/WA/0282).

Chapter 3

3 Results

In this section, the results of an investigation into the determinants of thrombin generation parameters in PPH patients are presented. The OBS+ cohort consisted of 521 women who experienced PPH, categorised by their primary cause of bleeding. Among these participants, 114 women met the specific inclusion criteria to have thrombin generation measured.

Four thrombin generation parameters, including lag time (min), peak value (nM), endogenous thrombin potential (nM*min), and velocity index (nM/min), were analysed in order to gain insights into the coagulation dynamics during PPH. Additionally, some study participants had multiple study blood samples taken due to continuous bleeding or clinical concerns, leading to the total number of samples analysed exceeding the number of participants in this project. However, careful data analysis and exclusion of samples with repeated errors were performed to ensure the accuracy and reliability of the findings. Through statistical analysis, the aim is to shed light on the underlying mechanisms of coagulopathy in PPH, ultimately contributing to an improved understanding and management of women with excess of bleeding after childbirth.

3.1 Descriptive analyses

The following table summarises the descriptive analyses, including, age, BMI, mode of delivery, gestation, twins, blood loss at study entry, and the total blood loss. Analyses parameters include median, 25th and 75th percentiles. Four cohorts were analysed:

1. The entire OBS+ PPH cohort (521 patients)
2. The entire MPhil PPH cohort (114 patients)
3. The MPhil PPH cohort subdivided into primary cause of bleed (114 patients)
4. The pregnant non-bleeding control cohort (35 patients)

Apart from the defined cohorts, this project also includes one woman who experienced an amniotic fluid embolus, who will be discussed separately in section 3.3 to explore this unique clinical scenario in detail.

Table 4. Descriptive analysis: OBS+ cohort vs. the pregnant control (PC) and postpartum haemorrhage cohort.

				Postpartum Haemorrhage Study Group					
		OBS+ Cohort	**PC**	**Total**	**Ab-ruption**	**Atony**	**Placenta praevia/ accreta**	**Retained products**	**Surgical/ trauma**
Count		521	35	114	21	10	10	15	58
Age	Median	31.2	NA	32	28	35	36	31	33
	25th percentile	28	NA	28	24	33.25	32	29	28
	75th percentile	35	NA	35	33	36.5	35.25	34.5	35
BMI	Median	27.5	NA	25	24	27.45	23	25	26.5
	25th percentile	23	NA	23	23	23.25	20.5	24	24
	75th percentile	31	NA	29	25	35	28.5	30.5	30
Mode of Delivery n (%)	Unassisted vaginal	167 (32)	0 (0.0)	28 (24.6)	4 (19.0)	1 (10.0)	0 (0.0)	12 (80.0)	11 (19.0)
	Instrumental vaginal	130 (25)	0 (0.0)	29 (25.4)	3 (14.3)	1 (10.0)	0 (0.0)	2 (13.3)	23 (39.7)
	Routine caesarean section	80 (15)	35 (100.0)	17 (14.9)	1 (4.8)	6 (60.0)	4 (40.0)	0 (0.0)	6 (11.3)
	Emergency caesarean section	144 (28)	0 (0.0)	40 (35.1)	13 (61.9)	2 (20.0)	6 (60.0)	1 (6.7)	18 (31.0)
Blood loss at study entry	Median	1198	0	1200	975	1400	1000	1115.5	1300
	25th percentile	1000	0	1000	600	1300	985	1000	1000
	75th percentile	1400	0	1552.5	1175	1465.5	1102	1475	1674
Total blood loss (ml)	Median	1500	NA	2050	1200	2040	3525	2250	2125
	25th percentile	1200	NA	1600	900	2005	2425	1950	1825
	75th percentile	1800	NA	2500	1500	2308.5	5125	2450	2500

NA stands for not applicable meaning not recorded. Pregnant controls (PC).

3.2 Thrombin generation results

This section focuses on the analyses of thrombin generation results (lag time, peak value, endogenous thrombin potential, and velocity index). Per thrombin generation parameter, results are reported for the first blood sample during the PPH for each PPH participant at study entry, i.e., at the start of bleeding (or clinical concern of bleeding) and the most abnormal thrombin generation result during the bleed. The following four cohorts were analysed:

1. The entire MPhil PPH cohort (114 patients)
2. The MPhil PPH cohort subdivided into primary cause of bleed (114 patients)
3. The non-pregnant female control cohort (20 participants)
4. The pregnant non-bleeding control cohort (35 patients)

In the PPH group, a total of 106 first blood samples were collected for analysis. However, during the thrombin generation assessment, 11 samples encountered error messages and could not be included in the analysis. For this reason, three samples from abruption had to be removed, one sample from atony, two from placenta praevia/accreta, three in retained placenta/products, and two from the surgical/trauma group. As a result, the final analysed dataset consisted of 95 first blood samples from the PPH group.

The patient suffering from amniotic fluid embolus during PPH will be reported separately.

3.2.1 Lag time

3.2.1.1 First blood samples

Lag time corresponds to the initiation of coagulation. Thus, the end of lag time indicates the time plasma clots start to be formed. Table 5 shows the descriptive analysis of the first blood sample taken during postpartum haemorrhage and the results for the pregnant and non-pregnant controls.

Table 5. Descriptive analysis: lag time (min) in the first samples during postpartum haemorrhage and the control groups.

	Count	Min	Max	Mean	SD	Median	IQR
Non-pregnant control	20	2.00	4.65	3.20	0.73	3.07	0.80
Non-bleeding pregnant control	34	2.11	6.11	3.08	0.76	3.00	0.66
Postpartum haemorrhage (first blood samples)	96	1.83	6.67	2.96	0.74	2.94	0.88
Postpartum haemorrhage (all blood samples)	240	1.44	6.67	2.83	0.77	2.67	0.84

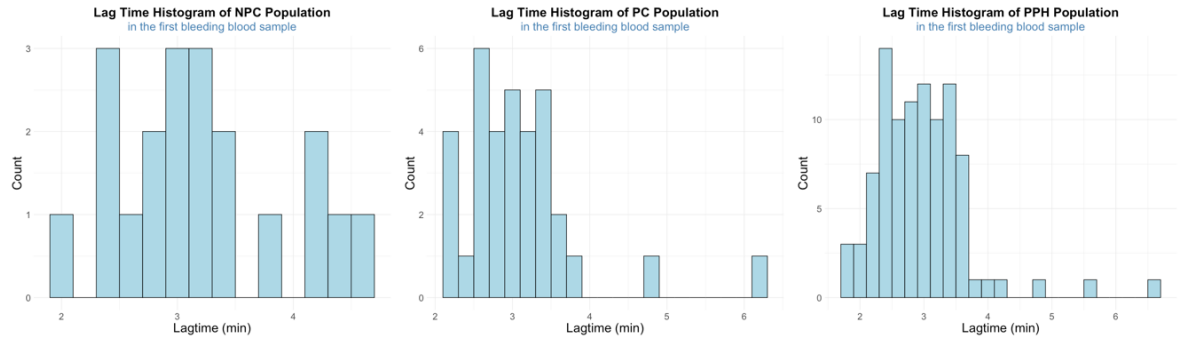

Figure 4. Lag time (min) histograms for the non-pregnant (NPC) and non-bleeding pregnant control (PC) groups and the first blood samples taken during postpartum haemorrhage (PPH).

Due to small sample sizes, specifically in the non-pregnant and non-bleeding pregnant group, the distribution of lag time prior to choosing the appropriate statistical method needed to be investigated. To check for normality visually, histograms (see Figure 4) were produced and Shapiro-Wilk Tests performed, which showed that the distribution of lag time departed significantly from normality in the non-bleeding pregnant control (W = 0.80, p < 0.01 [$2.44e^{-05}$]) and PPH group (W = 0.87, p < 0.01 [$8.522e^{-08}$]). The Shapiro-Wilk results in the non-pregnant control group did not show evidence of non-normality (W = 0.95, p = 0.33).

Based on this outcome, a Kruskal-Wallis test was used to test for any significant differences in lag time between the first blood sample taken during PPH compared with the non-bleeding pregnancy and non-pregnant controls and thus to see if time to clot formation is affected by pregnancy or disturbed early during PPH. As the p is not significant ($H(2)$ = 1.93, p = 0.38), there is no statistically significant difference in lag time in the first blood sample taken during PPH and the control groups.

To further understand lag time within the PPH group, the first blood samples during the PPH were analysed by subgrouping them into their primary causes of bleeding and the descriptive analysis is summarised in Table 6. Figure 5 compares the results in a box and whisker plot.

Table 6. Descriptive analysis: lag time (min) in first blood samples taken during postpartum haemorrhage.

Primary cause of bleed	Count	Min	Max	Mean	SD	Median	IQR
Abruption	18	2.17	5.62	3.21	0.80	3.17	0.93
Atony	7	1.84	3.67	2.77	0.59	2.72	0.59
Placenta praevia/accreta	8	2.00	3.67	2.81	0.60	2.94	0.90
Retained placenta/ products	12	2.67	3.67	3.19	0.36	3.11	0.59
Surgical/trauma	50	1.83	6.67	2.87	0.80	2.79	0.84

The median lag time ranges from 2.72 min in the atony to 3.17 min in the abruption group. Prior to running significance testing, Shapiro-Wilk Tests were performed on each PPH subgroup: Abruption ($W = 0.88$, $p < 0.05$ [$p = 0.023$]), atony ($W = 0.99$, $p = 0.997$), placenta praevia/accreta ($W = 0.94$, $p = 0.64$), retained placenta/products ($W = 0.92$, $p = 0.31$), surgical/trauma ($W = 0.81$, $p < 0.01$ [$p = 1.073e^{-06}$]). Since distribution departed significantly from normality in some groups, a Kruskal-Wallis test was run to check for significance. The p is not significant ($H(2) = 7.72$, $p = 0.1$), thus despite a difference of 0.445 min in median lag time between the lowest and highest median lag time across PPH subgroups, there is no significant difference in lag time in the first blood sample across PPH subgroups.

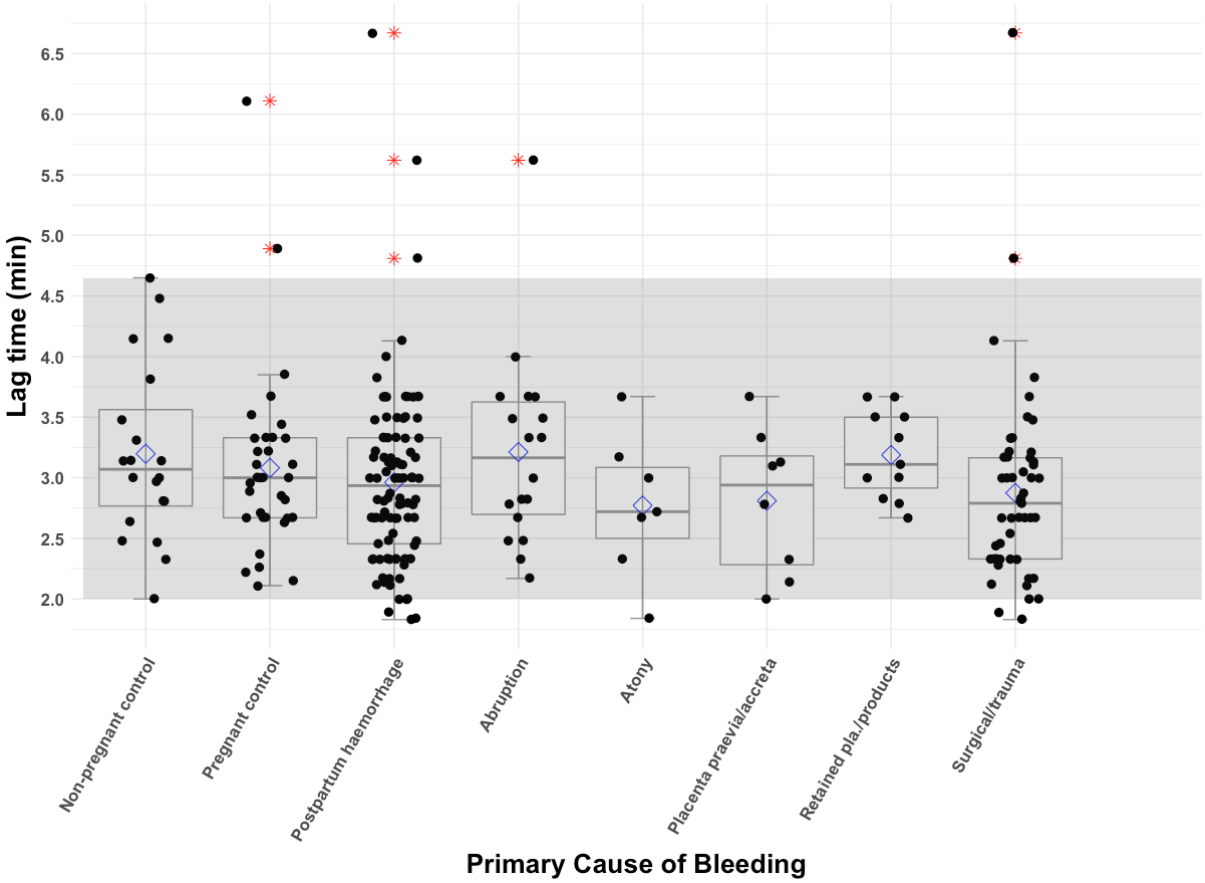

Figure 5. Box plot: lag time (min) in first blood samples taken during postpartum haemorrhage.

Blue diamond represents the mean value. The red stars show outliers, and the grey area indicates the normal non-pregnant range.

3.2.1.2 Sample with longest lag time during postpartum haemorrhage

However, the first blood sample during PPH may not be the most abnormal. Therefore, analyses on the longest lag time during PPH for each patient were performed as a sign of most significant coagulopathy and these are reported in the following table:

Table 7. Descriptive analysis: lag time (min) of blood samples with the longest lag time during postpartum haemorrhage.

Primary cause of bleed	Count	Min	Max	Mean	SD	Median	IQR
Abruption	21	2.33	5.62	3.31	0.82	3.04	0.89
Atony	10	1.84	4.11	2.91	0.64	2.80	0.42
Placenta praevia/accreta	10	2.00	4.33	2.84	0.71	2.73	0.95
Retained placenta/products	14	2.67	3.78	3.19	0.37	4.06	0.63
Surgical/trauma	57	1.89	6.67	3.01	0.99	2.67	0.96

By focussing on the longest lag time for each woman, median lag time results range from 2.67 min in the surgical/trauma to 4.06 min in the retained placenta/products groups. As done with the first blood sample during PPH, Shapiro-Wilk Tests were performed on each PPH subgroup prior to testing for significant differences across the subgroups: abruption (W = 0.89, p <0.05 [p = 0.023]), atony (W = 0.96, p = 0.79), placenta praevia/accreta (W = 0.92, p = 0.38), retained placenta/products (W = 0.91, p = 0.17), surgical/trauma (W = 0.83, p < 0.01 [p = 1.278e^{-06}]).

Since distribution departed significantly from normality in some groups, a Kruskal-Wallis Test was performed to check for any significant differences. The p is not significant ($H(2)$ = 7.61, p = 0.12), there is no significant difference in lag time in the blood sample with the longest lag time across PPH subgroups.

3.2.1.3 Lag time of all samples taking during postpartum haemorrhage

This section includes the analysis of all samples collected during PPH. As mentioned earlier, a single woman may contribute multiple samples to this cohort. As done previously, Shapiro-Wilk Tests was performed on each subgroup: non-pregnant control (W = 0.95, p = 0.33), non-bleeding pregnant control (W = 0.80, p < 0.01 [p = 2.44e^{-05}]), PPH (W = 0.88, p < 0.01 [p = 2.205e^{-11}]), abruption (W = 0.87, p < 0.01 [p = 0.0004]), atony (W = 0.97, p = 0.90]), placenta praevia/accreta (W = 0.85, p < 0.01 [p = 0.0005]), retained placenta/products (W = 0.96, p = 0.31]), surgical/trauma (W = 0.80, p < 0.01 [p = 2.169e^{-09}]).

Since distribution departed significantly from normality in some groups, a Kruskal-Wallis Test was performed to check for any significant differences. Since the p ($H(2)$ = 35.44, p = 9.229e^{-06}) is less than the significance level 0.05, there are statistically significant differences in lag time between the PPH and the control groups.

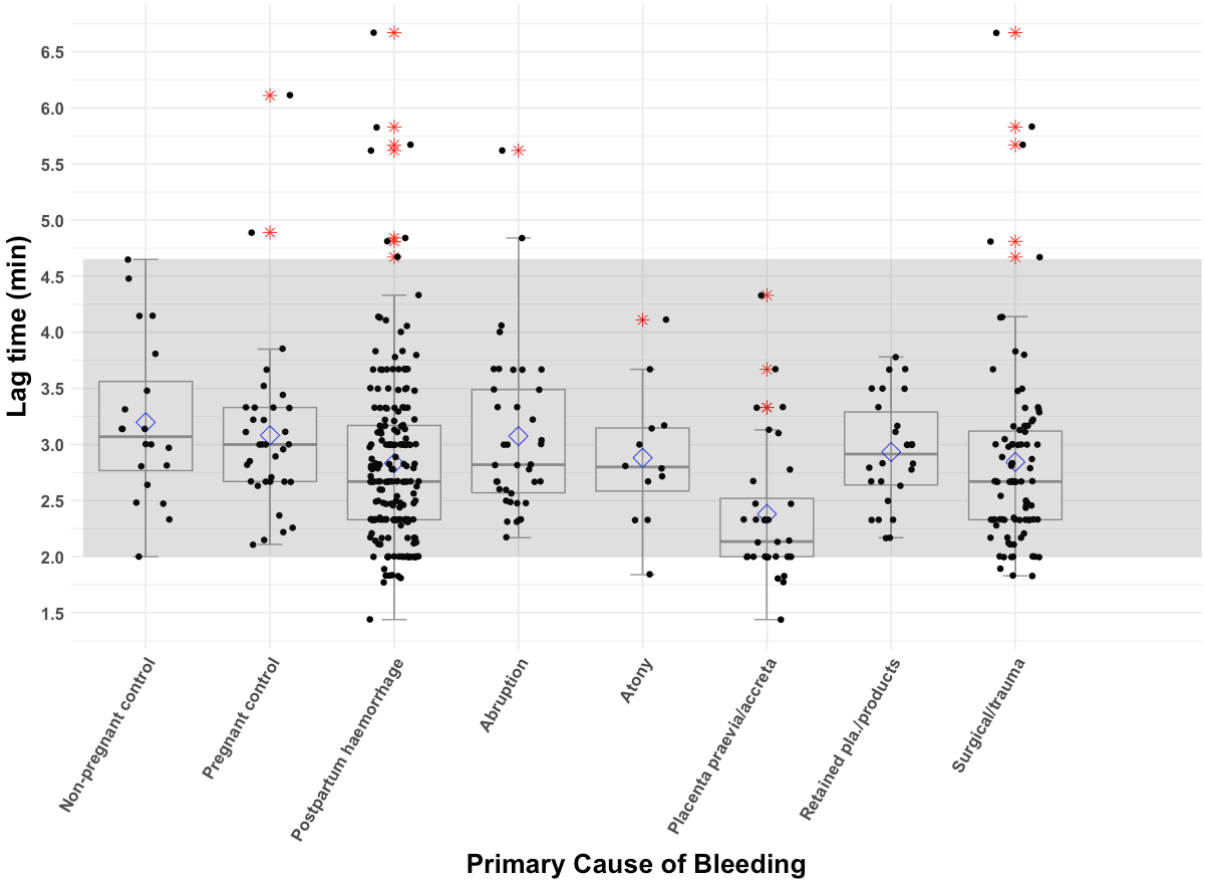

Figure 6. Box plot: lag time (min) in all samples taken during postpartum haemorrhage. Blue diamond represents the mean. The red stars show outliers, and the grey area indicates the normal non-pregnant range.

Post-hoc Wilcoxon rank sum tests using the conservative Bonferroni-adjusted method were used to compare all pairs of groups with each other. The pairwise comparison shows that placenta praevia/accrete lag time was statistically significantly shorter than that of non-pregnant controls (p = 0.002), pregnant controls (p = 0.0005), abruption (p = 0.0002), retained placenta/products (p = 0.003) and surgical/trauma (p =0.019). Changing the adjustment method to the more powerful Benjamin & Hochberg method does not change the number of significant pairs. Figure 6 supports visually the statistics, the mean and median of group placenta praevia/accreta is noticeably lower in comparison to all other PPH subgroups and control groups. A shorter lag time means that coagulation is activated more rapidly and is not associated with haemostatic impairment. This means that, whilst the differences are statistically significant, they are not likely to be clinically significant.

The primary endpoint of the study was to determine the proportion of women exhibiting abnormal thrombin generation, and, in the majority of cases, the lag time observed in

the PPH group remained below the top of the normal range for both non-bleeding pregnant and non-pregnant control groups. There were 7/114 (6.14%) women with PPH who had lag times above the top of the normal range of the non-pregnant control (4.65 min) at some time during the bleed suggesting the possibility of haemostatic impairment. However, 1/114 (0.88%) had a lag above the top of the normal range of non-bleeding pregnant controls (6.11 min). This suggests that despite the PPH, women in this cohort did not show signs of haemostatic impairment compared to other pregnant women. The number of women with a lag time above the range for normal pooled plasma (5.78 mins) was 2/114 (1.75%) suggesting that the thrombin generation, as measured by lag time, would not have been improved by FFP infusion in the large majority of women with PPH.

The seven women who triggered the primary endpoint are described in more detail in section 3.3.1.

3.2.1.4 Lag time during postpartum haemorrhage dependent on measured blood loss

In the linear regression analyses conducted for different subgroups of primary cause of bleeding, the relationship between measured blood loss and lag time was investigated. Figure 7 and Figure 8 show the graphical correlation with regression lines grouped per PPH subgroup. The abruption and atony groups show an overall visual increase in lag time with increasing blood loss, whereas no visual pattern can be observed in groups retained placenta/praevia and surgical/trauma. A visual decrease in lag time with bleeding volume is observed in the group placenta praevia/accreta. The regression line for placenta praevia/accreta is strongly influenced by two patients (study numbers 239 & 611). Both patients were young (25 and 23 years old), underwent a category 2 caesarean section, i.e., the health of the mother or baby are at danger but not immediate life threatening, and a caesarean section needs to be carried out between 30-60 minutes, and had normal BMIs (20 & 23). At the time of the first tests conducted during PPH, patient 239 had a normal PT (11.3 sec), a reduced aPTT (23.3 sec), and a fibrinogen that was reduced for pregnancy (3.3 g/L). Unfortunately, no data on measured blood loss was recorded for this initial sample. The first instance of measured blood loss associated with a study sample

occurred at 3 litres blood loss, correlating with a normal PT (11.8 sec), a normal aPTT (27.5 sec), and a fibrinogen of 5.5 g/L after receiving fibrinogen transfusion. Two additional coagulation samples were obtained between the first sample and the first study sample where MBL data was available. However, as these were regular coagulation samples and not study samples, it was not possible to assess thrombin generation. These two samples did reveal a further decrease in fibrinogen levels to 2.4 g/L, prompting the initiation of fibrinogen infusion, which ultimately raised the fibrinogen level to 5.5 g/L. On the other hand, the first blood sample taken for patient 611 was at 2320mL and showed a normal PT (12.2 sec), a reduced aPTT (<22 sec), and a low level of fibrinogen of 2.5 g/L. Ultimately, both patients had shorter lag times (2.14 and 2.33 minutes) than normal pooled plasma (4.02 minutes) suggesting FFP would not have improved haemostasis based on lag time parameter.

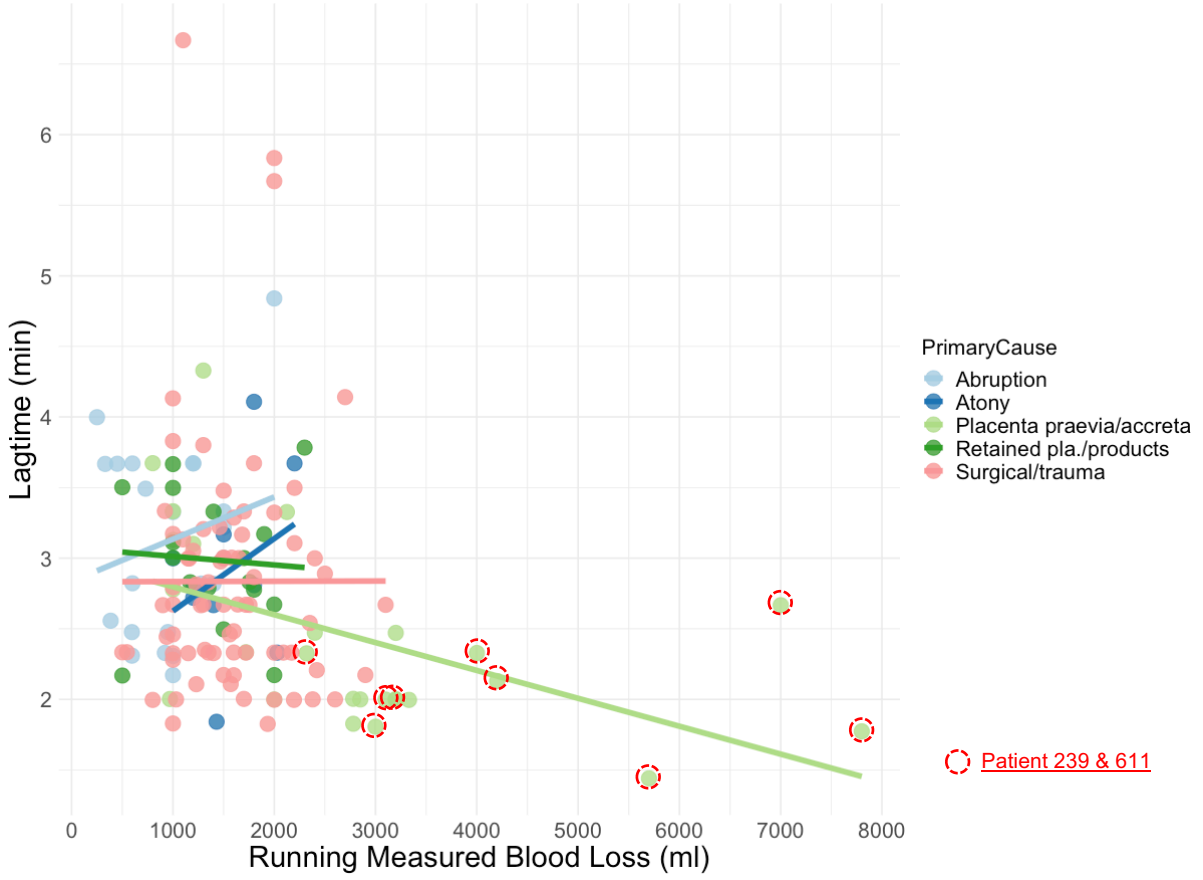

Figure 7. Lag time (min) – running measured blood loss (ml) correlation grouped by primary cause of bleed with regression lines excluding standard errors.

Linear regression analyses results are summarised in Table 8. For abruption, the analysis showed a negligible and statistically non-significant association ($F(1, 20) = 0.85$, $p = 0.37$). Similarly, in atony, there was no significant relationship between these variables ($F(1, 8) = 0.86$, $p = 0.38$). In contrast, the placenta praevia/accreta subgroup demonstrated a weak relationship ($F(1, 24) = 8.75$, $p = 0.007$), suggesting that shorter lag time was associated with increased MBL. Conversely, for the retained placenta/products and surgical/trauma subgroups, lag time did not show a significant effect ($F(1, 15) = 0.069$, $p = 0.80$ and $F(1, 74) = 0.0001$, $p = 0.99$, respectively). Overall, the R^2 values were low, indicating that the models had limited explanatory power for the relationship between lag time and measured blood loss across all subgroups.

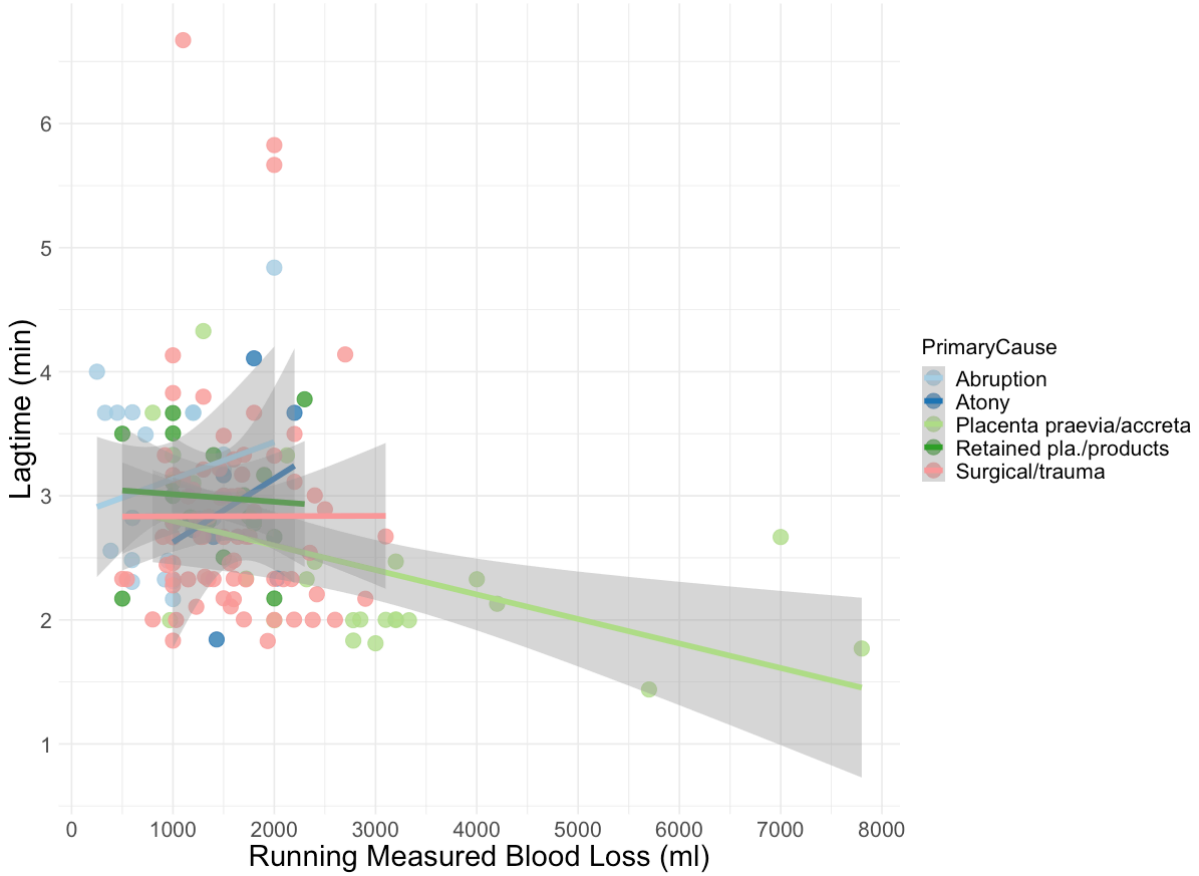

Figure 8. Lag time (min) – running measured blood loss (ml) correlation grouped by primary cause of bleed with regression lines including standard errors in grey.

Table 8. Linear regression analysis for lag time.

	Abruption	Atony	Placenta praevia/accreta	Retained placenta/products	Surgical/ trauma
Lag time estimate	136.4	189.5	-1353.1	-75.69	0.76
R^2	0.041	0.097	0.27	0.0046	$1.419e^{-06}$
Adjusted R^2	-0.007	-0.016	0.24	-0.062	-0.014
F-statistic	0.85	0.86	8.75	0.069	0.0001
p	0.37	0.38	0.007	0.80	0.99

3.2.2 Peak thrombin generation

3.2.2.1 First blood sample

As previously described, peak thrombin value represents the maximum concentration of thrombin generated. Table 9 shows the descriptive analysis of the first blood and all samples taken during PPH, as well as the peak thrombin results of the non-pregnant and the non-bleeding pregnant controls.

Table 9. Descriptive analysis: peak thrombin generation (nM) in the first samples during postpartum haemorrhage and the control groups.

	Count	Min	Max	Mean	SD	Median	IQR
Non-pregnant control	20	110.19	408.18	273.18	73.97	266.10	108.05
Non-bleeding pregnant control	34	194.49	532.48	394.19	71.87	397.34	93.58
Postpartum haemorrhage (first blood samples)	96	117.16	672.97	382.43	84.16	387.69	99.39
Postpartum haemorrhage (all blood samples)	240	117.16	672.97	362.17	85.49	359.88	97.23

To determine the distribution of peak thrombin across the three groups and to check for normality visually, histograms were produced (see Figure 9) and Shapiro-Wilk Tests performed, which did not show evidence of non-normality of peak values across all groups: non-pregnant controls ($W = 0.96$, $p = 0.58$), non-bleeding pregnant controls ($W = 0.98$, $p = 0.66$), and PPH ($W = 0.97$, $p = 0.06$). Homogeneity of variance was tested by running a Levene's test. For peak thrombin values, the variances were similar for non-pregnant controls, non-bleeding pregnant controls, & PPH ($F(8, 237) = 0.43$, $p = 0.90$).

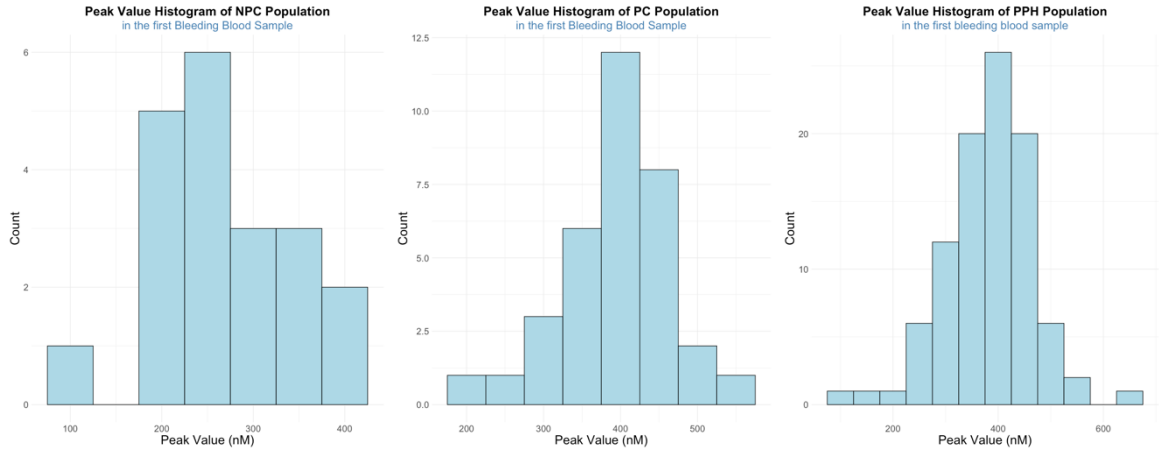

Figure 9. Peak thrombin generation histograms for the non-pregnant (NPC) and non-bleeding pregnant control groups (PC) and the first blood samples taken during postpartum haemorrhage (PPH).

Based on this outcome, and after visual examination of the histograms, a parametric test was used to investigate the first blood samples during PPH and the control groups. A one-way ANOVA was performed and revealed that there was a statistically significant difference in peak thrombin between the three groups (*F*-value = 17.24, *p* = $1.88e^{-07}$). Thus, a multiple pairwise-comparison (Tukey Honest Significant Differences) was performed. This found that the mean of peak values was significantly higher in the non-bleeding pregnant control group compared to the non-pregnant controls (*p* = 0.000001, 95% C.I. = [67.45, 174.57]). Similarly, the peak values for the first samples taken during PPH were higher than non-pregnant controls (*p* = 0.0000004, 95% C.I. = [62.54, 155.98]). There was no statistically significant difference between the PPH and the non-bleeding pregnant group (*p* = 0.74).

To further understand the peak thrombin within the PPH group, first blood samples taken during the postpartum haemorrhage were analysed by subgrouping them into primary cause of bleeds and the descriptive analysis is summarised in Table 10.

Table 10. Descriptive analysis: peak thrombin generation (nM) in first blood samples taken during postpartum haemorrhage.

Primary cause of bleed	Count	Min	Max	Mean	SD	Median	IQR
Abruption	18	265.00	672.97	410.03	99.17	389.79	108.03
Atony	7	295.29	560.67	376.45	91.00	361.92	79.05
Placenta praevia/ accreta	8	284.34	447.78	358.85	59.42	346.35	93.11
Retained placenta/ products	12	137.58	456.19	348.70	95.75	375.95	89.18
Surgical/trauma	50	117.16	506.43	383.17	78.24	393.40	88.17

The median peak value ranges from 346 nM in the placenta praevia/accreta group to 393 nM in the surgical/trauma group. Prior to running significance testing, Shapiro-Wilk Tests were performed on each PPH subgroup: abruption ($W = 0.94$, $p = 0.28$]), atony ($W = 0.84$, $p = 0.10$), placenta praevia/accreta ($W = 0.93$, $p = 0.53$), retained placenta/products ($W = 0.89$, $p = 0.14$), and surgical/trauma ($W = 0.94$, $p < 0.05$ [$p = 0.012$]).

Figure 10 shows that the non-bleeding pregnant controls and all groups of women experiencing PPH had higher peak thrombin generation compared to the non-pregnant controls. To investigate whether the peak thrombin varied dependent on the cause of the bleed, these groups were compared. Given the small sample sizes of the PPH subgroups and that the p is < 0.05 in the surgical/trauma group, normality could not be assumed. Thus, a Kruskal-Wallis test was performed to test for any statistical significant differences across PPH subgroups. The p of 0.43 shows that despite a difference of 47 nM in median peak value between the lowest and highest median peak thrombin across PPH subgroups groups, there is no significant difference between the groups.

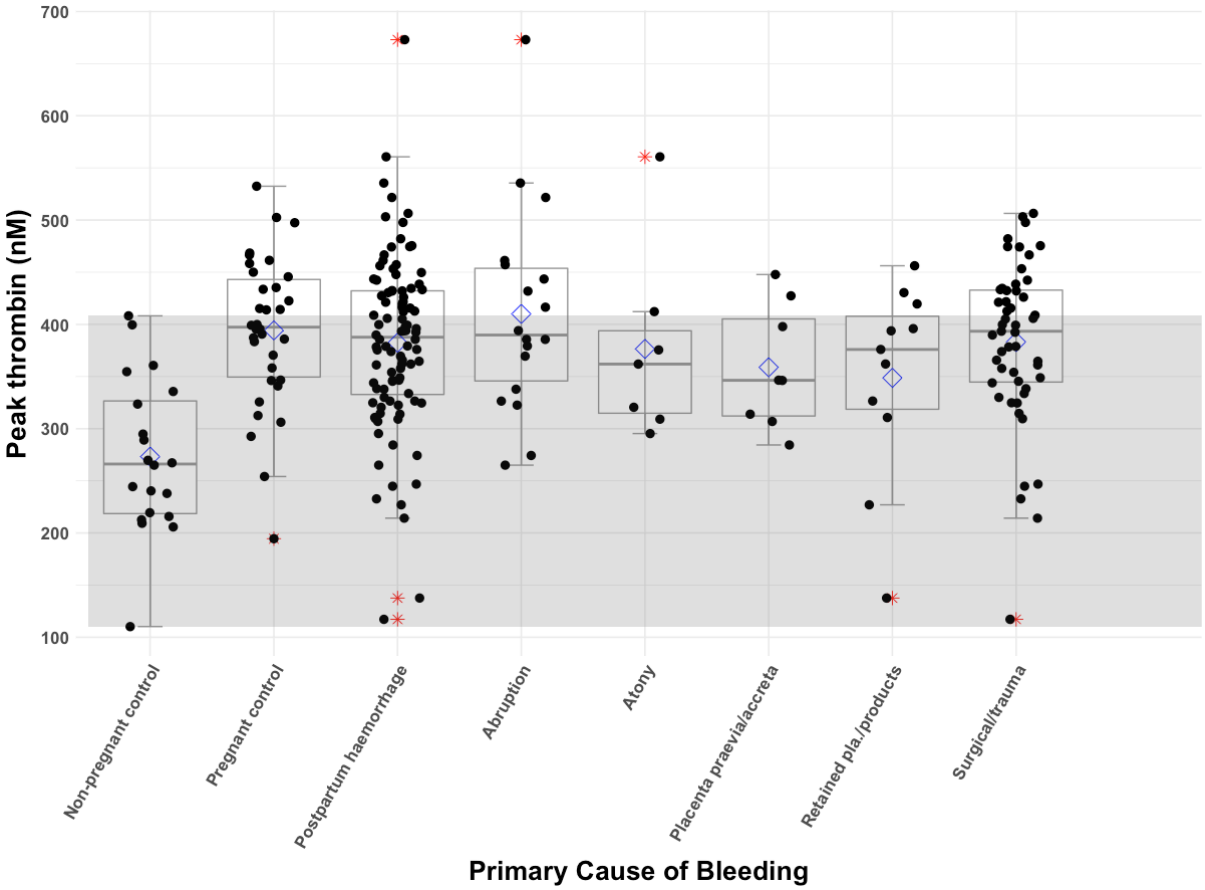

Figure 10. Box plot: peak thrombin generation (nM) in first blood samples taken during postpartum haemorrhage.

Blue diamond represents the mean. The red stars show outliers, and the grey area indicates the normal non-pregnant reference.

Interestingly, 57 out of 95 first blood samples taken during PPH are above the NPP range of 368.62 nM, indicating that despite the bleeding, almost 60% of patients reach higher maximum thrombin concentrations than would be expected for FFP. When comparing the results to the reference range of our non-bleeding pregnant control group (194.49-532.48 nM), two first blood samples from this PPH cohort fell below this reference range. One patient from the surgical/trauma subgroup exhibited a thrombin peak concentration of 117.16 nM, while another patient with retained placenta/products had a thrombin peak concentration of 137.58 nM.

Figure 10 shows that almost 2/3 of women with PPH have higher peak thrombin generation in the first blood sample than non-pregnant controls. These high peak values become even more apparent in Figure 11, which shows all blood samples taken during PPH.

3.2.2.2 Sample with lowest peak thrombin generation during postpartum haemorrhage

The first blood sample during postpartum haemorrhage may not be representative of the lowest peak thrombin generation during the bleed. Therefore, I extracted the lowest peak values for each woman, and these are shown in the following descriptive analyses:

Table 11. Descriptive analysis: peak thrombin generation (nM) of blood samples with the lowest peak thrombin during postpartum haemorrhage.

Primary cause of bleed	Count	Min	Max	Mean	SD	Median	IQR
Abruption	21	264.31	566.46	364.67	83.07	353.55	71.45
Atony	10	295.29	601.39	407.95	114.02	368.71	175.04
Placenta praevia/ accreta	10	161.04	397.85	291.42	77.19	298.89	69.80
Retained placenta/ products	14	120.43	417.57	342.76	86.24	376.55	87.51
Surgical/trauma	57	117.16	547.46	361.34	83.36	365.68	96.26

The median peak values range from 298.89 nM in placenta praevia/accreta to 376.55 nM in retained placenta/products. Shapiro-Wilk Tests were performed on each PPH subgroup prior to testing for any significant difference from normality in peak value across the subgroups: abruption (W = 0.91, p = 0.057]), atony (W = 0.89, p = 0.08), placenta praevia/accreta (W = 0.95, p = 0.70), retained placenta/products (W = 0.81 p < 0.01 [p = 0.007]), and surgical/trauma (W = 0.982, p = 0.55).

Since the distribution departed significantly from normality in one group (retained placenta/products) and sample sizes are <20 in three groups, a non-parametric test, i.e., Kruskal-Wallis Test, was used to investigate for any significant differences. The p is not significant ($H(2)$ = 7.01, p = 0.22), thus there is no significant difference in the lowest peak thrombin generation across PPH subgroups.

Two women in this sub analysis exhibited peak thrombin generation levels below the reference range of normal pooled plasma (137.89 nM). The first woman, who suffered from surgical/trauma and was previously discussed for having lag times outside the NPP range, had a thrombin peak of 117.2 nM. The second woman, who experienced retained placenta/products, had two samples with thrombin peaks below the NPP

reference: 120.4 nM (second blood sample) and 137.6 nM (first blood sample). The measured blood loss was not known for the lower peak thrombin generation, but her total blood loss was 3000 ml, and her fibrinogen level for that sample was below 2 g/L, measuring at 1.7 g/L [126]. Notably, she did not receive any blood products during the study.

A total of four blood samples taken during PPH exhibited peak thrombin generation levels below the reference range observed in non-bleeding pregnant controls (194.49 nM). Among these samples were the two women mentioned in the previous paragraph, who suffered from surgical/trauma and retained placenta/products, with thrombin peaks of 117.2 nM and 120.4 nM, respectively. Additionally, two women with placenta praevia/accreta showed thrombin peaks of 161.04 nM and 192.97 nM.

3.2.2.3 Peak thrombin generation of all samples taking during postpartum haemorrhage

Figure 11 shows a box plot of all peak thrombin generation results taken during PPH. A Shapiro-Wilk Tests was performed on each group: non-pregnant control ($W = 0.96$, $p = 0.58$), non-bleeding pregnant control ($W = 0.98$, $p = 0.66$), PPH ($W = 0.99$, $p = 0.07$), abruption ($W = 0.93$, $p < 0.05$ [$p = 0.017$]), atony ($W = 0.82$, $p < 0.05$ [$p = 0.018$]), placenta praevia/accreta ($W = 0.98$, $p = 0.70$), retained placenta/products ($W = 0.87$, $p < 0.01$ [$p = 0.004$]), and surgical/trauma ($W = 0.98$, $p = 0.34$).

Since distribution departed significantly from normality in some groups, a Kruskal-Wallis Test was performed to investigate for any significant differences. Since the p ($H(2) = 46.44$, $p = 7.169e^{-08}$) is less than the significance level 0.05, I can conclude that there are statistically significant differences in peak thrombin between the groups.

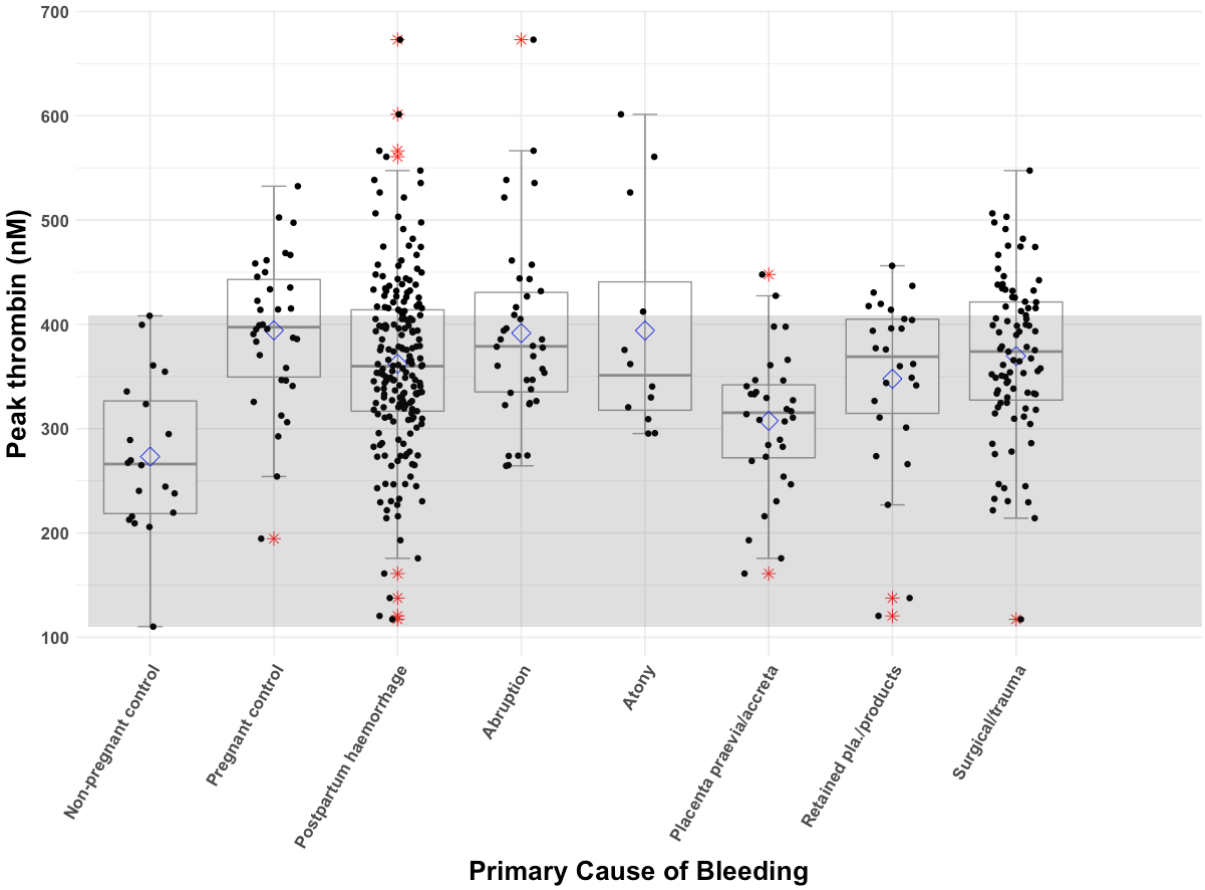

Figure 11. Box plot: peak thrombin generation (nM) in all blood samples taken during postpartum haemorrhage.
Blue diamond represents the mean. The red stars show outliers, and the grey area indicates the non-pregnant normal range.

Post-hoc Wilcoxon rank sum tests using the Benjamin & Hochberg adjusted method were used to compare all pairs of groups with each other. Table 12 shows the *p value*s of the pairwise comparison. It shows that the non-pregnant control group had lower peak thrombin generation than non-bleeding pregnant controls and all groups of women with PPH. The non-bleeding pregnant control group had higher peak thrombin generation than the combined PPH group and this was mainly related to the effect of lower peak thrombin levels seen in placenta praevia/accreta relative to other causes of bleeding.

Changing the adjustment method to the more conservative Bonferroni method changes the result, so that for retained placenta/products vs placenta praevia/accreta, where the *p* was 0.33, therefore suggesting there is no statistically significant difference between those groups.

Table 12. Post-hoc Wilcoxon rank sum test for peak thrombin generation across non-pregnant, non-bleeding pregnant, postpartum haemorrhage and its subgroups.

Non pregnant control	vs	non-bleeding pregnant control	*p*	=	$3.1e^{-05}$
Non pregnant control	vs	postpartum haemorrhage	*p*	=	$9.7e^{-05}$
Non pregnant control	vs	abruption	*p*	=	$7.8e^{-05}$
Non pregnant control	vs	atony	*p*	=	0.002
Non pregnant control	vs	retained placenta/products	*p*	=	0.003
Non pregnant control	vs	surgical/trauma	*p*	=	$8.8e^{-05}$
Non-bleeding pregnant control	vs	postpartum haemorrhage	*p*	=	0.033
Non-bleeding pregnant control	vs	placenta praevia/accreta	*p*	=	$4.2e^{-05}$
Abruption	vs	placenta praevia/accreta	*p*	=	0.0002
Atony	vs	placenta praevia/accreta	*p*	=	0.042
Retained placenta/products	vs	placenta praevia/accreta	*p*	=	0.03
Surgical/trauma	vs	placenta praevia/accreta	*p*	=	0.0002

The primary endpoint of this project was to investigate the prevalence of abnormal thrombin generation in women experiencing PPH. The analysis revealed that 0/114 (0%) of the women exhibited peak thrombin generation levels below the lower reference limit of 110.19 nM of non-pregnant controls during the bleeding. The lowest peak thrombin level in the non-pregnant control group is not a statistical outlier but when considering ETP this sample is a statistical outlier. In post hoc analysis this control was excluded, and this resulted in 6/114 (5.3%) women having a peak thrombin below this revised normal range and based on this stricter definition, could be considered to have evidence of haemostatic impairment.

Furthermore, 4/114 women had a total of six samples exhibiting peak thrombin values below the lower limit of non-bleeding pregnant controls (194.49 nM) but only two women (2/114, 1.8%) had a total of three samples with peak thrombin values below the lower limit of normal pooled plasma (137.89 nM). Based on these findings, there was minimal evidence of haemostatic impairment in this cohort, concerning this specific thrombin generation parameter, and only two women could have potentially benefitted from FFP.

3.2.2.4 Peak thrombin generation during postpartum haemorrhage dependent on volume of blood loss

Figure 12 and Figure 13 were generated to examine the correlation between peak thrombin generation levels and measured blood loss at the time of blood sampling. Regression lines were grouped into primary cause of bleeds. Table 13 summarises the regression analyses. Visually only the group placenta praevia/accreta shows a decrease in peak thrombin associated with increasing measured blood loss. Out of the total of 10 women in the placenta praevia/accreta group, only two women experienced severe PPHs larger than 4000 ml, resulting in the regression line being predominantly influenced by these two cases alone. The results for individual women with placenta praevia/accreta are shown in Figure 14. Of note is that the peak thrombin for the woman who had the bleed 8000 ml increased after FFP had been infused after about 6000 ml.

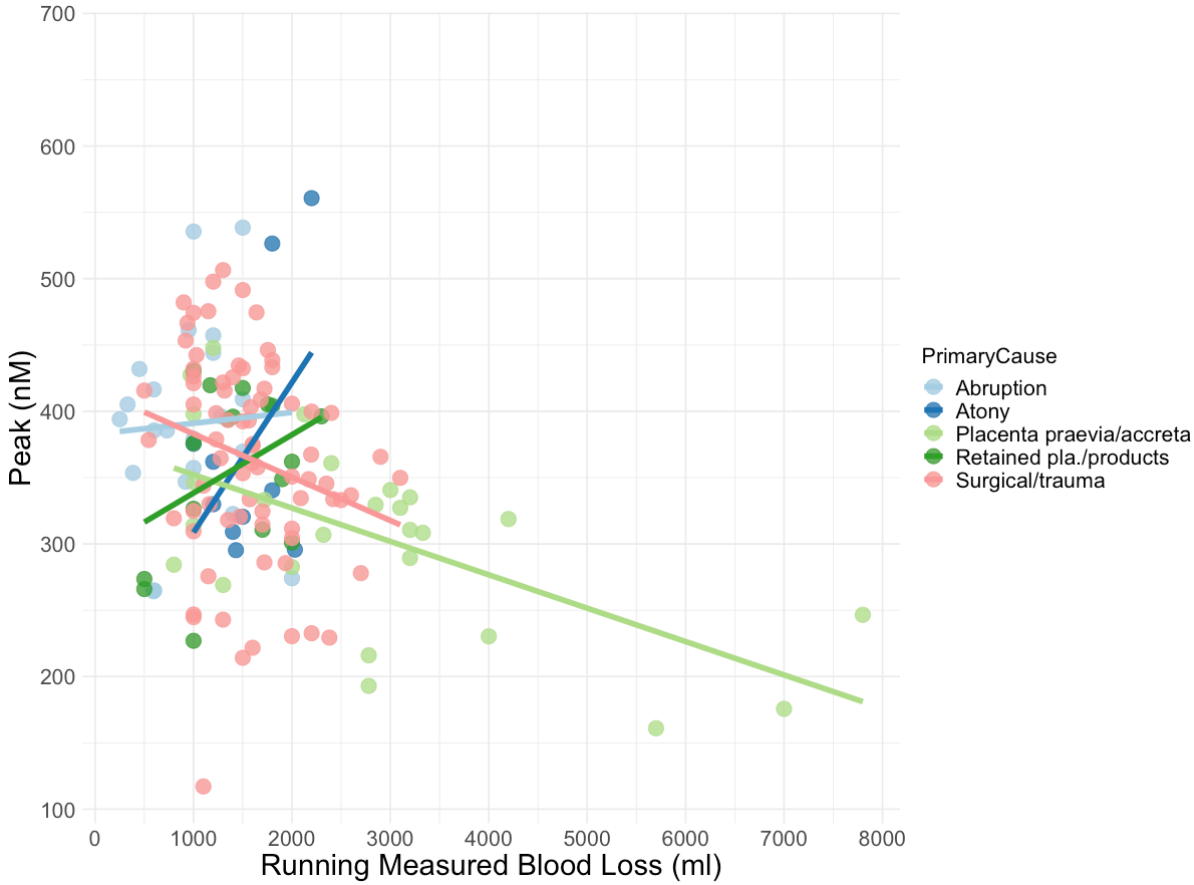

Figure 12. Peak thrombin generation (nM) – running measured blood loss (ml) correlation grouped by primary cause of bleed with regression lines excluding standard errors.

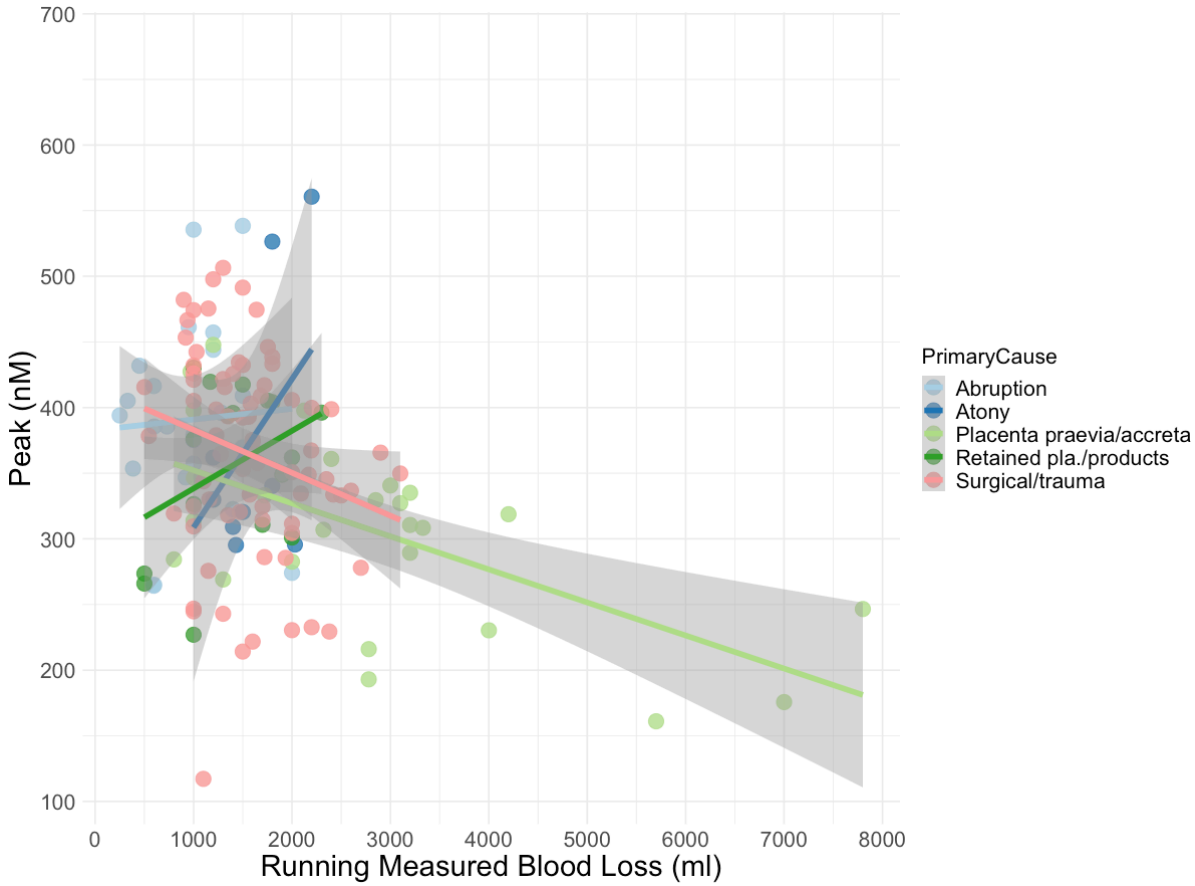

Figure 13. Peak thrombin generation (nM) – running measured blood loss (ml) correlation grouped by primary cause of bleed with regression lines including standard errors in grey.

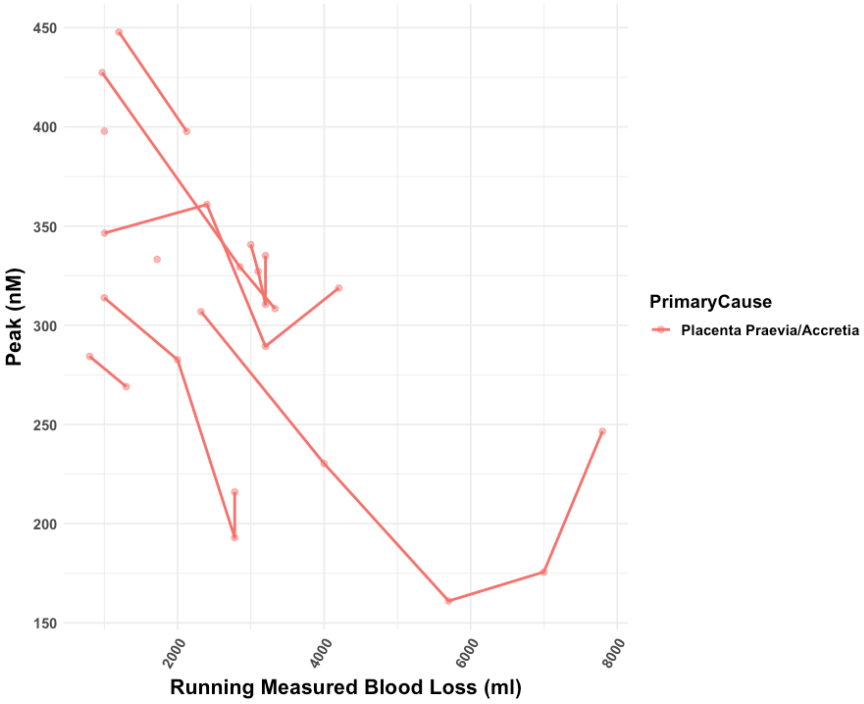

Figure 14. Peak thrombin generation (nM) in each patient suffering from placenta praevia/accreta dependent on measured blood loss (ml).

Table 13. Linear regression analysis for peak thrombin generation.

	Abruption	Atony	Placenta praevia/accreta	Retained placenta/products	Surgical/ trauma
Peak estimate	0.32	1.91	-15.31	3.24	-1.61
R^2	0.0026	0.22	0.39	0.14	0.05
Adjusted R^2	-0.05	0.12	0.36	0.09	0.04
F-statistic	0.05	2.21	15.05	2.49	4.11
p	0.8	0.18	0.0007	0.14	0.046

Summarising Table 13, the correlations between measured blood loss and peak thrombin generation vary across subgroups. The placenta praevia/accreta subgroup shows that peak thrombin falls as measured blood loss increases with 39% of the change in peak thrombin being associated with blood loss. However, in the other subgroups (surgical/trauma, atony, retained placenta/products, and abruption), the correlations are not statistically significant, indicating that there is no significant linear relationship between MBL and peak thrombin in these subgroups. The R^2 values indicate that the proportion of variability in MBL explained by peak thrombin is generally very low in these subgroups.

3.2.3 Endogenous thrombin potential

3.2.3.1 First Blood Sample taken during postpartum haemorrhage

Endogenous thrombin potential corresponds to the total amount of thrombin generated in a sample after activation. Table 14 shows the descriptive analysis for ETP in the first blood samples taken during a PPH. Data are shown for the first and all PPH samples and for the non-pregnant and non-bleeding pregnant control groups.

*Table 14. Descriptive analysis: endogenous thrombin potential (nM*min) in the first samples during postpartum haemorrhage and the control groups.*

	Count	Min	Max	Mean	SD	Median	IQR
Non-pregnant control	20	628.11	2643.82	1636.74	417.40	1642.12	306.42
Non-bleeding pregnant control	34	1273.25	3008.13	2130.51	405.18	2066.95	417.69
Postpartum haemorrhage (first blood samples)	96	819.13	3834.48	2160.50	546.32	2115.31	695.02
Postpartum haemorrhage (all blood samples)	240	819.33	3834.48	2065.90	502.51	1979.60	524.16

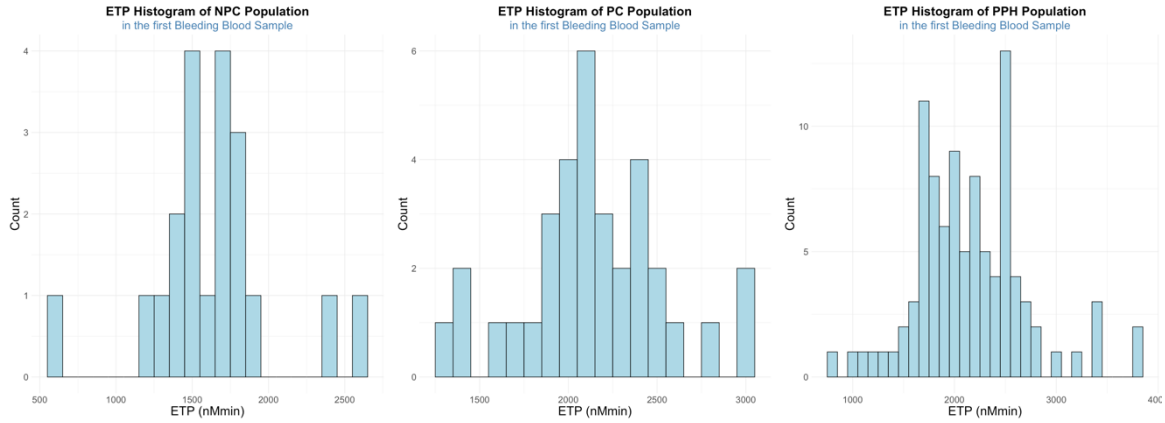

Figure 15. Endogenous thrombin potential (ETP) histograms for the non-pregnant (NPC) and non-bleeding pregnant control (PC) groups and the first blood samples taken during postpartum haemorrhage (PPH).

Considering Figure 15, normality was questionable for all groups, but in particular for the postpartum haemorrhage samples. Shapiro-Wilk Tests were performed, which confirmed that the distribution of ETP in the PPH group departed significantly from normality ($W = 0.96$, $p < 0.01$ [$p = 0.009$]). The Shapiro-Wilk results in the NPC ($W = 0.92$, $p = 0.11$) and PC group ($W = 0.98$, $p = 0.69$) did not show statistical evidence of non-normality. Homogeneity of variance was investigated by running a Levene's test. For ETP value, the variances were similar for NPC, PC, & PPH, $F(8, 237) = 1.27$, ns ($p = 0.26$).

Based on these results, a Kruskal-Wallis test was used to test for any significant differences in ETP values in the first blood sample in the PPH group and the two control groups. The p is significant ($H(2) = 19.52$, $p = 5.78e^{-05}$), therefore there is a significant difference in endogenous thrombin potential across those 3 groups. Running a multiple pairwise-comparison with a Benjamin & Hochberg adjustment method shows that ETP is higher in non-bleeding pregnant controls than non-pregnant controls ($p = 5.2e^{-05}$). The ETP in first PPH samples was higher than non-pregnant controls ($p = 5.2e^{-05}$). There was no difference between non-bleeding pregnant control and PPH first sample ($p = 0.99$) (Figure 16).

To further understand endogenous thrombin potential within the PPH group, we analysed the first blood samples during postpartum haemorrhage by subgrouping it into primary cause of bleeds and the descriptive analysis is summarised in Table 15 whereas Figure 16 compares the results in a box and whisker plot.

*Table 15. Descriptive analysis: endogenous thrombin potential (nM*min) in first blood samples taken during postpartum haemorrhage.*

Primary cause of bleed	Count	Min	Max	Mean	SD	Median	IQR
Abruption	18	1227.8	3834.48	2262.79	682.39	2200.09	612.46
Atony	7	1572.37	2464.96	2044.87	367.42	2203.86	616.41
Placenta praevia/accreta	8	1734.21	2549.64	2198.70	366.73	2239.29	627.79
Retained placenta/ products	12	1146.1	2215.03	1824.31	341.35	1813.74	460.01
Surgical/ trauma	50	819.13	3434.82	2202.77	563.06	2122.76	710.94

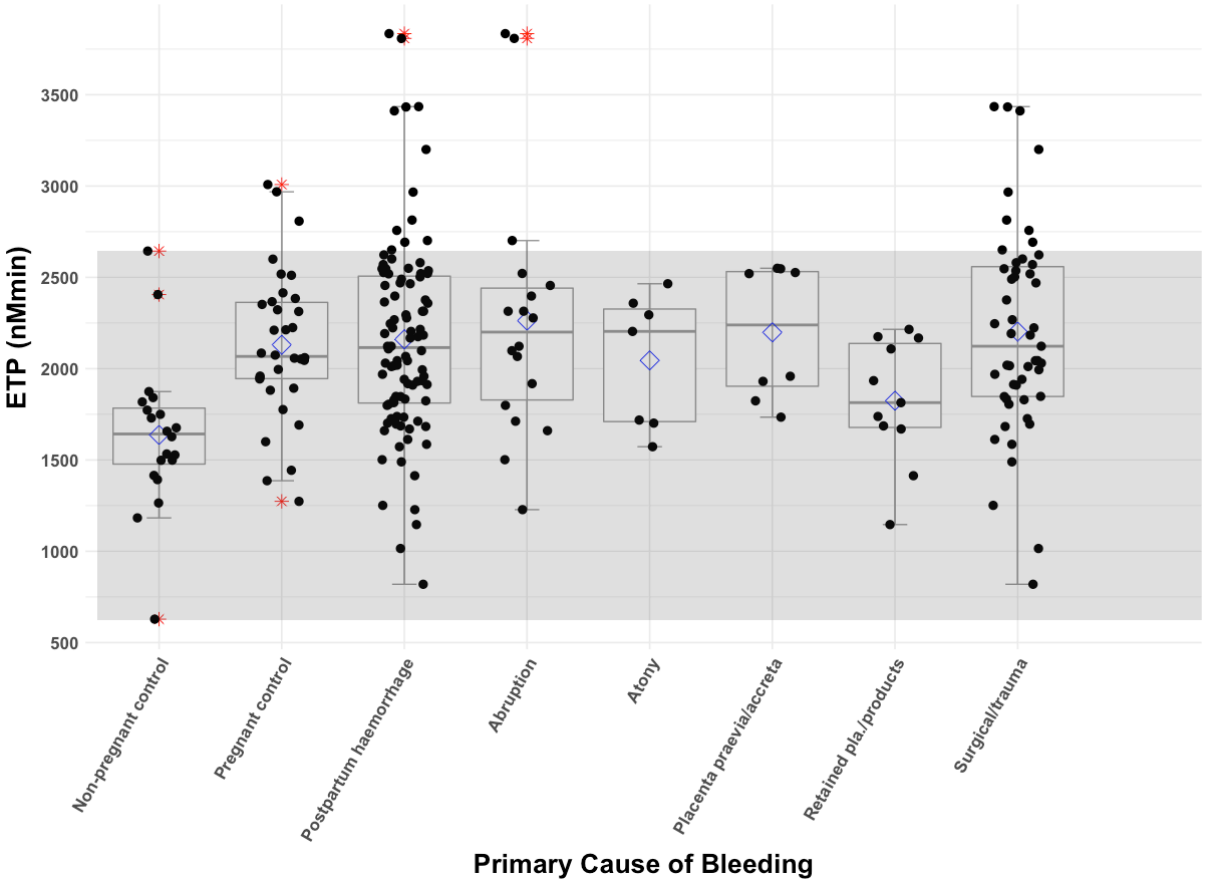

*Figure 16. Box plot: endogenous thrombin potential (ETP) (nM*min) in first blood samples taken during postpartum haemorrhage.*

Blue diamond represents the mean. The red stars show outliers, and the grey area indicates the non-pregnant normal range.

The median ETP ranges from 1813.74 nM*min in the retained placenta/products subgroup to 2239.29 nM*min in the placenta praevia/accreta group. A Shapiro-Wilk test was performed on each group: abruption ($W = 0.89$, $p < 0.05$ [$p = 0.039$]), atony ($W = 0.869$, $p = 0.169$), placenta praevia/accreta ($W = 0.78$, $p < 0.05$ [$p = 0.02$]), retained placenta/products ($W = 0.92$, $p = 0.35$), and surgical/trauma ($W = 0.98$, $p = 0.35$). Since distribution departed significantly from normality in some of the groups, a Kruskal-Wallis test was run to investigate for statistical significance between the groups. The p is not significant ($H(2) = 6.295$, $p = 0.18$), thus I conclude that there is no statistical significant difference between the groups.

The non-pregnant controls reference range was 628.11-2643.82 nM*min. None of the first blood samples taken during PPH fell below the lower limit of this range (628.11 nM*min), i.e., 0% of women suffering from PPH have ETP in their first blood sample below the normal range of healthy non-pregnant female controls. Interestingly, 12 out of 95 first blood samples exceeded the upper limit of the NPC range, indicating that despite the presence of bleeding, more than 12% of patients exhibited higher ETP levels compared to the NPC reference range at the onset of bleeding.

When comparing the first blood samples during PPH to the reference range of the non-bleeding pregnant control group (1273.25 - 3008.13 nM*min), five samples fell below this reference range. Specifically, three samples from the surgical/trauma subgroup displayed ETP values of 819.13, 1015.01, and 1251.16 nM*min, one sample from the retained placenta/products subgroup had an ETP value of 1146.10 nM*min, and one sample from the abruption subgroup showed an ETP value of 1227.80 nM*min. Among those patients, only two received blood products. The first patient had an ETP of 819.13 nM*min at the time of first study sampling and experienced a running MBL of 1500 ml at the time of the sample. After the sample was taken, this patient received 10 units of fibrinogen and had a total blood loss of 2800 ml. The second patient had an ETP of 1227.8 nM*min and a total blood loss of 1300 ml. This patient received 4 units of fibrinogen. Notably, this patient was also the one previously mentioned with a prolonged lag time of 5.62 minutes. These will be discussed in more detail in section 3.3.3.

3.2.3.2 Lowest endogenous thrombin potential measured during postpartum haemorrhage

The lowest ETP for each patient during the bleed was extracted and these are reported in following descriptive analyses:

*Table 16. Descriptive analysis: endogenous thrombin potential (nM*min) of blood samples with the lowest endogenous thrombin potential during postpartum haemorrhage.*

Primary cause of bleed	Count	Min	Max	Mean	SD	Median	IQR
Abruption	21	1223.61	3107.87	1989.55	492.16	1917.80	602.15
Atony	10	1572.37	3666.57	2190.16	630.79	2040.95	685.97
Placenta praevia/accreta	10	1305.13	2526.28	1820.25	351.54	1797.45	391.18
Retained placenta/ products	14	1146.10	2261.22	1786.58	351.12	1809.38	504.45
Surgical/trauma	57	819.13	3434.82	2041.88	539.38	1993.77	780.45

Analysing the lowest ETP results showed that the median ETP values range from 1797.45 nM*min in placenta praevia/accreta to 2040.95 nM*min in atony. Shapiro-Wilk tests were performed on the worst ETP results of each PPH subgroup: Abruption (W = 0.97, p = 0.72), atony (W = 0.85, p = 0.05), placenta praevia/accreta (W = 0.96, p = 0.84), retained placenta/products (W = 0.96, p = 0.71), and surgical/trauma (W = 0.99, p = 0.88). Despite the p values indicating normal distribution, due to the small number of values in each group and skewed visualisations via histograms (see Figure 17), non-parametric tests were used.

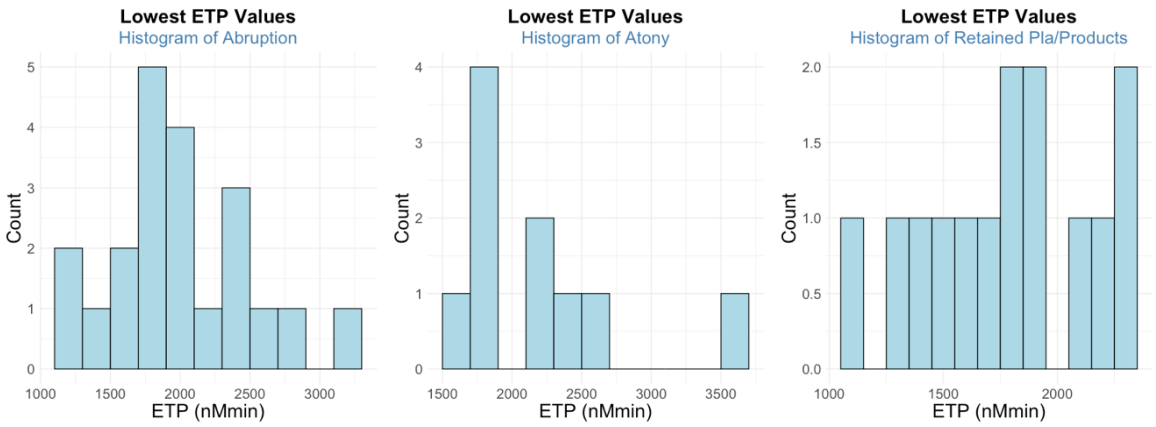

Figure 17. Exemplary endogenous thrombin potential (ETP) histograms of postpartum haemorrhage subgroups abruption, atony, and retained placenta/products.

The Kruskal-Wallis test showed a *p* of 0.31 (*H(2)* = 4.81) suggesting no significant difference in lowest ETP values across PPH subgroups. Furthermore, comparing this set of ETP results to the reference range of non-pregnant controls, no sample was below the minimum of 628.11 nM*min. This means that for the primary outcome 0/114 (0 %) women with PPH had an ETP below the non-pregnant reference range. This suggests that there was no evidence for haemostatic impairment in women with PPH based on the ETP parameter. However, the lowest ETP in the non-pregnant control group is a statistical outlier (Figure 18). In post hoc analysis this control was excluded, and this resulted in 3/114 women having an ETP below this revised normal range and, based on this stricter definition, could be considered to have evidence of haemostatic impairment.

Nine women had blood samples taken during PPH that were below the non-bleeding pregnant reference range of 1273.25 nM*min (see section 3.3.3). Among these samples, six patients were from the surgical/trauma group with ETP values of 819.13 nM*min, 1015.01 nM*min, 1145.01 nM*min, 1251.16 nM*min, 1255.46 nM*min, and 1258.14 nM*min. Additionally, one patient from the retained placenta/products group had an ETP of 1146.10 nM*min, and two with abruptions showed ETP values of 1223.61 and 1227.80 nM*min, respectively. The data above shows that only one of those patients (with an ETP of 819.13 nM*min), i.e., from the surgical/trauma group, had an ETP below that of normal pooled plasma. This suggests that the majority of women with PPH would not benefit from FFP treatment to improve haemostasis based on this parameter.

3.2.3.3 Endogenous thrombin potential of all samples taking during postpartum haemorrhage

An analysis was performed on all blood samples taken during PPH. Shapiro-Wilk tests were performed on each group: non-pregnant controls (W = 0.92, p = 0.11), non-bleeding pregnant controls (W = 0.98, p = 0.69), PPH (W = 0.96, p < 0.01 [$p = 1.775e^{-05}$]), abruption (W = 0.90, p < 0.01 [p = 0.002]), atony (W = 0.86, p < 0.05 [p = 0.046]), placenta praevia/accreta (W = 0.95, p = 0.17), retained placenta/products (W = 0.98, p = 0.83), and surgical/trauma (W = 0.98, p = 0.27).

Since distribution departed significantly from normality in some groups, a Kruskal-Wallis test was performed to investigate for any significant differences in ETP values. Since the p was ($H(2)$ = 29.39, p = 0.0001) is less than the significance level of 0.05, I can conclude that there are significant differences in ETP between groups.

Post-hoc Wilcoxon rank sum tests using the Benjamin & Hochberg method to compare all pairs of groups with each other show that the following are statistically significantly different ($p < 0.05$): The non-pregnant controls were lower than the non-bleeding pregnant control group (p = 0.0004). The non-pregnant controls were also lower than the combined PPH group (p = 0.0004), abruption (p = 0.0005), atony (p = 0.015), placenta praevia/accreta (p = 0.0045) and surgical/trauma (p = 0.0004). Retained placenta/products were lower than pregnant controls products (p = 0.045). Running a conservative Bonferroni adjusted method instead removes NPC vs atony (p = 0.088) and PC vs retained placenta/products (p = 0.31) from the statistically significantly different list.

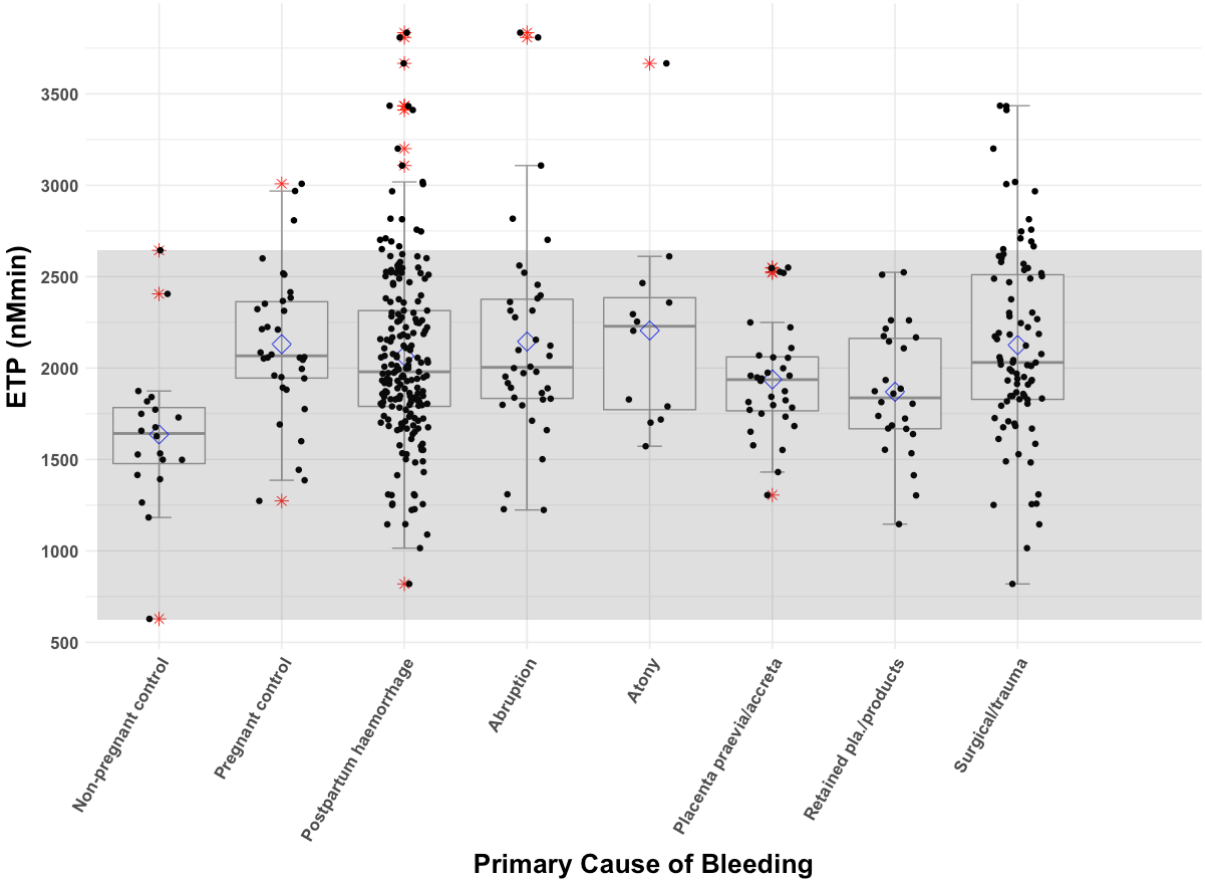

*Figure 18. Box plot: endogenous thrombin potential (ETP) (nM*min) in all samples taken during postpartum haemorrhage.*

Blue diamond represents the mean. The red stars show outliers, and the grey area indicates the non-pregnant normal range.

The primary endpoint of this project was to investigate the prevalence of abnormal thrombin generation in women experiencing PPH. The analysis revealed that 0/114 (0%) woman during PPH exhibited ETP thrombin generation levels below the lower reference limit of non-pregnant controls (628.11 nM*min), suggesting that there was no evidence of haemostatic impairment in this cohort, concerning endogenous thrombin potential. However, the lowest ETP in the non-pregnant control group is a statistical outlier. In post hoc analysis this control was excluded, and this resulted in 5/114 (4.4%) women having an ETP below this revised normal range and, based on this more strict definition, could be considered to have evidence of haemostatic impairment.

Furthermore, 9/114 (7.89%) had ETP values below the lower limit of non-bleeding pregnant control range (1273.25 nM*min), but only one woman (0.88%) had endogenous thrombin potential below the lower limit of normal pooled plasma (894.49 nM*min). Based on these findings, it was concluded that the ETP of the majority of women in this cohort would not have been improved by FFP infusion.

3.2.3.4 ETP during postpartum haemorrhage dependent on measured blood loss

Figure 19 and Figure 20 show the correlations between ETP (nM*min) and measured blood loss at the time of samples taken during PPH dependent on the primary cause of bleed.

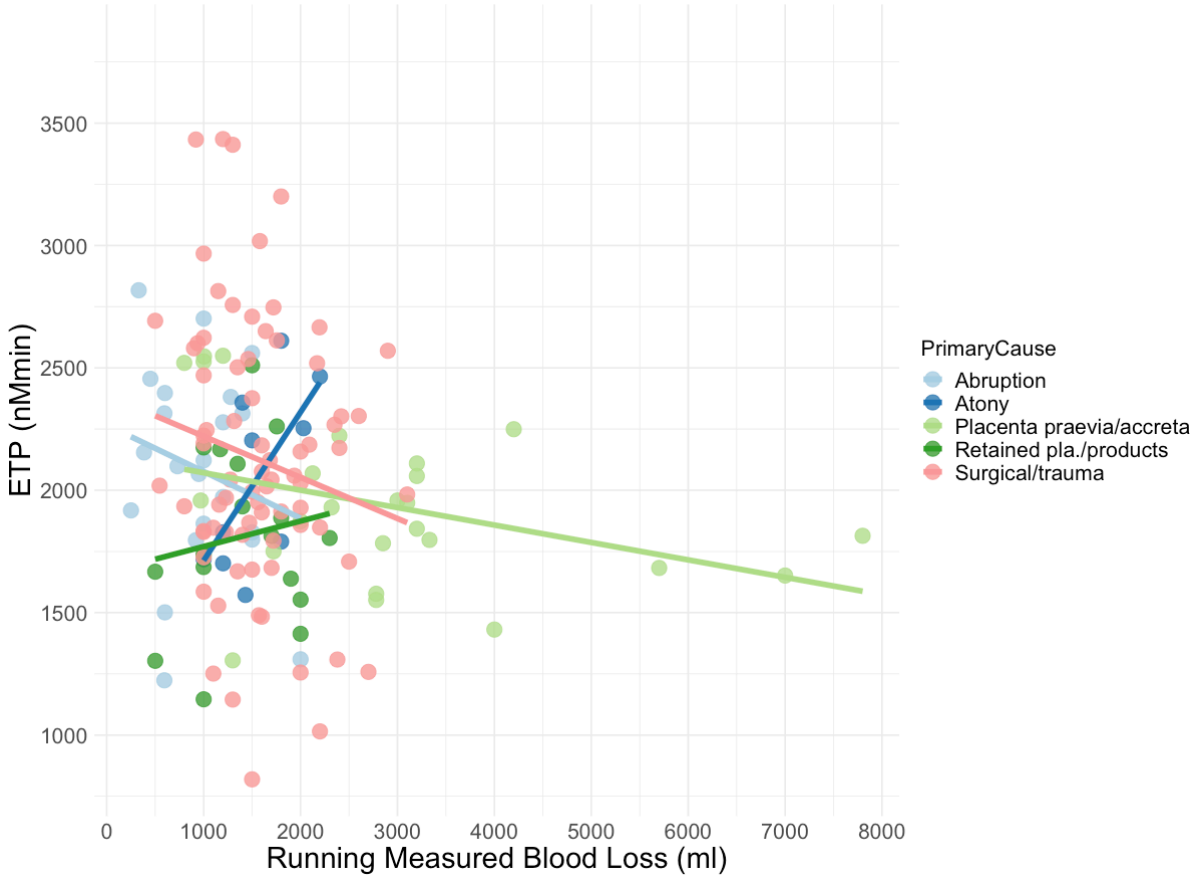

*Figure 19. Endogenous thrombin potential (ETP) (nM*min) – running measured blood loss (ml) correlation grouped by primary cause of bleed with regression lines excluding standard errors.*

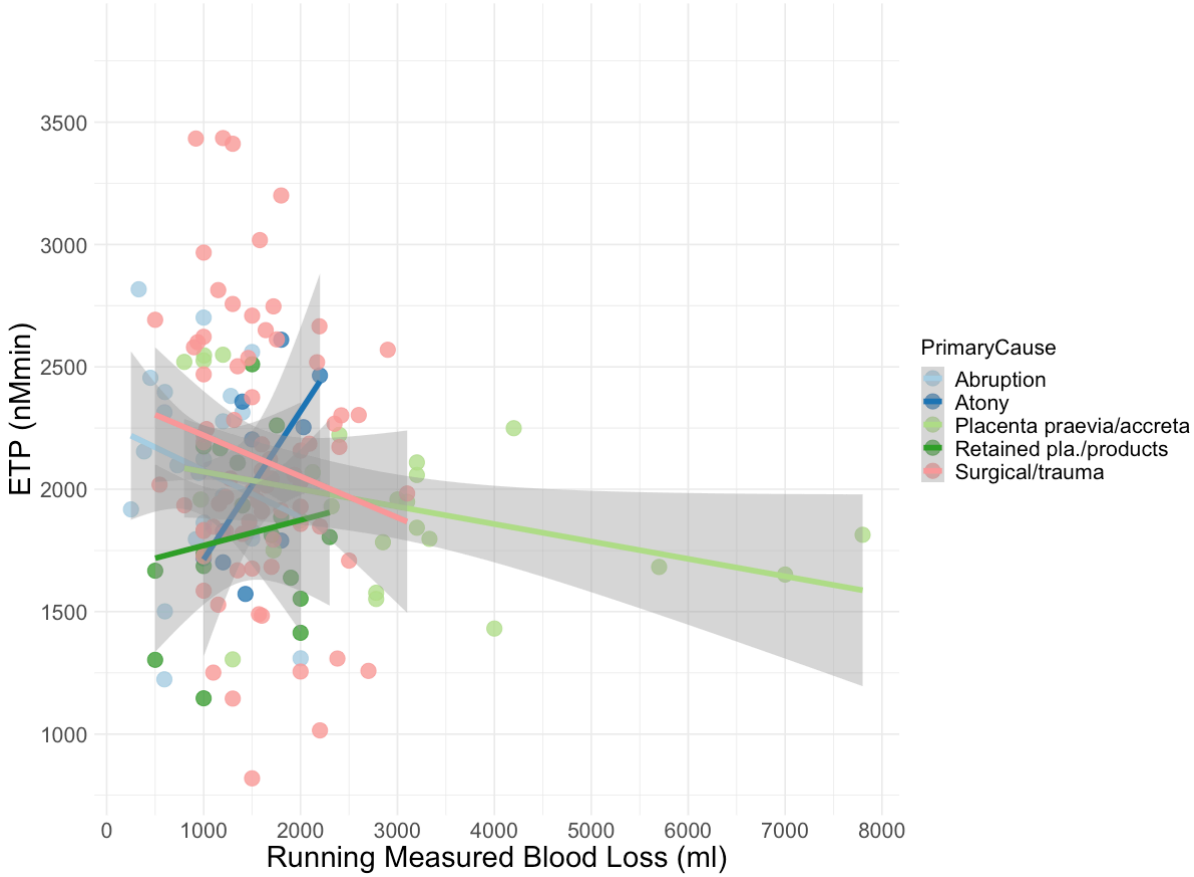

*Figure 20. Endogenous thrombin potential (ETP) (nM*min) – running measured blood loss (ml) correlation grouped by primary cause of bleed with regression lines including standard errors in grey.*

Table 17. Linear regression analysis for endogenous thrombin potential (ETP).

	Abruption	**Atony**	**Placenta praevia/accreta**	**Retained placenta/products**	**Surgical/ trauma**
ETP estimate	-0.23	0.68	-1.96	0.23	-0.17
R^2	0.04	0.41	0.14	0.02	0.03
Adjusted R^2	-0.003	0.34	0.10	-0.04	0.01
F-statistic	0.93	5.57	3.88	0.36	2.13
p	0.35	0.046	0.06	0.56	0.15

Regression lines were added for each PPH subgroup and regression analysis results are presented in Table 17. Visually in Figure 19 and Figure 20, placenta praevia/accreta, surgical/trauma and abruption may have a negative correlation, suggesting that as PPH progresses, ETP declines. Overall, the results suggest that there is a minimal relationship between endogenous thrombin potential and measured blood loss across different subgroups. Only in the atony subgroup, ETP shows a

moderate relationship with MBL and statistically significant, while in the other subgroups (abruption, placenta praevia/accreta, retained placenta/products, and surgical/trauma), the relationship is either not statistically significant or marginally not statistically significant.

3.2.4 Velocity index

3.2.4.1 Velocity index at the time of the first blood sample taken during postpartum haemorrhage

Velocity index corresponds to the rate of thrombin production. Table 18 shows the descriptive analysis of the first blood sample taken during PPH, all PPH samples, and the control groups.

Table 18. Descriptive analysis: velocity index (nM/min) in the first samples during postpartum haemorrhage and the control groups.

	Count	Min	Max	Mean	SD	Median	IQR
Non-pregnant control	20	38.93	199.77	97.66	41.55	86.54	49.60
Non-bleeding pregnant control	34	54.69	268.05	166.17	49.22	170.60	58.42
Postpartum haemorrhage (first blood samples)	96	25.11	403.78	174.05	54.85	176.03	61.88
Postpartum haemorrhage (all blood samples)	240	25.11	403.78	167.78	51.03	166.60	57.81

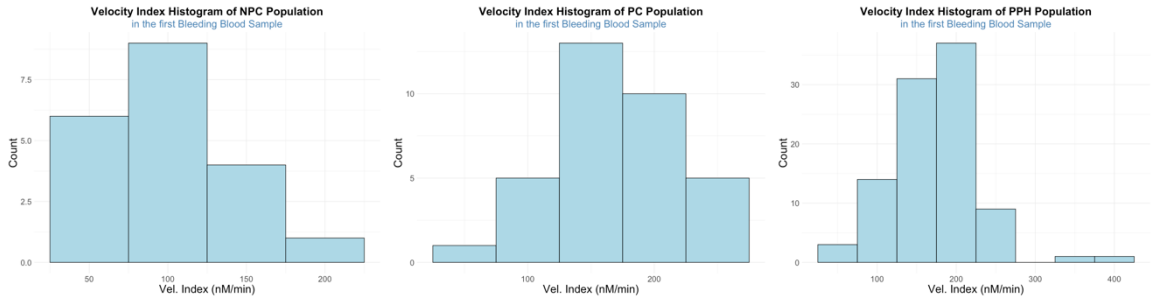

Figure 21. Velocity index (nM/min) histograms for the non-pregnant (NPC) and non-bleeding pregnant control (PC) groups and the first blood samples taken during postpartum haemorrhage (PPH).

Considering Figure 21, normality is visually questionable in all three groups. Shapiro-Wilk tests were performed, which confirmed that the distribution of velocity index in the PPH group departed significantly from normality ($W = 0.95$, $p < 0.01$ [$p = 0.00072$]). The Shapiro-Wilk results in the non-pregnant ($W = 0.94$, $p = 0.20$) and non-bleeding pregnant group ($W = 0.99$, $p = 0.99$) did not show statistical evidence of non-normality. Homogeneity of variance was investigated by running a Levene's test. For velocity index, the variances were similar for NPC, PC, & PPH ($F(8, 237) = 0.78$, $p = 0.62$).

Based on this outcome, a Kruskal-Wallis test was used to test for any significant differences in velocity index in the first blood sample during PPH in the groups NPC, PC, & PPH. The p ($H(2) = 28.905$, $p = 5.288e^{-07}$) shows that there is a significant difference in velocity index across those 3 groups. Irrespective what p adjustment method (Bonferroni vs Benjamin & Hochberg) is used while running a multiple pairwise-comparison, the velocity index in the non-bleeding pregnant control group is higher than in the non-pregnant group ($p = 1.2e^{-05}$, $p = 6.4e^{-06}$). In addition, the velocity index is higher in the PPH group than the non-pregnant controls ($p = 4.2e^{-07}$, $p = 4.2e^{-07}$). The p comparing non-bleeding pregnant controls and PPH is not statistically significant for both methods ($p = 1.00$, $p = 0.42$).

To further understand velocity index within the PPH group, I analysed the first blood samples during PPH by subgrouping them into primary cause of bleeds and the descriptive analysis is summarised in Table 19, whereas Figure 22 compares the results in a box and whisker plot.

Table 19. Descriptive analysis: velocity index (nM/min) in first blood samples taken during postpartum haemorrhage.

Primary Cause of Bleed	Count	Min	Max	Mean	SD	Median	IQR
Abruption	18	87.8	403.78	180.59	69.03	168.22	41.16
Atony	7	98.04	336.40	177.28	84.65	160.92	100.09
Placenta praevia/ accreta	8	71.08	213.69	151.99	43.38	155.19	34.81
Retained placenta/ products	12	31.92	227.72	158.95	56.71	181.00	68.89
Surgical/trauma	50	25.11	260.87	177.02	46.09	187.63	52.69

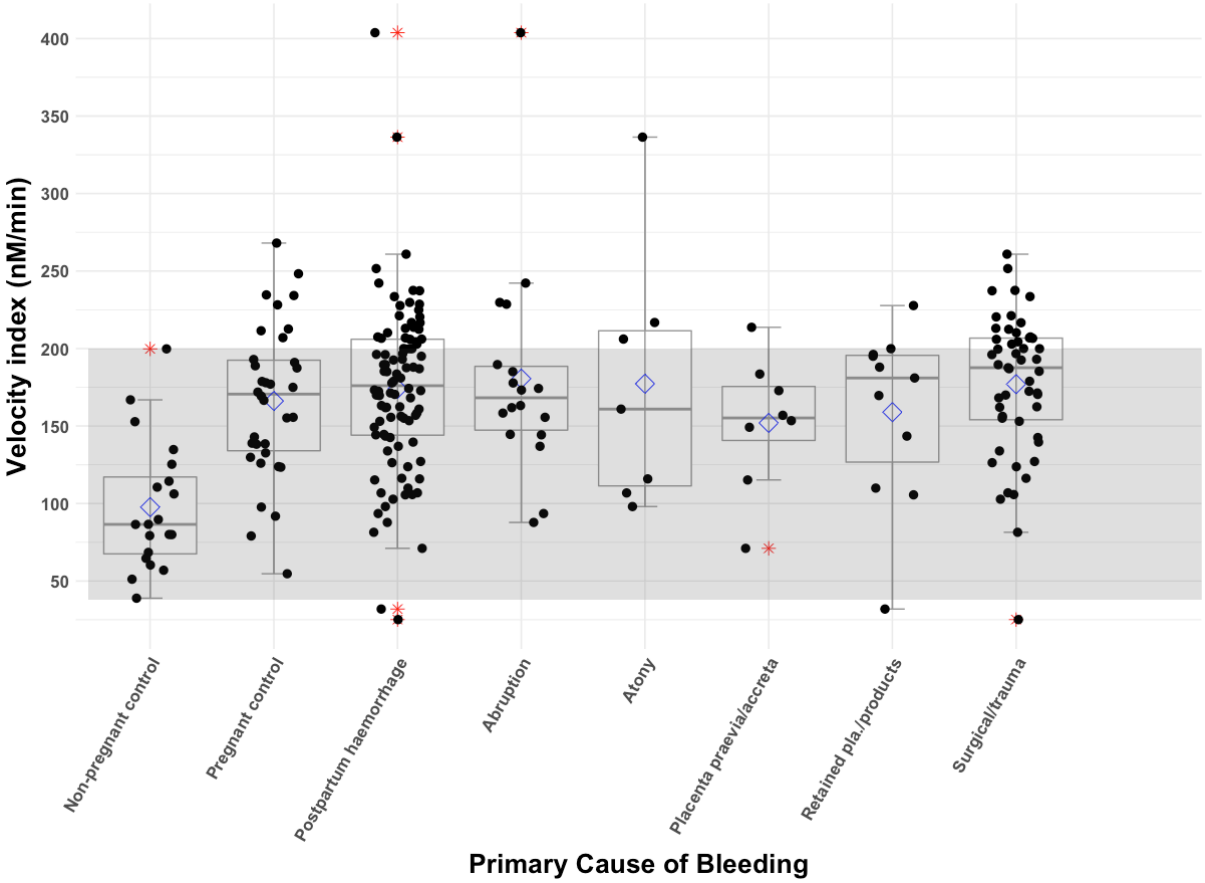

Figure 22. Box plot: velocity index (nM/min) in first blood samples taken during postpartum haemorrhage.

Blue diamond represents the mean. The red stars show outliers, and the grey area indicates the non-pregnant normal range.

The median velocity index ranges from 155.19 nM/min in the placenta praevia/accreta group to 187.63 nM/min in the surgical/trauma group. Prior to running a Kruskal-Wallis test, histograms were visualised (Figure 23) and Shapiro-Wilk tests were performed on each group: abruption ($W = 0.81$, $p < 0.05$ [$p = 0.0023$]), atony ($W = 0.88$, $p = 0.23$), placenta praevia/accreta ($W = 0.96$, $p = 0.78$), retained placenta/products($W = 0.89$, $p = 0.13$), and surgical/trauma ($W = 0.95$, $p < 0.05$ [$p = 0.039$]). Since distribution departed significantly from normality in some of the groups, a Kruskal-Wallis test was used to investigate for statistical significance. The p is not significant ($H(2) = 3.0867$, $p = 0.54$).

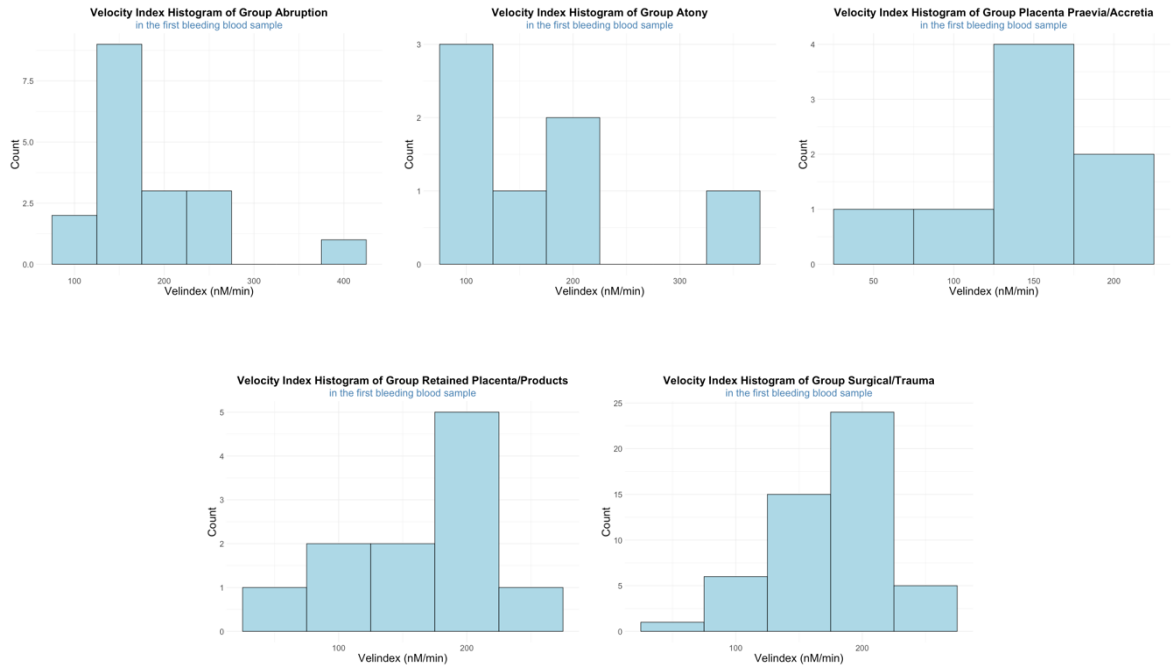

Figure 23. Velocity index (nM/min) histograms: PPH subgroups for the first blood sample taken during postpartum haemorrhage.

In the non-pregnant reference range for the velocity index, the values ranged from 38.06 to 199.77 nM/min, and two samples fell below the lower limit of 38.06 nM/min. Thus, 2.1% (2/95) of women have a first velocity index below the normal range of healthy non-pregnant female controls. This result did not change if the non-pregnant control who was a statistical outlier with respect to ETP was excluded. Interestingly, among the 95 first blood samples, 56 samples exceeded the upper limit of the non-pregnant reference range (169.06 nM/min). This finding indicates that nearly 60% of the patients, despite their bleeding condition, exhibited a velocity index higher than the upper limit of the reference range observed in non-pregnant individuals.

When comparing the velocity index values to the reference range of the non-bleeding pregnant control group (54.69 - 269.05 nM/min), the same two first blood samples as above fell below that reference range. These two samples came from patients in the surgical/trauma subgroup (with a velocity index of 25.11 nM/min) and the retained placenta/products subgroup (with a velocity index of 31.92 nM/min). Interestingly, these are the same patients mentioned earlier in the context of peak thrombin first blood samples.

3.2.4.2 Lowest velocity index during postpartum haemorrhage

The lowest velocity index during PPH for each patient was identified and the results are reported in the following descriptive analyses:

Table 20. Descriptive analysis: velocity index (nM/min) of blood samples with the lowest velocity index during postpartum haemorrhage.

Primary cause of bleed	Count	Min	Max	Mean	SD	Median	IQR
Abruption	21	78.49	283.23	158.50	48.41	155.63	25.02
Atony	10	93.54	336.4	193.99	84.72	183.51	129.03
Placenta praevia/ accreta	10	71.08	183.54	122.83	40.94	125.03	69.34
Retained placenta/ products	14	25.81	206.98	152.39	49.35	171.67	67.83
Surgical/trauma	57	25.11	273.73	169.63	47.29	170.40	64.75

The median lowest velocity index values range from 125.03 nM/min in placenta praevia/accreta to 183.51 nM/min in atony. Histograms showing skewness were produced (see Figure 24), and Shapiro-Wilk tests were performed on the lowest velocity index results of each PPH subgroup: abruption (W = 0.906, $p < 0.05$ [p = 0.0.045]), atony (W = 0.93, p = 0.48), placenta praevia/accreta (W = 0.91, p = 0.27), retained placenta/products(W = 0.85, $p < 0.05$ [p = 0.021]), and surgical/trauma (W = 0.98, p = 0.42). As some groups departed significantly from normality, a Kruskal-Wallis Test was performed, which produced a p of 0.047 ($H(2)$ = 9.643), thus indicating a significant difference in velocity index across PPH subgroups.

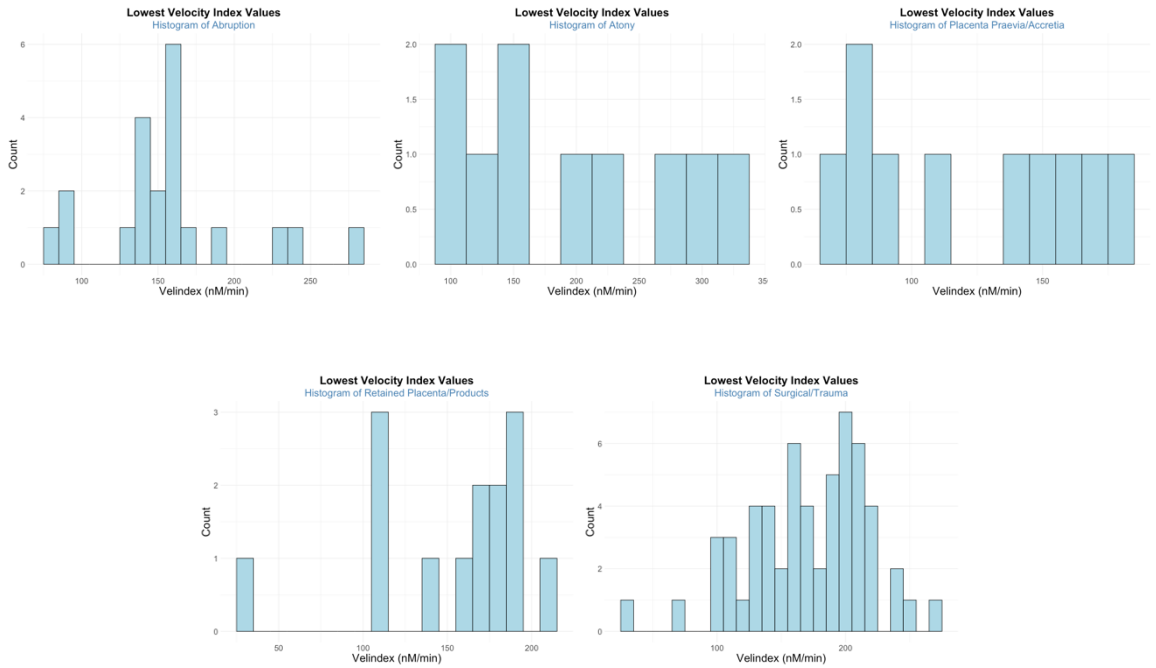

Figure 24. Velocity index (nM/min) histograms: postpartum haemorrhage subgroups for the lowest velocity index blood sample.

A multiple pairwise-comparison with no p adjustment method resulted in one significant result between placenta praevia/accreta and surgical/trauma (p = 0.0051), whereas adjusting with either Bonferroni or Benjamin & Hochberg method found no results of statistical significance. Thus, the velocity index did not vary dependent on the cause of bleeding.

Moreover, upon comparing the present set of all PPH blood samples to the reference range established for non-pregnant controls, it was observed that two women (total of three blood samples) exhibited velocity index values below 38.06 nM/min (see section 3.2.4.1). This means that the primary endpoint for velocity index was that 2/114 (1.75%) women had a result below the non-pregnant normal range. This result did not change if the non-pregnant control who was a statistical outlier with respect to ETP was excluded.

The data above shows that only two of those patients with velocity indices of 25.11 nM/min (surgical/trauma) and 25.18 nM/min (retained placenta/products) had velocity indices below that of normal pooled plasma. This suggests that the velocity index of the majority of women with PPH would not be improved by FFP infusion.

3.2.4.3 Velocity index of all samples taking during postpartum haemorrhage

Shapiro-Wilk tests were performed on each group: non-pregnant control ($W = 0.94$, $p = 0.20$), non-bleeding pregnant control ($W = 0.99$, $p = 0.99$), PPH ($W = 0.97$, $p < 0.01$ [$p = 0.00024$]), abruption ($W = 0.85$, $p < 0.01$ [$p = 0.00017$]), atony ($W = 0.91$, $p = 0.12$), placenta praevia/accreta ($W = 0.96$, $p = 0.37$), retained placenta/products ($W = 0.85$, $p < 0.01$ [$p = 0.0013$]), and surgical/trauma ($W = 0.98$, $p = 0.13$).

Since distribution departed significantly from normality in some groups, a Kruskal-Wallis test was used to investigate for any significant differences in velocity index values. The p ($H(2) = 43.492$, $p = 2.68e^{-07}$) indicates that there are significant differences in velocity index values between groups.

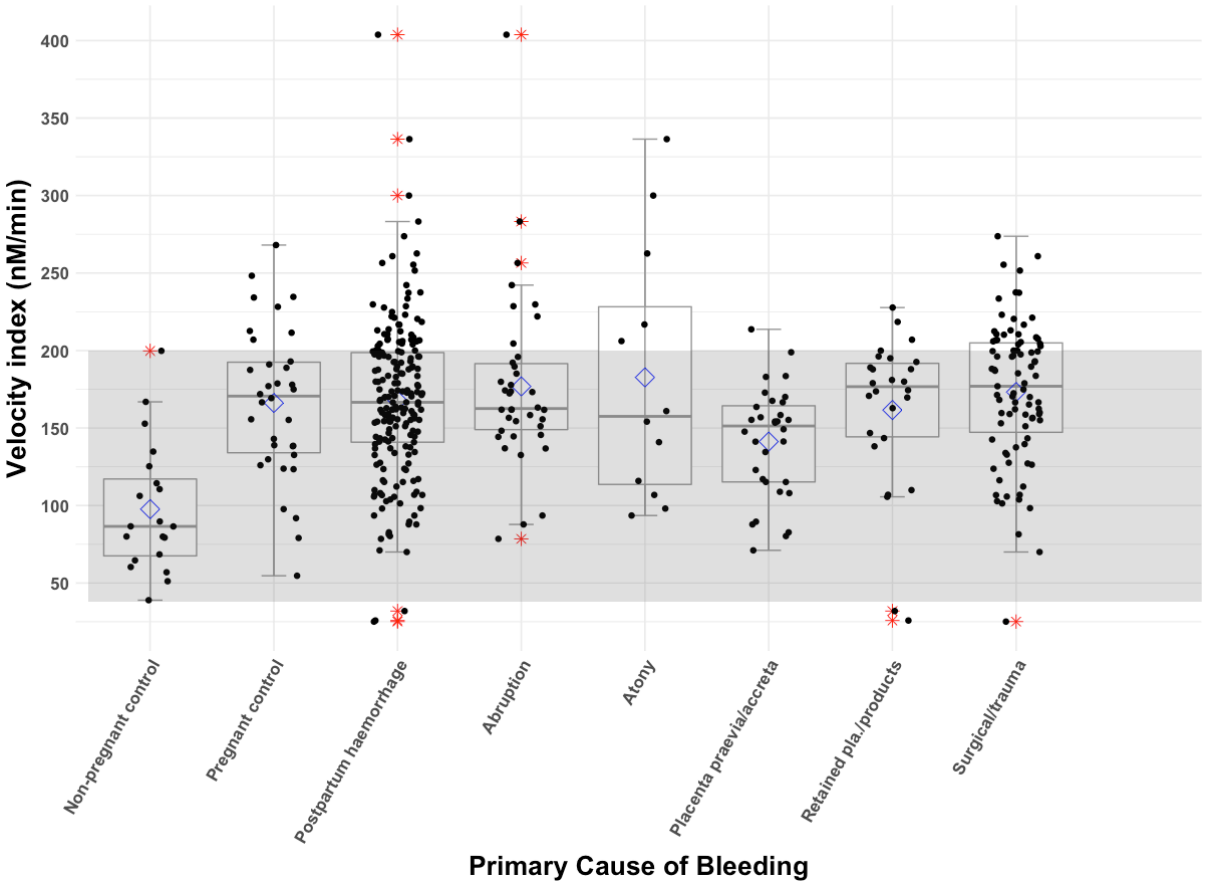

Figure 25. Box plot: velocity index (nM/min) in all samples taken during postpartum haemorrhage.

Blue diamond represents the mean. The red stars show outliers, and the grey area indicates the non-pregnant normal range.

Table 21 shows the statistically significant results from the post-hoc Wilcoxon rank sum tests using the Benjamin & Hochberg method to compare all pairs of groups with each other.

Table 21. Post-hoc Wilcoxon rank sum test for velocity index across non-pregnant, non-bleeding pregnant, PPH and its subgroups.

Non pregnant control	vs	non-bleeding pregnant control	*p*	=	$3.0e^{-05}$
Non pregnant control	vs	postpartum haemorrhage	*p*	=	$1.3e^{-06}$
Non pregnant control	vs	Abruption	*p*	=	$1.4e^{-06}$
Non pregnant control	vs	Atony	*p*	=	0.0018
Non pregnant control	vs	placenta praevia/accrete	*p*	=	0.0008
Non pregnant control	vs	retained placenta/products	*p*	=	0.0003
Non pregnant control	vs	surgical/trauma	*p*	=	$1.3e^{-06}$
Abruption	vs	placenta praevia/accrete	*p*	=	0.008
Retained placenta/products	vs	placenta praevia/accrete	*p*	=	0.026
Surgical/trauma	vs	placenta praevia/accrete	*p*	=	0.0008

The velocity index values obtained from non-pregnant controls were consistently lower than those observed in pregnant controls and across various subgroups, including all PPH samples combined, abruption, atony, placenta praevia/accreta, retained placenta/products, and surgical/trauma. Statistical analysis revealed significant differences between several pairs of subgroups, specifically, abruption vs placenta praevia/accreta, retained placenta/products vs placenta praevia/accreta, and surgical/trauma vs placenta praevia/accreta. However, employing a conservative Bonferroni-adjusted method resulted in the exclusion of abruption vs placenta praevia/accreta (p = 0.084) and retained placenta/products vs placenta praevia/accreta (p = 0.288) from the list of significantly different subgroups. These differences are not likely to be clinically significant as the velocity index was either within or above the non-pregnant normal range in almost all cases.

The primary endpoint of this project was to investigate the prevalence of abnormal thrombin generation in women experiencing PPH. The analysis revealed that 2 out of 114 women (1.75%) with PPH exhibited velocity indices in a total of three blood samples below the lower reference limit of non-pregnant controls (38.93 nM/min), non-bleeding pregnant controls (54.69 nM/min) and normal pooled plasma (40.08 nM/min).

This result did not change if the non-pregnant control who was a statistical outlier with respect to ETP was excluded.

Based on this finding, it was concluded that there was limited evidence of haemostatic impairment in this cohort, concerning velocity index and the majority would not have benefited from FFP infusion.

3.2.4.4 Velocity index during postpartum haemorrhage dependent on measured blood loss

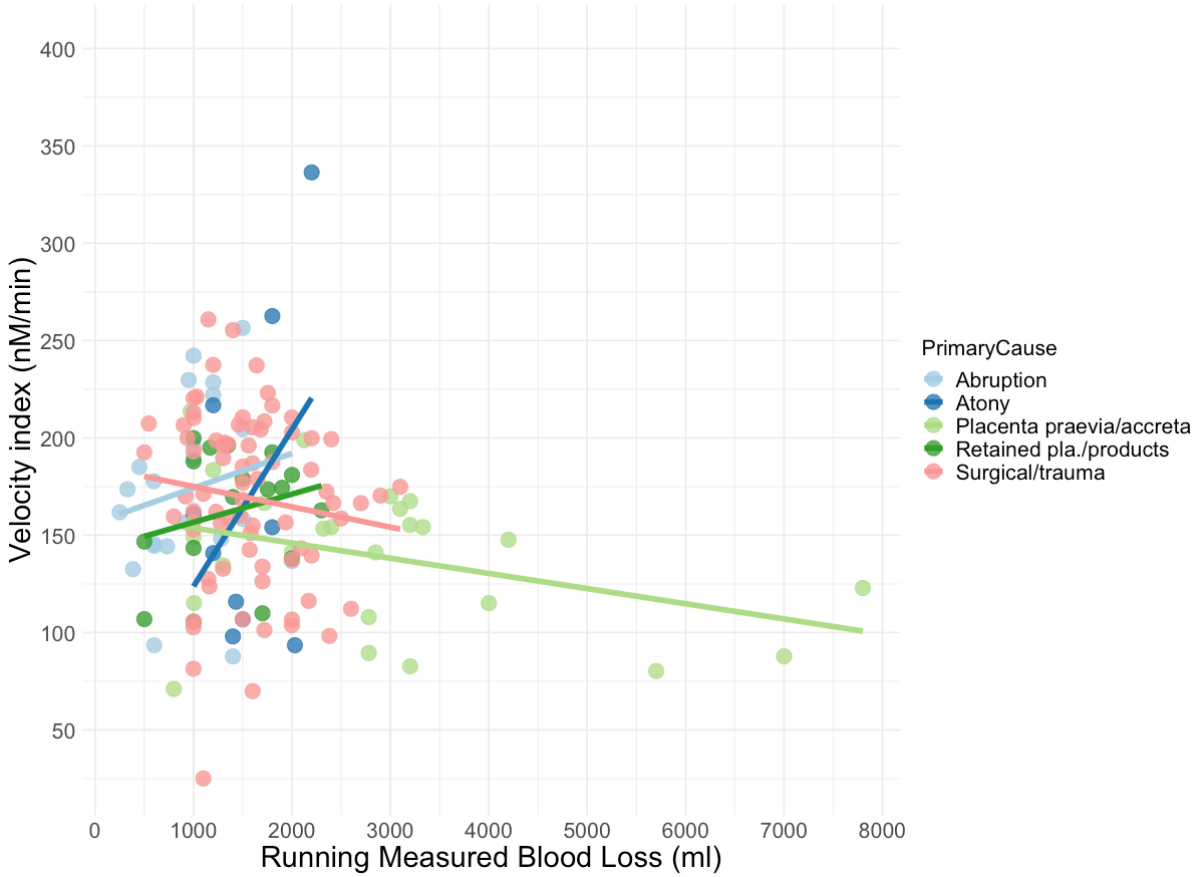

Figure 26. Velocity index (nM/min) – running measured blood loss (ml) correlation grouped by primary cause of bleed with regression lines excluding standard errors.

Figure 26 and Figure 27 show visual insights into the relationships between bleed size and velocity index within different bleeding categories. Additionally, Table 22 provides a comprehensive summary of the results obtained from the linear regression analysis, elucidating the statistical associations between bleed size and velocity index for each primary cause of bleeding. Overall, the results suggest that the relationships between

velocity index and measured blood loss is not statistically significant in any of the subgroups and the explanatory power of velocity index for measured blood loss is relatively low across all subgroups.

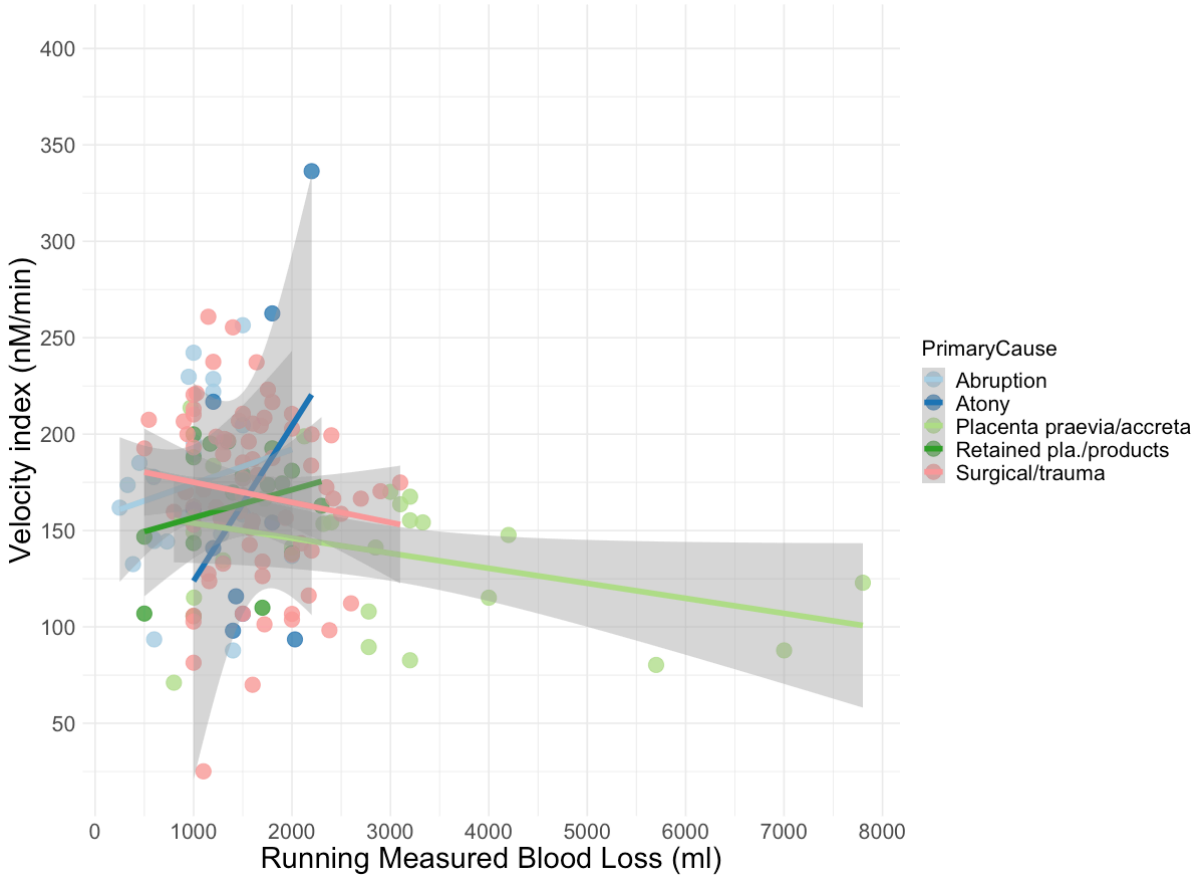

Figure 27. Velocity index (nM/min) – running measured blood loss (ml) correlation grouped by primary cause of bleed with regression lines including standard errors in grey.

Table 22. Linear regression analysis for velocity index.

	Abruption	Atony	Placenta praevia/accreta	Retained placenta/products	Surgical/ trauma
Velocity estimate	1.86	1.91	-18.08	3.96	-1.56
R^2	0.03	0.15	0.14	0.06	0.02
Adjusted R^2	-0.015	0.048	0.11	-0.005	0.003
F-statistic	0.68	1.46	3.94	0.91	1.22
p	0.42	0.26	0.059	0.36	0.27

3.2.5 Amniotic fluid embolus patient

The patient suffering from an amniotic fluid embolus (AFE) was a 36-year-old woman, gravida 5 para 3 with a BMI of 56 kg/m^2 (discussed in detail here [130]). She delivered by planned caesarean at 39 weeks' gestation. The patient had a history of three previous caesarean sections and her current pregnancy was complicated by polyhydramnios. Preadmission laboratory values showed a haemoglobin concentration of 113 g/L and a platelet count of 217 × 10^9/L. During delivery, the patient complained of feeling 'strange' with difficulty breathing. Soon after, she lost consciousness and suffered a cardiac arrest.

An AFE was part of the differential diagnosis, and the major obstetric haemorrhage protocol was activated in anticipation of bleeding. Intravenous tranexamic acid 1 g was administered. Blood samples were taken 20 min after the cardiac arrest, and included a full blood count, rotational thromboelastometry, and a coagulation screen comprising PT, activated partial aPTT, and Clauss fibrinogen. PPP was derived from this and stored for later thrombin generation analysis. Following the return of her circulation, the patient rapidly lost an estimated 1000 mL of blood (assessed by a gravimetric method) and subsequent management focussed on correcting the coagulopathy and managing major haemorrhage. The thrombin generation results are shown Table 23. All thrombin generation parameters are within the non-pregnant normal range suggesting that, despite the severe coagulopathy, as demonstrated by the reduced fibrinogen, adequate procoagulant factor levels were sustained throughout the bleed. This suggests that the coagulopathy of AFE is predominantly due to reduced fibrinogen and that there is no evidence for a generalised consumption of clotting factors.

Table 23. Summary of the AFE patient's blood loss, administered blood products and coagulation test results.

Minutes from collapse	**+ 20**	**+55**	**+110**
Quantitative blood loss (mL)	1310	1620	2300
Haemostatic treatment pre-sample	TXA 1 g	Fibrinogen concentrate 6 g TXA 1 g PRBC 1 unit	Fibrinogen concentrate 4 g FFP 4 units PRBC 1 unit
PT (s)	13.4	14.0	13.4
aPTT (s)	41.8	35.0	32.9
Fibrinogen (g/L)	1.6	2.1	2.4
Platelet count (x 10^9/L)	92	90	130
Factor II (IU/dL)	88	78	83
Factor V (IU/dL)	44	32	39
Factor VII (IU/dL)	141	128	139
Factor VIII (IU/dL)	140	95	98
Factor IX (IU/dL)	162	125	126
Factor X (IU/dL)	98	84	91
Factor XI (IU/dL)	83	65	68
Factor XIII (IU/dL)	50	46	57
Lag time (min)	2.44	2.61	2.61
Peak thrombin (nM)	393	350	386
ETP (nM*min)	2129	1950	1987
Velocity index (nM/min)	168	162	179

Tranexamic acid (TXA). Packed red blood cells (PRBC). Fresh frozen plasma (FFP).

3.3 Analyses of outliers in context of individual coagulation factor levels

3.3.1 Lag time

One patient had a lag time longer than the upper limit of non-bleeding pregnant control reference range of 6.11 min (see Table 24) and normal pooled plasma of 5.78 min. In post hoc analysis, the patient's lag time was within the non-bleeding pregnant control range if the two statistical outliers in the control group are excluded. The patient's ETP was 1251.16 nM*min and thus below the non-bleeding pregnant control reference range (1273.25 nM*min). Their peak thrombin value was 117.2 nM, i.e., significantly below the non-bleeding pregnant control reference range (194.49 nM) and the velocity index of 25.11 nM/min was half of the the non-bleeding pregnant control minimum

reference value (54.69 nM/min). Despite that the woman had a relatively small PPH with a measured blood loss of 1100 ml and did not receive any blood products. The patient had normal levels of all coagulation factors and the thrombin generation parameters were within the non-pregnant normal range suggesting that there was no evidence for haemostatic impairment. Additionally, one more woman had a longer lag time than the upper limit of normal pooled plasma. This suggests that this particular woman could potentially have benefitted from FFP infusion, however, the majority of women suffering from PPH would not.

In total, seven women had lag time values above the upper limit of the non-pregnant control reference range of 4.65 min and so triggered the primary endpoint. All of those women had ETP values above the lower end of the non-pregnant control reference range of 628 nM but three had values below the ETP of the non-bleeding pregnant control reference range (1273.25 nM*min). All the women maintained peak thrombin generation above the non-bleeding pregnant control reference range. Considering their coagulation profiles, all women had coagulation factor levels within or above the normal range and both PT and aPTT were normal. This suggests that FFP infusion would not have improved haemostasis. The finding of normal P, aPTT and coagulation factor levels means that the prolonged lag time cannot be explained based on deficiencies of these factors and the underlying mechanism remains unknown.

Table 24. Lag time outliers and their coagulation profiles.

Primary cause	TBL (ml)	Sam-ple	PT	aPTT	FI	FII	FV	FVII	FVIII	FIX	FX	FXI	Lag time (min)
Surgical/ trauma	1100 (running MBL 1.1L)	1st	13.1	28	5.9	70.3	53.1	60.7	283.3	120.5	89.7	73.9	6.67
Surgical/ trauma	2500 (running MBL 2L)	2nd	10.9	23.9	3.8	119.8	109.4	118.5	248.3	151.4	77.9	74.6	5.83
Surgical/ trauma	2500 (running MBL 2L)	2nd	10.8	28.2	9	113.4	125	131.7	419.3	139.6	166	49.3	5.67
Abrup-tion	1300 (running MBL unknown)	1st	10.2	23.2	2.0	140.5	78.0	177.9	228.1	161.3	186.6	88.3	5.62
Abrup-tion	2000 (running MBL 2L)	2nd	10.6	22.4	2.9	119.8	99.1	145.3	495.8	188.1	130.2	79.9	4.84
Surgical/ trauma	3350 (running MBL unknown)	1st	12.1	26.7	4.4	110.4	57.9	139	176.1	128.5	96	72.4	4.81
Surgical/ trauma	2300 (running MBL unknown)	2nd	11.5	24.1	6.1	145.3	96.7	160.5	377.8	231.3	165.3	81.5	4.67

PT and aPTT are shown in seconds, factor I (fibrinogen) levels are shown in g/L and other factor levels are shown in IU/dL. Normal range for factor levels are 50-150 IU/dL.

3.3.2 Peak thrombin generation

Six patient samples (or four patients) had peak thrombin values below the non-bleeding pregnant normal reference range of a minimum of 194.49 nM. None of them had a peak value below the non-pregnant control minimum of 110.19 nM and three had peak thrombin values below the lower limit of normal pooled plasma range of 137.89 nM. Their coagulation factors can be seen in Table 25. Low levels of factor II are likely cause of the reduced peak thrombin in most of the cases with low factor X and V contributing in some cases.

One patient with placenta praevia/accreta had also particular low levels of factor V and also low factor X. This patient had a total blood loss of 8.5 L and developed reduced peak thrombin secondary to decreased factor II, V and X as a result of dilution. She received 8 units of fibrinogen and 2 units of FFP which led to an increase in peak thrombin (Figure 14). Prior to her low peak thrombin value compared to non-bleeding pregnant control, her first and second study blood sample show peak values (306.9 nM and 230.33 nM, respectively) within non-pregnant and non-bleeding pregnant control reference range. Her corresponding fibrinogen levels were 2.3 g/L and 2.2 g/L and her factor II levels were within normal range (87.1 IU/dL and 60.5 IU/dL). These results can be best explained by dilutional coagulopathy related to her total blood loss and resuscitation.

Table 25. Peak thrombin outliers and their coagulation profiles.

Primary cause	TBL (ml)	Sam-ple	PT	aPTT	FI	FII	FV	FVII	FVIII	FIX	FX	FXI	Peak (nM)
Placenta praevia/ accreta	4000 (running MBL 2.78 L)	4th	13.4	33.7	1.7	43.4	34.7	100.2	230.8	97.7	42.1	55.1	192.97
Placenta praevia/ accreta	8500 (running MBL at 7 L)	4th	17.2	50.5	2.5	34.5	19.7	46.0	86.8	58.0	38.4	20.5	175.67
Placenta praevia/ accreta	8500 (running MBL at 5.7 L)	3rd	18.2	63.3	1.7	32.4	15.7	47.6	120.1	55.5	31.2	18.8	161.04
Retained pla./ products	3000 (running MBL unk-nown)	1st	12.7	46.1	1.8	37.7	49.6	77.7	432.9	68.0	56.8	31.9	137.58
Retained pla./ products	3000 (running MBL unk-nown)	2nd	13.2	48.2	1.7	30.9	42.7	70.1	163.8	50.1	53.8	26.6	120.43
Surgical/trauma	1100 (MBL 1.1 L)	1st	13.1	28	5.9	70.3	53.1	60.7	283.3	120.5	89.7	73.9	117.16

PT and aPTT are shown in seconds, factor I (fibrinogen) levels are shown in g/L and other factor levels are shown in IU/dL. Normal range for factor levels are 50-150 IU/dL.

There were 69 patient samples above the non-bleeding pregnant control reference range of 394.19 nM.

3.3.3 Endogenous thrombin potential

Nine patients had ETP values below the lower end of the non-bleeding pregnant control reference range of 1273.25 nM*min as shown in Table 26. Only one of those had an ETP below the normal reference range of normal pooled plasma (894.49 nM*min) and none of these patients had an ETP below the non-pregnant normal range (628.11 nM*min). However, if the non-pregnant control who was a statistically outlier is excluded then 3/114 women had an ETP below the non-pregnant normal range.

Out of these the nine women, three received blood products. One abruption with a total blood loss of 1.1 L received 10 gms of fibrinogen concentrate. Her fibrinogen level was 1.4 g/L at the first study blood sample with a MBL of 595 ml and at that time her ETP of 2314.38 nM*min was above non-pregnant normal range. Subsequently, her fibrinogen level improved to 2.3 g/L in the second sample with a stable MBL, but her ETP levels dropped to 1223.61 nM*min. Her third and last sample showed a stable fibrinogen of 2.5 g/L, stable coagulation factor levels and an ETP of 1863.5 nM*min, i.e., once again above the low non-bleeding pregnant control reference. At all times her ETP was above the non-pregnant normal range.

The second bleed that was treated with blood products was caused by an abruption, with a total blood loss of 1.3 L. The patient received 4 grams of fibrinogen concentrate. The fibrinogen level of 2.0 g/L and ETP of 1227.8 nM*min in the first sample improved to a fibrinogen of 2.5 g/L and ETP 2007.5 nM*min. Interestingly, the coagulation factor II dropped from 140.5 IU/dL to 112.7 IU/dL, however, was still in normal range. This patient's ETP was above the non-pregnant normal range throughout the bleed.

The third patient who received blood products was from the surgical/trauma group. Here, fibrinogen levels were initially 2.9 g/L and ETP of 819.13 nM*min. The fibrinogen then dropped to 1.5 g/L (unfortunately no ETP analysis was possible on this sample) with factor II within the normal range at of 81.9 IU/dL. The fibrinogen level then increased to 2.1 g/L with an ETP of 2303.4 nM*min and a factor II level stable at 79.9 IU/dL. This patient received in total 10 units of fibrinogen. The patient's first ETP was below the reference range of normal pooled plasma and so theoretically the ETP could have been improved with FFP, however, the actual coagulation factors levels were

normal or high at the time. In addition, the ETP increased to 2303 nM*min without FFP infusion and the initial ETP of 813 nM*min cannot be explained based on the information available.

Table 26. Endogenous thrombin potential (ETP) outliers and their coagulation profiles.

Primary cause	TBL (ml)	Sam-ple	PT	aPTT	FI	FII	FV	FVII	FVIII	FIX	FX	FXI	ETP (nM*min)
Surgical/ trauma	2800 (running MBL 1.5 L)	1st	9.5	22.3	2.9	113.0	74.9	196.5	284.0	167.9	127.4	100.9	819.13
Surgical/ trauma	2200 (running MBL 2.2 L)	1st	10.7	22.2	4.2	108.9	79.6	145.3	350.6	186.8	127.7	90.9	1015.0
Surgical/ trauma	1400 (running MBL 1.3 L)	2nd	11	26.8	4	96.8	91.9	109.5	175.4	145.5	78.7	69.4	1145.0
Retained pla./ products	2650 (running MBL 1 L)	1st	10.2	22.8	4.2	116.2	120.9	165.3	400.7	208.5	159.0	153.0	1146.1
Abruption	1100 (running MBL 595 ml)	2nd	12.8	28.9	2.3	76.9	22.9	170.1	56.8	100.3	103.3	88.0	1223.6
Abruption	1300 (running MBL unknown)	1st	10.2	23.2	2.0	140.5	78.0	177.9	228.1	161.3	186.6	88.3	1227.8
Surgical/ trauma	1100 (running MBL 1.1 L)	1st	13.1	28	5.9	70.3	53.1	60.7	283.3	120.5	89.7	73.9	1251.2
Surgical/ trauma	2500 (running MBL 2 L)	2nd	10.9	23.9	3.8	119.8	109.4	118.5	248.3	151.4	77.9	74.6	1255.5
Surgical/ trauma	2700 (running MBL 2.7 L)	3rd	10.9	26.4	4	77.5	73.8	155.6	207.1	110.7	75.9	47.9	1258.1

PT and aPTT are shown in seconds, factor I (fibrinogen) levels are shown in g/L and other factor levels are shown in IU/dL. Normal range for factor levels are 50-150 IU/dL.

There were 75 patient samples above the maximum non-bleeding pregnant control reference range of 2130.51 nM*min.

3.3.4 Velocity index

Three patient samples (or two patients) had velocity index values below the lower level of non-bleeding pregnant control reference range of 54.69 nM/min as shown in Table 27. In both cases, the velocity index was also below the non-pregnant normal range. All three samples were also below the low-level reference of normal pooled plasma (40.08 nM/min) and so, this haemostatic parameter could have been improved with FFP. None of the patients received blood products. The patient who had the 3000 mL blood loss had low levels of factor II and XI and this would explain the reduced velocity index. The other patient had normal coagulation factors and the reduced velocity index is unexplained.

Table 27. Velocity index outliers and their coagulation profiles.

Primary cause	TBL (ml)	Sam-ple	PT	aPTT	FI	FII	FV	FVII	FVIII	FIX	FX	FXI	Velocity index (nM/min)
Surgical/ trauma	1100	1st	13.1	28	5.9	70.3	53.1	60.7	283.3	120.5	89.7	73.9	25.11
Retained pla./ products	3000 (running MBL unknown)	1st	12.7	46.1	1.8	37.7	49.6	77.7	432.9	68.0	56.8	31.9	31.92
Retained pla./ products	3000 (running MBL unknown)	2nd	13.2	48.2	1.7	30.9	42.7	70.1	163.8	50.1	53.8	26.6	25.81

PT and aPTT are shown in seconds, factor I (fibrinogen) levels are shown in g/L and other factor levels are shown in IU/dL. Normal range for factor levels are 50-150 IU/dL.

Chapter 4

4 General Discussion

4.1 Recapitulating aims

This thesis investigated thrombin generation in obstetric patients suffering from postpartum haemorrhage. Current guidelines recommend empirical replacement of procoagulant clotting factors using FFP during PPH on the assumption that deficiencies of these factors are common [127] [128]. This practice is based on data derived from studies in major trauma where clotting factors deficiencies are common and occur early during the bleed [4]. Whether procoagulant clotting factors become deficient during PPH is debated [19] [119] [120]. Standard tests of coagulation, PT and aPTT, may not be the best way to assess clotting factor deficiencies because they do not reflect the cell-based model of haemostasis or are not good predictors of haemorrhagic risk [24] [25] [28] [44] [57] [129]. Thrombin generation is a method that can be used to investigate the integrated role of procoagulant factors in haemostasis [12]. This means that the assay can be used to assess whether there is any evidence for haemostatic impairment, secondary to clotting factor deficiencies.

The primary endpoint of the study was to investigate whether women experiencing severe PPH, or those most likely to have coagulopathy (placental abruption or AFE) had evidence of haemostatic impairment related to deficiencies of procoagulant factors. The specific endpoint chosen was to establish the proportion of women with PPH who had thrombin generation parameters outside the normal range of haemostatic competence for non-pregnant controls. Specifically, a lag time above the normal range or a peak thrombin, ETP or velocity index below the normal range was used. The results showed that 9/114 (7.9%) women had one or more thrombin generation parameters outside the normal range. Of note, most of the abnormal thrombin generation assays were a result of increased lag time and no women had a peak thrombin or ETP below the non-pregnant normal range.

Furthermore, the thrombin generation results were compared to normal pooled plasma, which was used as a model for FFP. The assumption was made that if a woman's thrombin generation was within or above the thrombin generation

haemostatic range of normal pooled plasma then the infusion of FFP would be unlikely to improve haemostasis. The results showed that 5/114 women had thrombin generation that could have potentially been improved by FFP.

In addition, thrombin generation in women with PPH was compared with two other groups, non-pregnant health women controls and non-bleeding term pregnant controls (samples taken immediately before an elective caesarean section). These data were used to investigate the effect of pregnancy on thrombin generation. The results confirmed that peak thrombin, ETP and velocity index were substantially increased in non-bleeding pregnant women supporting previous publications and confirming the prothrombotic state at term [10] [65] [69] [70]. The lag time was not different between non-pregnant and non-bleeding pregnant controls suggesting that this parameter is not sensitive to the prothrombotic state associated with pregnancy and hence may not be the best parameter to assess haemostatic impairment in this cohort of patients.

There was no clinically significant association between thrombin generation and the primary cause of bleeding, suggesting that secondary haemostasis is not affected by the aetiology of the bleed. This is discrepant to findings for fibrinogen where reduced levels are well recognised in cases of placental abruption [124] [127]. The implication is that depletion of fibrinogen during abruption is specific and not associated a generalised consumption of coagulation factors.

I also investigated whether the primary cause of bleed or the bleeding size would have an effect on the thrombin generation parameters and would this be reflected in the coagulation factors of the patients.

The following section provides conclusive statements while critically appraising their limitations and the influence this will have on future research.

4.2 Lag time

The primary endpoint of this project was to assess the proportion of women suffering from PPH with lag times above the normal range for healthy non-pregnant female controls. There were 7 out of 114 (6.14%) women with PPH had lag times above the

upper limit of the normal range for non-pregnant controls. However, none of the patients with a high lag time had any procoagulant factors below the normal range. This further supports the suggestion that lag time is not a good parameter to investigate haemostatic impairment during PPH. The cause of the prolonged lag time in these 7 cases remains unknown and further research is required.

Regarding the secondary endpoints, the study found that only 1 out of 114 (0.88%) women with PPH had a lag time above the upper limit of the normal range for non-bleeding pregnant controls and only 2 out of 114 (1.75%) women had a longer lag time than the upper limit of normal pooled plasma reference range. This implies that administering FFP would not be likely to enhance haemostasis in the vast majority of women experiencing PPH based on the lag time, and this is supported by the finding that coagulation factors were normal or raised in the women with high lag times.

Furthermore, in the comparative analysis of lag times between the postpartum haemorrhage cohort and the non-bleeding pregnant controls, no statistically significant difference was observed. This finding suggests the absence of any evident haemostatic impairment in women experiencing PPH compared to non-bleeding women at term. Consequently, it implies that additional factors must account for the occurrence of major haemorrhage following childbirth in certain women.

Among the various primary causes of bleeding in the PPH group, the subgroup characterised by placenta praevia/accreta displayed notably shorter lag times in comparison to other PPH subgroups, such as abruption, retained placenta/products, and surgical/trauma. The underlying reasons for this observation remain unclear and require further investigation. Furthermore, only the placenta praevia/accreta subgroup exhibited a relationship between lag time and measured blood loss. This finding is also unexpected and difficult to rationalise as it implies that as the bleed size increases the initiation of haemostasis becomes more rapid. This is a counter-intuitive finding and would need to be investigated further to establish whether it was a reproducible finding. However, any conclusion is limited by the low R^2 value, indicating that additional factors other than blood loss may influence lag time during PPH. Overall, the lag time does not appear to be a useful parameter to assess haemostatic impairment in this cohort of patients.

4.3 Peak thrombin generation

Significant differences were found between the non-pregnant control group and PPH patients, with PPH patients demonstrating higher peak thrombin values. In relation the primary endpoint 0 out of 114 patients had peak thrombin generation below the non-pregnant normal range. Furthermore, there was no significant difference in peak values between the pregnant control and PPH groups. These findings suggest that during PPH, the peak thrombin concentrations do not decrease in most cases and remain comparable to those observed in the non-bleeding pregnant control group, indicating that they are adequate for coagulation processes. The finding of the higher peak thrombin values means that, despite bleeding, the patients were on average more haemostatically competent than non-pregnant controls, supporting the view that FFP infusion would not enhance *in vivo* haemostasis in most cases.

Only two PPH women with a total of three samples exhibited peak thrombin generation levels (117.2 nM, 120.4 nM and 137.6 nM) below the reference range of normal pooled plasma (137.89 nM). One of these women was infused with FFP during a bleed of more than 8 L and the peak thrombin increased suggesting that in very large bleeds coagulation factors become depleted and FFP has a role. This is supported by the finding of reduced levels of factors II, V and XI in this patient.

Statistically significant differences were also showed between the non-bleeding pregnant and non-pregnant control groups, i.e., the non-bleeding pregnant control group exhibited significantly higher peak thrombin values, aligning with the physiological changes that occur in preparation for delivery of the foetus and placenta. This finding is most likely due to increased levels of procoagulant clotting factors at term as has been previously shown by many groups [36]. It shows that the peak thrombin levels are sensitive to the procoagulant changes associated with pregnancy, in contrast to lag time.

Considering bleed size correlations with peak thrombin generation – this project did find variations of correlations across the various PPH subgroups. The subgroup placenta praevia/accreta shows a significant negative correlation between peak thrombin and MBL, meaning that as the bleed size increased the peak thrombin

decreased. This suggests that haemostasis worsened as bleed size increased and is expected if coagulation factors become depleted. The finding is opposite that reported for lag time but is a plausible outcome for large bleeds. Other PPH subgroups lack statistically significant correlations possibly because they were not often associated with very big bleeds as is the case for placenta accreta and praevia.

4.4 Endogenous thrombin potential

The primary endpoint was to assess the proportion of women suffering from PPH with endogenous thrombin potential below the normal range for healthy non-pregnant female controls and this project did not identify any woman. This suggests a lack of evidence for haemostatic impairment during PPH. However, it was noted that one non-pregnant control had an ETP that was a statistical outlier. If that control was excluded, then 3/114 women had evidence of haemostatic impairment at some time during PPH. Although the three cases were identified in a post hoc analysis, the most cautious conclusion is that a small number of women with PPH have haemostatic impairment as assessed by ETP.

Ten postpartum haemorrhage patients had ETPs below the values of the non-bleeding pregnant control range, however, only one of those patients (819.13 nM*min) had an ETP below that of our laboratories normal pooled plasma range. This suggests, if assuming NPP can be used as a model for FFP, only 1 out of 114 patients (0.88%) could have benefitted from receiving FFP to support their total thrombin generation potential. Interestingly, this woman did not receive FFP but was given 10 units of fibrinogen and stopped bleeding with a total blood loss of 2800 ml.

Consistent with the peak thrombin, but not lag time, a visible increase in ETP was noted in non-bleeding term pregnant women when compared to non-pregnant controls. Notably, ETP values were significantly elevated in obstetric patients, irrespective of bleeding status, indicating that the body's procoagulable state is mirrored in the total amount of thrombin generated. This shows that, like peak thrombin, ETP is sensitive to the well-described procoagulant state associated with pregnancy. This is likely to be due to increased levels of procoagulant clotting factors, especially factor VIII at term [36]. The raised levels may act as a buffer to coagulation

factor depletion during bleeding because even women experiencing PPH had either raised ETP or ETP within the normal non-pregnant range.

Furthermore, there was no statistically significant difference in endogenous thrombin potential, between the non-bleeding pregnant controls and the PPH group showing that the bleeding associated with PPH does not affect the ability to produce thrombin in most cases. If considering a conservative adjustment method during pairwise comparison, there is no statistical difference between non-bleeding pregnant controls and any PPH group, suggesting individual bleed causes of bleeding do not cause a haemostatic impairment related to coagulation factors and secondary haemostasis.

Regarding bleed size and endogenous thrombin potential – as with lag time and peak thrombin, the relationship between ETP and measured blood loss varies among PPH subgroups. Only in the atony subgroup, ETP is positively associated with MBL and statistically significant, while in the other subgroups (abruption, placenta previa/ accreta, retained placenta/products, and surgical/trauma), the relationship is either not statistically significant or only marginally so. The explanatory power of ETP for MBL for ETP is also relatively low across all subgroups.

4.5 Velocity index

There were 2 out of 114 (1.75%) women suffering from postpartum haemorrhage who had velocity index below the normal range of healthy non-pregnant female controls (38.93 nM). These women also had velocity indices below the normal range of normal pooled plasma (40.08 nM). This suggests that in the majority of women, FFP infusion would not improve haemostasis based on the velocity index parameter.

Additionally, there was no statistically significant distinction observed between women experiencing PPH and non-bleeding pregnant controls. However, placenta previa/ accreta exhibited statistically significant differences compared to subgroups abruption, retained placenta/products, and surgical/trauma. Specifically, the velocity index in the placenta praevia/accreta subgroup tended to be lower, indicating slower thrombin production within this particular group.

The linear regression analysis between bleed size and velocity index showed that the relationship between velocity index and measured blood loss is not statistically significant in any PPH subgroup. This suggests that postpartum haemorrhage bleed size is unlikely to affect the velocity index or the rate of increased thrombin generation. It implies that velocity index is less sensitive to the effect of very large PPHs on haemostasis than peak thrombin.

4.6 Amniotic fluid embolus

The analysis of the AFE patient revealed that coagulation factors VIII, II, IX, VII, X, XI, and XIII were within the normal range thus confirming that there is no generalised consumption of clotting factors in this coagulopathy. Platelets levels also remained within a range that is adequate for haemostasis. However, the level of factor V was reduced to around 30% of the normal levels, resulting in a slight extension of the PT/aPTT. The reason for the isolated decrease in FV is not known but may be related to the action of activated protein C or plasmin [127] [128]. Nonetheless, this reduced level is typically considered sufficient for maintaining haemostasis. All thrombin generation parameters remained normal or increased throughout, confirming the adequacy of factor V levels and the absence of any deficiency in overall secondary haemostasis. The main cause of coagulopathy in this case was low functional Clauss fibrinogen level as previously described in AFE. This suggests that the initial coagulation disorder in AFE is driven by reduced fibrinogen whilst preserving thrombin generation potential and levels of all clotting factors within a haemostatic range.

4.7 Limitations

The current project has limitations that should be acknowledged. One limitation is the relatively small sample size, which limited detailed sub-cohort analyses based on the primary cause of bleeding. This may mean that differences between the groups may not have been identified. As noted in the introduction, different causes of postpartum haemorrhage may have distinct pathophysiological mechanisms and may require tailored treatment approaches. However, conducting a study with a larger sample size would have been labour-intensive and would not have been feasible within the scope of this project. Furthermore, the challenging sample collection and storage, especially

outside of routine hours, meant that not all women experiencing PPH had adequate study samples taken or sampling was inadequate and thus had to be excluded. Furthermore, thrombin generation was performed on a subgroup of cases selected for bleed severity and likelihood of coagulopathy. This causes obvious limitations, i.e., the results cannot be extrapolated to all women suffering from PPH. Furthermore, the results may not be applicable to other centres as women in this cohort were treated using the OBS Cymru protocol based on of early recognition and treatment with fibrinogen [126].

Another limitation is the lack of stratification for obesity in the analysis. Previous research has shown that obesity is associated with altered thrombin generation parameters, such as longer lag time and higher endogenous thrombin potential and peak height, which may be attributed to low-grade inflammation observed in obese individuals [64]. Although not directly investigated in this study, a comparison of median BMI values reveals that the OBS+ cohort had a higher median BMI of 30, whereas the women investigated for PPH in this project had lower median BMI values: all PPH cases had a median BMI of 25, median BMI for abruption was 24, for atony was 27.45, for placenta praevia/ accreta was 23, for retained placenta/ products was 25, and for surgical/ trauma was 26.5.

Moreover, to gain a more comprehensive understanding of the dynamic changes in thrombin generation, it would have been advantageous to assess the coagulation profiles of patients both before and after delivery, as demonstrated in previous studies [1] [68] [70]. However, this would have required recruitment of all women giving birth because the ones who went on to experience PPH could not be predicted.

In light of these limitations, it is imperative to conduct a larger-scale longitudinal prospective study that examines the coagulation profiles, including coagulation factors and thrombin generation, of women throughout the entirety of pregnancy and at multiple time points before and after delivery. This expanded investigation would provide valuable insights into the factors influencing thrombin generation during the peripartum period, as well as shed light on how individual coagulation profiles may be influenced by the occurrence of postpartum haemorrhage.

4.8 Conclusion and clinical relevance

In conclusion, this project provides valuable insights into thrombin generation dynamics during PPH, highlighting the need for individualised treatment approaches. The limited evidence indicating secondary haemostatic failure in PPH suggests that empirical fresh frozen plasma administration is generally unnecessary for the majority of PPH cases, distinguishing it from trauma-induced coagulopathy.

However, it is important to note that FFP administration may still be warranted for a small subset of PPH cases with abnormal thrombin generation, specifically those with placenta praevia/accrete or those with very large bleeds. Nevertheless, it is essential to state that the results obtained from this study may not be universally applicable to other healthcare centres, as the University Hospital of Wales follows the OBS Cymru protocol, which emphasises early recognition and treatment with fibrinogen.

The thrombin generation parameter that appeared to best measure the prothrombotic state at term and identify low levels of coagulation factors was peak thrombin. The lag time appeared to have limited utility in the assessment of haemostasis during PPH although ETP and velocity indices have value. Further work is required to confirm these conclusions.

Although thrombin generation assays can be used to provide insight into haemostasis during PPH in a research setting, they cannot be considered to be a clinical useful assay because they are only established in a small number of centres and results cannot be made available in a timely fashion.

In conclusion, further research is essential to comprehensively understand the complex relationship between thrombin generation, bleeding severity, and treatment responses in the context of PPH. A greater understanding of these factors will enable more effective management strategies in PPH cases.

5 Bibliography

[1] K. C. McLean, I. M. Bernstein and K. E. Brummel-Ziedins, “Tissue factor–dependent thrombin generation across pregnancy,” *American Journal of Obstetrics and Gynecology,* vol. 207, no. 2, pp. 135-e1, 2012.

[2] M. E. Cross and E. V. Plunkett, Physics, pharmacology and physiology for anaesthetists: key concepts for the FRCA, Crambridge University Press, 2014.

[3] P. Thornton and J. Douglas, “Coagulation in pregnancy,” *Best practice & research Clinical obstetrics & gynaecology,* vol. 24, no. 3, pp. 339-352, 2010.

[4] G. P. Dobson, H. L. Letson, R. Sharma, F. R. Sheppard and A. P. Cap, “Mechanisms of early trauma-induced coagulopathy: The clot thickens or not?,” *Journal of Trauma and Acute Care Surgery,* vol. 79, no. 2, pp. 301-309, 2015.

[5] D. Bevan and B. Sørensen, “Hemostasis: Principles of investigation,” in *Blood and Bone Marrow Pathology E-Book*, Elsevier Health Sciences, 2011, pp. 473-490.

[6] A. J. Gale, “Continuing education course #2: current understanding of hemostasis,” *Toxicologic pathology,* vol. 39, no. 1, pp. 273-280, 2011.

[7] N. Tynngård, T. L. Lindahl and S. Ramström, “Assays of different aspects of haemostasis - what do they measure?,” *Thombosis journal,* vol. 13, no. 1, pp. 1-10, 2015.

[8] E. H. Uchikova and I. I. Ledjev, “Changes in haemostasis during normal pregnancy,” *European Journal of Obstetrics & Gynecology and Reproductive Biology,* vol. 119, no. 2, pp. 185-188, 2005.

[9] V. Thiruvenkatarajan, A. Pruett and S. D. Adhikary, “Coagulation testing in the perioperative period,” *Indian journal of anaesthesia,* vol. 58, no. 5, p. 565, 2014.

[10] D. Katz and Y. Beilin, “Disorders of coagulation in pregnancy,” *BJA: British Journal of Anaesthesia,* vol. 115, no. suppl_2, pp. ii75-ii88, 2015.

[11] M. Van de Wouwer, D. Collen and E. M. Conway, “Thrombomodulin-protein C-EPCR system: integrated to regulate coagulation and inflammation,”

Arteriosclerosis, thrombosis, and vascular biology, vol. 24, no. 8, pp. 1374-1383, 2004.

[12] T. Baglin, “The measurement and application of thrombin generation,” *British journal of haematology,* vol. 130, no. 5, pp. 653-661, 2005.

[13] B. Patri, A. A. Sahu, S. Pal and S. Chakravarty, “The cell basedmodel of in-vivo coagulation: a work in progress,” *International Blood Research & Reviews,* vol. 6, no. 1, pp. 1-10, 2016.

[14] K. E. Brummel, S. G. Paradis, S. Butenas and K. G. Mann, “Thrombin functions during tissue factor–induced blood coagulation,” *Blood, The Journal of the American Society of Hematology,* vol. 100, no. 1, pp. 148-152, 2002.

[15] H. M. Monroe DM, “What does it take to make the perfect clot?,” *Arteriosclerosis, thrombosis, and vascular biology,* vol. 26, no. 1, pp. 41-8, 2006.

[16] M. Metze, M. Platz, C. Pfrepper and P. S., “Gerinnungsdiagnostik im klinischen Alltag - Teil 1,” *Die Innere Medizin,* vol. 63, no. 6, pp. 619-30, 2022.

[17] E. M. Keohane, C. N. Otto and J. M. Walenga, “Rodak’s Hematology-E-Book: Clinical Principles and Applications,” Elsevier Health Sciences, 2019.

[18] M. Maegele, P. C. Spinella and H. Schöchl, “The acute coagulopathy of trauma: mechanisms and tools. of risk stratification,” *Shock,* vol. 38, no. 5, pp. 450-458, 2012.

[19] R. E. Collis and P. W. Collins, “Haemostatic management of obstetric haemorrhage,” *Anaesthesia,* vol. 70, pp. 70-e28, 2015.

[20] P. W. Collins, S. F. Bell, L. De Lloyd and R. E. Collis, “Management of postpartum haemorrhage: from research into practice, a narrative review of the literature and the Cardiff experience,” *International journal of obstetric anaesthesia,* vol. 37, pp. 106-117, 2019.

[21] R. Collis and E. Guasch, “Managing major obstetric haemorrhage: Pharmacotherapy. and transfusion,” *Best practice & Research Clinical Anaesthesiology,* vol. 31, no. 1, pp. 107-124, 2017.

[22] L. de Lloyd, P. V. Jenkins, S. F. Bell, N. Mutch, J. F. Pereira, P. Badenes, D. James, A. Ridgeway, L. Cohen, T. Roberts and V. Field, “Acute obstetric coagulopathy during postpartum hemorrhage is caused by hyperfibrinolysis

and dysfibrinogenemia: an observational cohort study," *Journal of Thrombosis and Haemostasis,* vol. 21, no. 4, pp. 862-79, 2023.

[23] C. L. Balduini and P. Noris, "Platelet count and aging," *Haematologica,* vol. 99, no. 6, p. 953, 2014.

[24] A. H. Kamal, A. Tefferi and R. K. Pruthi, "How to interpret and pursue an abnormal prothrombin time, activated partial thromboplastin time, and bleeding time in adults," *Mayo Clinic Proceedings,* vol. 82, no. 7, pp. 864-873, 2007.

[25] C. Kitchens, "To bleed or not to bleed? Is that the questions for the PTT?," *Journal of Thrombosis and Haemostasis,* vol. 3, no. 12, pp. 2607-2611, 2005.

[26] R. C. F. Duarte, C. N. Ferreira, D. R. A. Rios, H. J. d. Reis and M. d. G. Carvalho, "Thrombin generation assays for global evaluation of the hemostatic system: perspectives and limitations," *Revista brasileira de hematologia e hemoterapia,* vol. 39, pp. 259-265, 2017.

[27] J. P. Antovic and M. Blombäck, "Essential guide to blood coagulation," Wiley Online Library, 2013.

[28] M. Roshal and M. Reyes Gil, "Activated Partial Thromboplastin Time," in *Transfusion Medicine and Hemostasis - Clinical and Laboratory Aspects*, Elsevier Science, 2019.

[29] V. H. Flood and J. P. Scott, "Bleeding and Thrombosis," in *Nelson Pediatric Symptom-Based Diagnosis*, Elsevier, 2018, pp. 682-700.

[30] M. M. Flanders, R. Crist and G. M. Rodgers, "Comparison of five thrombin time reagents," *Clinical chemistry,* vol. 49, no. 1, pp. 169-172, 2003.

[31] B. J. Bain, I. Bates and M. A. Laffan, "Dacie and Lewis Practical Haematology E-Book," Elsevier Health Sciences, 2016.

[32] I. J. Mackie, S. Kitchen, S. J. Machin and G. D. Lowe, "Guidelines on fibrinogen assays," *British journal of haematology,* vol. 121, no. 3, pp. 396-404, 2003.

[33] R. A. Newman, A. Mehboob and J. H. Chung, "Chapter 9: Anticoagulation in Pregnancy," in *Cardio-Obstetrics - A Practical Guide to Care for Pregnant Cardiac Patients*, CRC Press, 2020.

[34] B. Brenner, “Haemostatic changes in pregnancy,” *Thrombosis research,* vol. 114, no. 5-6, pp. 409-414, 2004.

[35] K. A. Bremme, “Haemostatic changes in pregnancy,” *Best practice & research Clinical haematology,* vol. 16, no. 2, pp. 153-168, 2003.

[36] C. Solomon, R. E. Collis and P. W. Collins, “Haemostatic monitoring during postpartum haemorrhage and implications for management,” *British journal of anaesthesia,* vol. 109, no. 6, pp. 851-863, 2012.

[37] U. Nowak-Göttl, V. Limperger, G. Kenet, F. Degenhardt, R. Arlt, J. Domschikowski, H. Clausnizer, J. Liebsch, R. Junker and D. Steppat, “Developmental hemostasis: a lifespan from neonates and pregnancy to the young and elderly adult in a European white population,” *Blood Cells, Molecules, and Diseases,* vol. 67, pp. 2-13, 2017.

[38] G. Hron, P. A. Kyrle, A. Kaider, K. Phillipp, I. Pabinger, M. Kollars and S. Eichinger, “ProCGlobal and endogenous thrombin potential during pregnancy,” *American journal of obstetrics and gynecology,* vol. 203, no. 5, pp. 463-e1, 2010.

[39] M. Abbassi-Ghanavati, L. G. Greer and F. G. Cunningham, “Pregnancy and laboratory studies: a reference table for clinicians,” *Obstretrics & Gynecology,* vol. 114, no. 6, pp. 1326-1331, 2009.

[40] A. T. Hansen, B. H. Andreasen, J. D. Salvig and A.-M. Hvas, “Changes in fibrin D-dimer, fibrinogen, and protein S during pregnancy,” *Scandinavian journal of clinical and laboratory investigation,* vol. 71, no. 2, pp. 173-176, 2011.

[41] T. M. Hackeng and J. Rosing, “Protein S as cofactor for TFPI,” *Arte-riosclerosis, thrombosis, and vascular biology,* vol. 29, no. 12, pp. 2015-2020, 2009.

[42] A. Rousseau, R. Favier and P. Van Dreden, “Elevated circu- lating soluble thrombomodulin activity, tissue factor activity and circulating procoagulant phospholipids: new and useful markers for pre-eclampsia?,” *European Journal of Obstetrics & Gynecology and Reproductive Biology,* vol. 146, no. 1, pp. 46-49, 2009.

[43] J. C. Chapin and K. A. Hajjar, “Fibrinolysis and the control of blood coagulation,” *Blood reviews,* vol. 29, no. 1, pp. 17-24, 2015.

[44] B. H. Shaz, C. D. Hillyer, M. Roshal and C. S. Abrams, Transfusion Medicine and Hemostasis: Clinical and Laboratory Aspects, Newnes: Elsevier, 2013.

[45] R. Hughes, E. Aitken, J. Anderson, C. Barry and M. Benton, “Antenatal care for uncomplicated pregnancies,” *National Institute for Health and Care Excellence,* vol. 5, 2008.

[46] F. Boehlen, P. Hohlfeld, P. Extermann, T. V. Perneger and P. De Moerloose, “Platelet count at term pregnancy: a reappraisal of the threshold,” *Obstetrics & Gynecology,* vol. 95, no. 1, pp. 29-33, 2000.

[47] L. Hammerova, J. Chabada, J. Drobny and A. Batorova, “Longitudinal evaluation of markers of hemostasis in pregnancy,” *Bratislavske lekarske listy,* vol. 115, no. 3, pp. 140-144, 2014.

[48] P. Srimala, I. Khan and P. S. Hari, “Estimation of prothrombin time in pregnancy compared with normal controls,” *Journal of Evolution of Medical and Dental Sciences,* vol. 2, no. 2, pp. 72-79, 2013.

[49] A. Dielis, E. Castoldi, H. Spronk, R. Van Oerle, K. Hamulyak, H. Ten Cate and J. Rosing, “Coagulation factors and the protein C system as determinants of thrombin generation in a normal population,” *Journal of Thrombosis and Haemostasis,* vol. 6, no. 1, pp. 125-131, 2008.

[50] W. F. Bahou, “Chapter 9: Thrombin receptors,” in *Platelets*, Elsevier, 2007.

[51] E. Di Cera, “Chapter 4: Thrombin as an anticoagulant,” in *Progress in molecular biology and translational science*, vol. 99, Elsevier, 2011.

[52] B. F. Le Bonniec, Thrombin, Elsevier, 2013, pp. 2915-2932.

[53] E. Di Cera, “Thrombin interactions,” *Chest,* vol. 124, no. 3, pp. 11S-17S, 2003.

[54] J. A. Huntington, “How Na+ activates thrombin – a review of the functional and structural data,” *Biological chemistry,* vol. 389, no. 8, pp. 1025-1035, 2008.

[55] J. Crawley, S. Zanardelli, C. Chion and D. Lane, “The central role of thrombin in hemostasis,” *Journal of thrombosis and haemostasis,* vol. 5, pp. 95-101, 2007.

[56] E. Castoldi and J. Rosing, “Thrombin generation tests,” *Thrombosis research,* vol. 127, pp. S21-S25, 2011.

[57] C. Ping, J. Jani, M. B. Streiff, G. Zheng and T. S. Kickler, “Evaluation of global hemostatic assays in response to factor VIII inhibitors,” *Clinical and Applied Thrombosis/Hemostasis,* vol. 25, pp. 1-10, 2019.

[58] J. Kintigh, P. Monagle and V. Ignjatovic, “A review of commercially available thrombin generation assays,” *Research and practice in thrombosis and haemostasis,* vol. 2, no. 1, pp. 42-48, 2018.

[59] A. Tripodi, “Thrombin generation assay and its application in the clinical laboratory,” *Clinical chemistry,* vol. 62, no. 5, pp. 699-707, 2016.

[60] H. H. Coenraad, P. Giesen, R. Al Dieri, V. Regnault, E. De Smedt, R. Wagenvoord, T. Lecompte and S. Bégun, “Calibrated automated thrombin generation measurements in clotting plasma,” *Pathophysiology of haemostasis and thrombosis,* vol. 33, no. 1, pp. 4-15, 2003.

[61] H. C. Hemker, R. Al Dieri and S. Bégun, “Thrombin generation assays: accruing clinical relevance,” *Current opinion in hematology,* vol. 11, no. 3, pp. 170-175, 2004.

[62] H. C. Hemker and R. Kremers, “Data management in thrombin generation,” *Thrombosis research,* vol. 131, no. 1, pp. 3-11, 2013.

[63] R. Hudák, E. G. Székely, K. R. Kovács, A. Nagy, G. Hofgárt, E. Berényi, L. Csiba, J. Kappelmayer and Z. Bagoly, “Low thrombin generation predicts poor prognosis in ischemic stroke patients after thrombolysis,” *PLOS One,* vol. 12, no. 7, 2017.

[64] P. van Paridon, M. Panova-Noeva, R. van Oerle, A. Schulz, I. Hermanns, J. Prochaska, N. Arnold, H. Binder, I. Schmidtmann, M. Beutel and N. Pfeiffer, “Thrombin generation in cardiovascular disease and mortality–results from the Gutenberg Health Study,” *Haematologica,* vol. 105, no. 9, p. 2327, 2020.

[65] M. Macey, S. Bevan, S. Alam, L. Verghese, S. Agrawal, S. Beski, R. Thuraisingham and P. MacCallum, “Platelet activation and endogenous thrombin potential in pre-eclampsia,” *Thrombosis Research,* vol. 125, no. 3, pp. e76-e81, 2010.

[66] C. Cimenti, A. Schlagenhauf, B. Leschnik, M. Schretter, G. Tschakert, W. Gröschl, F. Seibert, P. Hofmann and W. Muntean, “Low endogenous thrombin potential in trained subjects,” *Thrombosis Research,* vol. 131, no. 6, pp. e281-e285, 2013.

[67] H. Haidl, C. Cimenti, B. Leschnik, D. Zach and W. Muntean, “Age-dependency of thrombin generation measured by means of calibrated automated thrombography (CAT),” *Thrombosis and Haemostasis,* vol. 95, no. 5, pp. 772-775, 2006.

[68] S. Eichinger, A. Weltermann, K. Philipp, E. Hafner, A. Kaider, E.-M. Kittl, B. Brenner, C. Mannhalter, K. Lechner and P. A. Kyrle, “Prospective evaluation of hemostatic system activation and thrombin potential in healthy pregnant women with and without factor V Leiden.,” *Thrombosis and haemostasis,* vol. 82, no. 10, pp. 1232-1236, 1999.

[69] Y. Dargaud, S. Hierso, L. Rugeri, C. Battie, P. Gaucherand, C. Negrier and C. Trzeciak, “Endogenous thrombin potential, prothrombin fragment 1+ 2 and D-dimers during pregnancy,” *Thrombosis and Haemostasis,* vol. 103, no. 2, pp. 469-71, 2010.

[70] A. Rosenkranz, M. Hiden, B. Leschnik, W. Eva-Christine, D. Schlembach, U. Lang, S. Gallistl and W. Muntean, “Calibrated automated thrombin generation in normal uncomplicated pregnancy,” *Thrombosis and Haemostasis,* vol. 99, no. 2, pp. 331-337, 2008.

[71] C. Bagot, E. Leishman, C. Onyiaodike, F. Jordan and D. Freeman, “Normal pregnancy is associated with an increase in thrombin generation from the very early stages of the first trimester,” *Thrombosis Research,* vol. 157, pp. 49-54, 2017.

[72] K. Dallaku, H. Shakur-Still, D. Beaumont, I. Roberts, S. Huque, M. Delius, S. Holdenrieder, O. Gliozheni and U. Mansmann, “No effect of tranexamic acid on platelet function and thrombin generation (ETAPlaT) in postpartum haemorrhage: a randomised placebo-controlled trial,” *Wellcome Open Research,* vol. 4, no. 21, 2019.

[73] J. Coleman, S. Fabbri, M. Anderson, E. Moore, M. Cohen, J. Hadley, A. Ghasabyan, J. Chandler, M. Kelher, K. Freeman and Z. Miller, “Beyond uterine atony: characterizing postpartum hemorrhage coagulopathy,” *American Journal of Obstetrics & Gynecology MFM,* vol. 5, no. 3, 2023.

[74] L. Say, D. Chou, A. Gemmill, Ö. Tunçalp, A.-B. Moller, J. Daniels, A. M. Gülmezoglu, M. Temmerman and L. Alkema, “Global causes of maternal

death: a WHO systematic analysis," *The Lancet global health,* vol. 2, no. 6, pp. e323-e333, 2014.

[75] S. Kinsella, "Anaesthetic deaths in the CMACE (Centre for Maternal and Child Enquiries) Saving Mothers' Lives report 2006-08," *Anaesthesia,* vol. 66, no. 4, pp. 243-246, 2011.

[76] Royal College of Obstetricians and Gynaecologists, "Postpartum Haemorrhage, Prevention and Management (Green-top 52)," Royal College of Obstetricians and Gynaecologists, London, 2009.

[77] J. J. Zwart, J. R. Dupuis, A. Richters, F. Öry and J. van Roosmalen, "Obstetric intensive care unit admission: a 2-year nationwide population-based cohort study," *Intensive care medicine,* vol. 36, no. 2, pp. 256-263, 2010.

[78] "WHO recommendations for the prevention and treatment of postpartum haemorrhage," World Health Organization, 2017. [Online]. Available: https://apps.who.int/iris/bitstream/handle/10665/75411/97?sequence=1. [Accessed 27 10 2022].

[79] A. M. Gülmezoglu, WHO guidelines for the management of postpartum haemorrhage and retained placenta, World Health Organization Press, 2009.

[80] C. L. Kempton and K. Woods, "Bleeding Disorders in Pregnancy," in *Transfusion Medicine and Hemostasis - Clinical and Laboratory Aspects*, Elsevier Science, 2019.

[81] E. Chandraharan and A. Krishna, "Diagnosis and management of postpartum haemorrhage," *BMJ,* p. 358, 2017.

[82] N. J. Kassebaum, R. M. Barber, Z. A. Bhutta, L. Dandona, P. W. Gething, S. I. Hay, Y. L. H. Kinfu, X. Liang, S. Lim and A. Lopez, "Global, regional, and national levels of maternal mortality, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015," *The Lancet,* vol. 388, no. 10053, pp. 1775-1812, 2016.

[83] M. Knight, W. M. Callaghan, C. Berg, S. Alexander, M.-H. Bouvier-Colle, J. B. Ford, K. Joseph, G. Lewis, R. Liston, C. Roberts and J. Oats, "Trends in postpartum hemorrhage in high resource countries: a review and recommendations from the International Postpartum Hemorrhage Collaborative Group," *BMC pregnancy and childbirth,* vol. 9, no. 1, pp. 1-10, 2009.

[84] J. Rossen, I. Økland, O. B. Nilsen and T. M. Eggebø, “Is there an increase of postpartum hemorrhage, and is severe hemorrhage associated with more frequent use of obstetric interventions?,” *Acta obstetricia et gynecologica Scandinavica,* vol. 89, no. 10, pp. 1248-1255, 2010.

[85] E. M. Fyfe, J. Thompson, N. H. Anderson, K. M. Groom and L. M. McCowan, “Maternal obesity and postpartum haemorrhage after vaginal and caesarean delivery among nulliparous women at term: a retrospective cohort study,” *BMC pregnancy and childbirth,* vol. 12, no. 1, pp. 1-8, 2012.

[86] A. Evensen, J. M. Anderson and P. Fontaine, “Postpartum hemorrhage: prevention and treatment,” *American family physician,* vol. 95, no. 7, pp. 442-449, 2017.

[87] G. Carroli, C. Cuesta, E. Abalos and A. M. Gulmezogly, “Epidemiology of postpartum haemorrhage: a systematic review.,” *Best practice & research Clinical obstetrics & gynaecology,* vol. 22, no. 6, pp. 999-1012, 2008.

[88] S. F. Bell, A. Watkins, M. John, E. Macgillivray, T. L. Kitchen, D. James, C. Scarr, C. Bailey, K. Kelly, K. James and J. Stevensens, “Incidence of postpartum haemorrhage defined by quantitative blood loss measurement: a national cohort,” *BMC Pregnancy and Childbirth,* vol. 20, no. 1, pp. 1-9, 2020.

[89] N. Sloan, J. Durocher, T. Aldrich, J. Blum and B. Winikoff, “What measured blood loss tells us about postpartum bleeding: a systematic review,” *BJOG: An International Journal of Obstetrics & Gynaecology,* vol. 117, no. 7, pp. 788-800, 2010.

[90] G. Forbes, S. Akter, S. Miller, H. Galadanci, Z. Qureshi, S. Fawcus, G. Hofmeyr, N. Moran, M. Singata-Madliki, F. Dankishiya and G. Gwako, “Factors influencing postpartum haemorrhage detection and management and the implementation of a new postpartum haemorrhage care bundle (E-MOTIVE) in Kenya, Nigeria, and South Africa,” *Implementation Science,* vol. 18, no. 1, p. 1, 2023.

[91] H. A. Mousa, J. Blum, G. Abou El Senoun, H. Shakur and Z. Alfirevic, “Treatment for primary postpartum haemorrhage,” *Cochrane database of systematic reviews,* 2014.

[92] J. McClure, G. Cooper, T. Clutton-Brock and C. f. M. a. C. Enquiries, “Saving mothers’ lives: reviewing maternal deaths to make motherhood safer: 2006–8: a review,” *British journal of anaesthesia,* vol. 107, no. 2, pp. 127-132, 2011.

[93] L. T. Nyfløt, I. Sandven, B. Stray-Pedersen, S. Pettersen, I. Al-Zirqi, M. Rosenberg, A. F. Jacobsen and S. Vangen, “Risk factors for severe postpartum hemorrhage: a case-control study,” *BMC pregnancy and childbirth,* vol. 17, no. 1, pp. 1-9, 2017.

[94] S. Wray, “Insights into the uterus,” *Experimental physiology,* vol. 92, no. 4, pp. 621-631, 2007.

[95] M. Baggish, “Anatomy of the uterus,” in *Hysteroscopy: Visual perspectives of uterine anatomy, physiology and pathology*, London, Lippincott Williams&Wilkins, 2007.

[96] S. Wray, T. Burdyga, D. Noble, K. Noble, L. Borysova and S. Arrowsmith, “Progress in understanding electro-mechanical signalling in the myometrium,” *Acta Physiologica,* vol. 213, no. 2, pp. 417-431, 2015.

[97] M. Muñoz, J. Stensballe, A.-S. Ducloy-Bouthors, M.-P. Bonnet, E. De Robertis, I. Fornet, F. Goffinet, S. Hofer, W. Holzgreve, S. Manrique and J. Nizard, “Patient blood management in obstetrics: prevention and treatment of postpartum haemorrhage. A NATA consensus statement,” *Blood Transfusion,* vol. 17, no. 2, p. 112, 2019.

[98] I. Al-Zirqi, S. Vangen, L. Forsén and B. Stray-Pedersen, “Effects of onset of labor and mode of delivery on severe postpartum hemorrhage,” *American journal of obstetrics and gynecology,* vol. 201, no. 3, pp. 273-e1, 2009.

[99] B. T. Bateman, M. F. Berman, L. E. Riley and L. R. Leffert, “The epidemiology of postpartum hemorrhage in a large, nationwide sample of deliveries,” *Anesthesia & Analgesia,* vol. 110, no. 5, pp. 1368-1373, 2010.

[100] M. Driessen, M.-H. Bouvier-Colle, C. Dupont, B. Koshnood, R.-C. Rudigoz and C. Deneux-Tharaux, “Postpartum hemorrhage resulting from uterine atony after vaginal delivery: factors associated with severity,” *Obstetrics and gynecology,* vol. 117, no. 1, p. 21, 2011.

[101] G. C. Smith, Y. Cordeaux, I. R. White, D. Pasupathy, H. Missfelder-Lobos, J. P. Pell, D. S. Charnock-Jones and M. Fleming, “The effect of delaying

childbirth on primary cesarean section rates," *PLoS medicine,* vol. 5, no. 7, p. e144, 2008.

[102] A. K. Agten, D. Passweg, S. von Orelli, N. Ringel, R. Tschudi and B. Tutschek, "Temporal trends of postpartum haemorrhage in Switzerland: a 22-year retrospective population-based cohort study," *Swiss Medical Weekly,* vol. 45, 2017.

[103] A. Briley, P. T. Seed, G. Tydeman, H. Ballard, M. Waterstone, J. Sandall, L. Poston, R. M. Tribe and S. Bewley, "Reporting errors, incidence and risk factors for postpartum haemorrhage and progression to severe PPH: a prospective observational study," *BJOG: An International Journal of Obstetrics & Gynaecology,* vol. 121, no. 7, pp. 876-888, 2014.

[104] J. Zeitlin, A. Mohangoo, M. Delnord, M. Cuttini and E.-P. S. Committee, "The second European Perinatal Health Report: documenting changes over 6 years in the health of mothers and babies in Europe," *J Epidemiol Community Health,* vol. 67, no. 12, pp. 983-985, 2013.

[105] M. Tikkanen, "Placental abruption: epidemiology, risk factors and consequences," *Acta obstetricia et gynecologica Scandinavica,* vol. 90, no. 2, pp. 140-149, 2010.

[106] J. F. von Schmidt auf Altenstadt, C. W. Hukkelhoven, J. van Roosmalen and K. W. Bloemenkamp, "Pre-eclampsia increases the risk of postpartum haemorrhage: a nationwide cohort study in the Netherlands," *PLoS One,* vol. 8, no. 12, 2013.

[107] L. Han, X. Liu, H. Li, J. Zou, Z. Yang, J. Han, W. Huang, L. Yu, Y. Zheng and L. Li, "Blood coagulation parameters and platelet indices: changes in normal and preeclamptic pregnancies and predictive values for preeclampsia," *PLoS One,* vol. 9, no. 12, 2014.

[108] L. M. Dusse, D. R. Rios, M. B. Pinheiro, A. J. Cooper and B. A. Lwaleed, "Pre-eclampsia: relationship between coagulation, fibrinolysis and inflammation," *Clinica Chimica Acta,* vol. 412, no. 1-2, pp. 17-21, 2011.

[109] B. C. Young, R. J. Levine and S. A. Karumanchi, "Pathogenesis of preeclampsia," *Annual Review of Pathology: Mechanisms of Disease,* vol. 5, pp. 173-192, 2010.

[110] M. T. Tanjung, H. D. Siddik, H. Hariman and S. C. Koh, “Coagulation and fibrinolysis in preeclampsia and neonates,” *Clinical and applied thrombosis/hemostasis,* vol. 11, no. 4, pp. 467-473, 2005.

[111] M. Pinheiro, K. Gomes and L. Dusse, “Fibrinolytic system in preeclampsia,” *Clinica chimica acta,* vol. 416, pp. 67-71, 2013.

[112] M. Flood, S. J. McDonald, W. Pollock, F. Cullinane and M.-A. Davey, “Incidence, trends and severity of primary postpartum haemorrhage in Australia: A population-based study using Victorian Perinatal Data Collection data for 764 244 births,” *Australian and New Zealand Journal of Obstetrics and Gynaecology,* vol. 59, no. 2, pp. 228-234, 2019.

[113] S. Ononge, F. Mirembe, J. Wandabwa and O. M. Campbell, “Incidence and risk factors for postpartum hemorrhage in Uganda,” *Reproductive health,* vol. 13, no. 1, pp. 1-7, 2016.

[114] M. Sato, E. Kondoh, T. Iwao, S. Hiragi, K. Okamoto, H. Tamura, H. Mogami, Y. Chigusa, T. Kuroda, M. Mandai and I. Konishi, “Nationwide survey of severe postpartum hemorrhage in Japan: an exploratory study using the national database of health insurance claims,” *The Journal of Maternal-Fetal & Neonatal Medicine,* vol. 32, no. 21, pp. 3537-3542, 2019.

[115] W. M. Callaghan, E. V. Kuklina and C. J. Berg, “Trends in post- partum hemorrhage: United States, 1994–2006,” *American journal of obstetrics and gynecology,* vol. 202, no. 4, pp. 353-e1, 2010.

[116] M. S. Kramer, C. Berg, H. Abenhaim, M. Dahhou, J. Rouleau, A. Mehrabadi and K. Joseph, “Incidence, risk factors, and temporal trends in severe postpartum hemorrhage,” *American journal of obstetrics and gynecology,* vol. 209, no. 5, pp. 449-e1, 2013.

[117] J. Ford, C. Roberts, J. Simpson, J. Vaughan and C. Cameron, “Increased postpartum hemorrhage rates in Australia,” *International Journal of Gynecology & Obstetrics,* vol. 98, no. 3, pp. 237-243, 2007.

[118] J. Lutomski, B. Byrne, D. Devane and R. Greene, “Increasing trends in atonic postpartum haemorrhage in Ireland: an 11-year population-based cohort study,” *BJOG: An International Journal of Obstetrics & Gynaecology,* vol. 119, no. 3, pp. 306-314, 2012.

[119] J. Tachil and T. Cheng-Hock, “Disseminated intravascular coagulation in obstetric disorders and its acute haematological management,” *Blood reviews,* vol. 23, no. 4, pp. 167-176, 2009.

[120] P. Collins, R. Abdul-Kadir and J. Thachil, “Management of coagulopathy associated with postpartum hemorrhage: guidance from the SSC of the ISTH,” *Journal of Thrombosis and Haemostasis: JTH,* vol. 14, no. 1, pp. 205-210, 2015.

[121] M. Cortet, C. Deneux-Tharaux, C. Dupont, C. Colin, R. Rudigoz, M. Bouvier-Colle and C. Huissoud, “Association between fibrinogen level and severity of postpartum haemorrhage: secondary analysis of a prospective trial,” *British journal of anaesthesia,* vol. 108, no. 6, pp. 984-989, 2012.

[122] L. De Lloyd, R. Bovington, A. Kaye, R. E. Collis, R. Rayment, J. Sanders, A. Rees and P. W. Collin, “Standard haemostatic tests following major obstetric haemorrhage,” *International journal of obstetric anesthesia,* vol. 20, no. 2, pp. 135-141, 2011.

[123] P. W. Collins, G. Lilley, D. Bruynseels, D. B.-S. Laurent, R. Cannings-John, E. Precious, V. Hamlyn, J. Sanders, R. Alikhan, R. Rayment and A. Rees, “Fibrin-based clot formation as an early and rapid biomarker for progression of postpartum hemorrhage: a prospective study,” *Blood, The Journal of the American Society of Hematology,* vol. 124, no. 11, pp. 1727-1736, 2014.

[124] H. McNamara and S. Mallaiah, “Managing coagulopathy following PPH,” *Best Practice & Research Clinical Obstetrics & Gynaecology,* vol. 61, pp. 106-120, 2019.

[125] A. Gillissen, T. Van Den Akker, C. Caram-Deelder, D. D. Henriquez, K. W. Bloemenkamp, J. J. Van Roosmalen, J. Eikenboom and J. G. Van Der Bom, “Association between fluid management and dilutional coagulopathy in severe postpartum haemorrhage: a nationwide retrospective cohort study,” *BMC pregnancy and childbirth,* vol. 18, no. 1, pp. 1-9, 2018.

[126] OBS Cymru Quality and Safety Sub Group of Maternity Network Wales, “All Wales Guideline - Prevention and Management of Postpartum Haemorrhage,” NHS Wales, 2017.

[127] S. Bell, R. Collis, C. Bailey, K. James, M. John, K. Kelly, T. Kitchen, C. Scarr, E. Macgillivray and P. Collins, “The incidence, aetiology, and coagulation

management of massive postpartum haemorrhage: a two-year national prospective cohort study," *International Journal of Obstetric Anesthesia,* vol. 47, 2021.

[128] S. Bell, R. Collis and P. Collins, "Comparison of haematological indices and transfusion management in severe and massive postpartum haemorrhage: analysis of a two-year national prospective observational study," *International Journal of Obstetric Anesthesia,* vol. 50, 3033.

[129] N. M. de Lange, M. D. Lancé, R. de Groot, E. A. Beckers, Y. M. Henskens and H. C. Scheepers, "Obstetric hemorrhage and coagulation: an update. Thromboelastography, thromboelastometry, and conventional coagulation tests in the diagnosis and prediction of postpartum hemorrhage," *Obstetrical & gynecological survey,* vol. 67, no. 7, pp. 426-435, 2012.

[130] C. Oliver, J. Freyer, M. Murdoch, L. De Lloyd, P. Jenkins, R. Collis and P. Collins, "A description of the coagulopathy characteristics in amniotic fluid embolism: a case report," *International Journal of Obstetric Anesthesia,* vol. 51, 2022.