Haemostasis during pregnancy, labour and postpartum haemorrhage

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Cover illustration: TEG® profile in a woman with normal bleeding postpartum and in a woman with estimated blood loss 2500 mL.

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ABSTRACT

Background: Haemostatic disorders are common in obstetric complications and may result in more severe complications if not detected. There is limited knowledge about viscoelastic methods, fibrinogen and Factor XIII and how they are related to each other during pregnancy and postpartum haemorrhage. The aims of this thesis were (I) to obtain knowledge about physiological changes in thromboelastography (TEG®) variables and how they relate to haemostatic laboratory methods during normal pregnancy and 8 weeks postpartum, (II) to describe changes in Factor XIII activity, fibrinogen concentration, platelet count and their respective associations to clot strength during normal pregnancy, (III) to compare TEG® and laboratory analyses during major obstetric haemorrhage and (IV) to assess whether fibrinogen concentration at admission, before labour, is associated with severe postpartum haemorrhage.

Methods: In two prospective observational studies, TEG® and haemostatic laboratory analyses were studied longitudinally during normal pregnancy and postpartum. In one prospective study, the same methods were used during postpartum haemorrhage. Finally, fibrinogen concentration was determined before delivery and postpartum in order to assess whether there was any association to bleeding postpartum.

Results: TEG® demonstrated increased coagulability and decreased fibrinolysis during pregnancy. Factor XIII activity and platelet count were lower during pregnancy, while fibrinogen concentration was higher. Clot strength was higher and correlated with fibrinogen concentration and platelet count, but not with Factor XIII activity. During major obstetric haemorrhage (>2000 mL), impaired haemostasis was demonstrated with both TEG® and laboratory analyses. TEG® provided faster results, advantageous in the setting of ongoing obstetric haemorrhage. Fibrinogen concentration did not decrease during normal labour. Fibrinogen concentration at admission, before labour, did not predict the severity of postpartum haemorrhage. Excessive postpartum bleeding was mainly due to obstetric complications.

Conclusion: During normal pregnancy, increased coagulability and decreased fibrinolysis were observed. During postpartum haemorrhage, haemostasis was rapidly impaired. Prepartal fibrinogen concentration did not predict bleeding postpartum. Monitoring haemostasis in cases of obstetric complications is fundamental for providing good obstetric care.

Keywords: pregnancy, labour, postpartum haemorrhage, thromboelastography, haemostatic laboratory analyses, fibrinogen, Factor XIII

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SAMMANFATTNING PÅ SVENSKA

Hemostas under graviditet och förlossning samt vid blödningskomplikation.

Bakgrund: Rubbningar i hemostasen (blodstillning och upplösning av koagel) är vanliga vid förlossningskomplikationer och kan bidra till allvarligare förlopp om de inte upptäcks. Det finns begränsad kunskap om viskoelastiska metoder (tromboelastografi), fibrinogen (faktor I), faktor XIII och deras relation till varandra under graviditet och förlossning samt vid blödningskomplikationer. Syftet med denna avhandling var (I) att få kunskap om tromboelastografi (viskolelastisk metod) och dess relation till hemostatiska laboratorieanalyser under normal graviditet och 8 veckor efter förlossningen, (II) att beskriva förändringar hos faktor XIII aktivitet, fibrinogen koncentration och antalet blodplättar och deras relation till styrkan hos blodkoaglet under normal graviditet, (III) att vid stor förlossningsblödning jämföra tromboelastografi och laboratorieanalyser för att bedöma hemostasen, (IV) att undersöka om fibrinogen koncentrationen vid ankomst till förlossningen påverkar storleken på blödningen i samband med förlossning.

Metoder: I två observationsstudier har vi följt tromboelastografi och hemostatiska laboratorieanalyser under normal graviditet och förlossning. I den tredje studien har vi använt samma metoder för att studera hemostasen vid blödningskomplikation efter förlossningen. I den sista studien studerade vi om fibrinogenkoncentrationen vid ankomsten till förlossningen hade någon association till blödning efter förlossning.

Resultat: Tromboelastografi visade en ökad koagulationsförmåga och en sänkt förmåga att lösa upp koaglet under graviditet. Faktor XIII aktivitet och antalet blodplättar var lägre under graviditeten medan fibrinogen koncentrationen var högre. Styrkan av blodkoaglet var starkare och korrelerade med fibrinogen koncentration och med antalet blodplättar men inte med faktor XIII aktivitet. Under stor förlossningsblödning (>2000 ml) visade både tromboelastografi och laboratorieanalyser att hemostasen är försämrad. Tromboelastografi visade resultaten snabbare, vilket är en fördel vid pågående stor förlossningsblödning. Fibrinogen koncentrationen sjönk inte under normal förlossning. Fibrinogen koncentrationen vid ankomst till förlossningen förutsa inte storleken på blödningen efter förlossningen. Stora blödningar i samband med förlossning är i huvudsak orsakade av förlossningskomplikationer.

Slutsats: Hemostasen genomgår stora förändringar under graviditet och förlossning, i form av ökad koagulationsförmåga och minskad upplösningsförmåga av koaglet. Vid förlossningskomplikationer, t.ex. blödning, försämras blodstillningsförmågan avsevärt. Fibrinogen före förlossningen förutsäger inte storleken på blödning efter förlossning. Kunskap om dessa förändringar är viktiga och kontroll av hemostasen vid komplikationer är grundläggande för god och säker vård av den nyblivna mamman.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. Karlsson O, Sporrong T, Hillarp A, Jeppsson A, Hellgren M.
Prospective longitudinal study of Thromboelastography and standard hemostatic laboratory tests in healthy women during normal pregnancy.
Anesth Analg 2012;115:890-8.

II. Karlsson O, Jeppsson A, Hellgren M.
A longitudinal study of Factor XIII activity, fibrinogen concentration, platelet count and clot strength during normal pregnancy.
Thromb Res 2014;134:750-752

III. Karlsson O, Jeppsson A, Hellgren M.
Major obstetric haemorrhage: monitoring with thromboelastography, laboratory analyses or both?
Int J Obstet Anesth 2014;23:10-17.

IV. Karlsson O, Jeppsson A, Thornemo M, Lafrenz H, Rådström M, Hellgren M.
Fibrinogen plasma concentration before delivery is not associated with postpartum haemorrhage: a prospective observational study.
Submitted

CONTENT

ABBREVIATIONS

AFLP	acute fatty liver of pregnancy
APC	activated protein C
APTT	activated partial thromboplastin time
AT	antihrombin
BMI	body mass index
CI	confidence interval
EBL	estimated blood loss
F	Factor
GP	glycoprotein
GW	gestational week
Hb	haemoglobin
HELLP	haemolysis-elevated liver enzymes-low platelet syndrome
INR	international normalized ratio
MOH	major obstetric haemorrhage
PAI-1	plasminogen activator inhibitor 1
PAI-2	plasminogen activator inhibitor 2
PPH	postpartum haemorrhage
PT	prothrombin
SD	standard deviation
TEG	thromboelastography
TEM	thromboelastometry
TF	tissue factor
t-PA	tissue plasminogen activator
u-PA	urokinase plasminogen activator
VWF	von Willebrand Factor

1 INTRODUCTION

Haemostatic changes are pronounced during pregnancy and the puerperium [1-4], especially during obstetric complications such as postpartum haemorrhage (PPH), thromboembolism, preeclampsia, haemolysis-elevated liver enzymes-low platelet syndrome (HELLP) and acute fatty liver of pregnancy (AFLP) [5-7]. PPH is a common cause of morbidity and mortality in the obstetric population [8, 9]. However, there is limited knowledge about viscoelastic methods and how they are related to haemostatic laboratory analyses (e.g. fibrinogen concentration and Factor XIII (FXIII) activity) during pregnancy and PPH [10-13]. Knowledge of haemostasis and how it changes is fundamental in the care of patients with obstetric complications, including PPH [14].

1.1 Haemostasis

Normal haemostasis is a series of complex processes aimed at maintaining blood flow to the tissues and instantly reacting to vascular injury by sealing the vessel wall defect. These processes involve endothelial cells, platelets, clotting factors and inhibition of coagulation and fibrinolysis, all to promote the right balance and appropriate location of clot formation in the injured vessels [15, 16]. A small part of the processes are described in Figure 1.

Haemostasis is traditionally divided into three stages:

- Primary haemostasis
- Blood coagulation (secondary haemostasis)
- Fibrinolysis

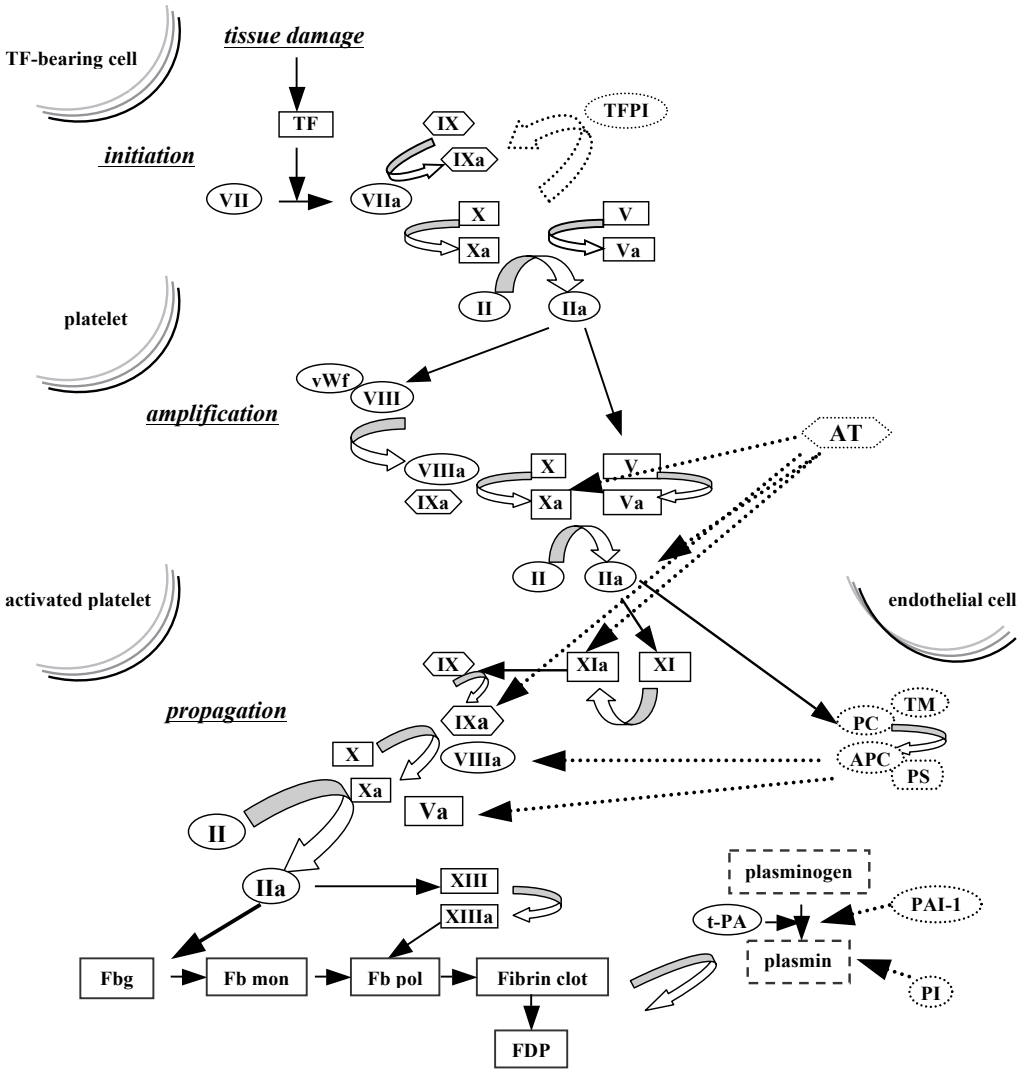

Figure 1. Simplified presentation of coagulation and fibrinolysis. Full arrow - activation or stimulation, dotted arrow – inhibition or degradation, TF – tissue factor, TFPI – tissue factor pathway inhibitor, AT – antithrombin, VWF – von Willebrand factor, IIa – thrombin, Fbg – fibrinogen, Fb mon – fibrin monomer, Fb pol – fibrin polimer, TM – thrombomodulin, PC – protein C, PS – protein S, t-Pa – tissue plasminogen activator, PAI-1 – plasminogen activator inhibitor, PI – plasmin inhibitor, FDP – fibrin degradation products. With permission from Aleksandra Antovic, Karolinska University Hospital.

1.1.1 Primary haemostasis

The final step for primary haemostasis is the formation of a platelet plug (Figure 2). Primary haemostasis includes vasoconstriction, platelet adhesion, platelet activation and platelet aggregation [15].

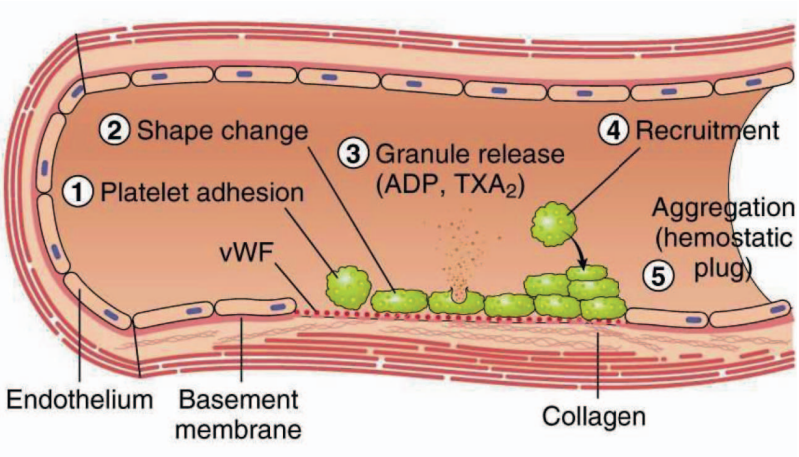

Figure 2. Primary haemostasis: vWF = von Willebrand Factor, ADP = Adenosine diphosphate, TXA2 = Thromboxane A2. Free download from MBBS Medicine.

The endothelium contains factors that inhibit and promote haemostatic reactions [17]. Vascular injury leads to vasoconstriction and exposure of sub-endothelial collagen, to which platelets bind, directly by platelet surface receptors (glycoprotein VI (GPVI) and integrin $\alpha_2\beta_1$), and indirectly by von Willebrand factor (VWF) and GPIb. The platelets are activated in connection with platelet adhesion [18]. Their shape changes, new receptors mobilize and granules (dense bodies and alfa-granules) release active substances. Dense bodies release, among other substances, thromboxane, serotonin and ADP, while VWF, Factor V (FV), FXIII and fibrinogen are among the substances released by the alfa-granules. More platelets become activated and more platelets aggregate by the action of GPIIb/IIIa receptors, together with fibrinogen, forming a platelet plug. However, the newly formed plug is unstable and must be stabilized.

1.1.2 Blood coagulation

T*he final step in blood coagulation is formation of a stable fibrin clot (Figure 3).* Traditionally, blood coagulation has been divided into extrinsic and intrinsic pathways; this division does not, however, occur in vivo. Instead, modern literature describes an initiation phase and a propagation phase in blood coagulation in vivo [15]. All circulating coagulation factors are inactive, except a small amount of active Factor VII (FVIIa).

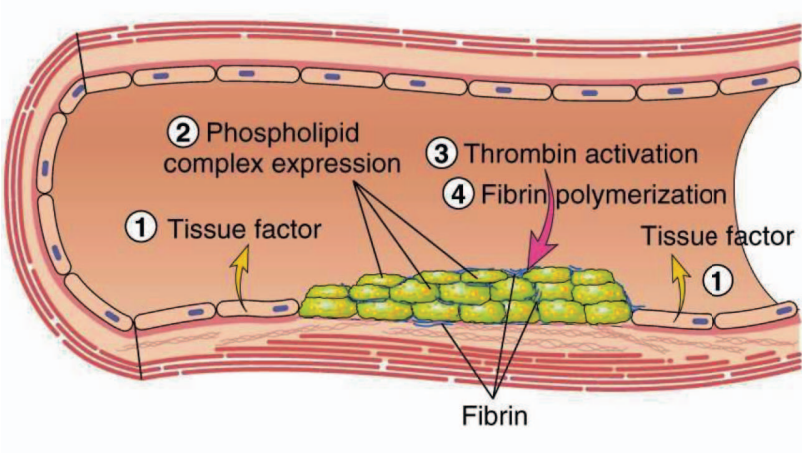

Figure 3. Blood coagulation, free download from MBBS Medicine.

The initiation phase starts when tissue factor (TF), an endothelial membrane protein, significantly potentiates FVIIa activity on contact with this factor [19]. The FVIIa/TF complex activates Factor IX (FIX). FIXa activates Factor X (FX), which, together with Factor V (FV), activates a small amount of prothrombin (Factor II) to thrombin (FIIa).

During the propagation phase, the small amount of thrombin activates and amplifies the coagulation process on the activated platelets' surface in the platelet plug. Thrombin formation is accelerated by positive feedback, in which it activates FV, Factor VIII (FVIII) and Factor XI (FXI), through FIX and FX, resulting in a burst of thrombin activity [20]. Finally, thrombin activates fibrinogen, forming fibrin. However, the fibrin strands are unstable and are stabilized by FXIII, which generates covalent bonds between fibrin γ-chains [21, 22]. The platelet plug then becomes a stable clot.

1.1.3 Fibrinogen

Fibrinogen (Factor I) plays a critical role in achieving haemostasis during haemorrhage, but also acts as an acute phase protein [15]. Fibrinogen (Figure 4) is a glycoprotein, synthesized in the liver, with a mean half-life of 3.7 days (range 3.0-4.1 days) [23]. It is a dimer consisting of three pairs of polypeptide chains [24]. During coagulation, thrombin cleaves fibrinogen to fibrin monomers that form a network of fibrin molecules. These fibrin strands are unstable until FXIII stabilizes them, as mentioned above. The stability of the fibrin network depends on fibrinogen concentration, fibrin network architecture and FXIII-induced cross-linking [15, 25].

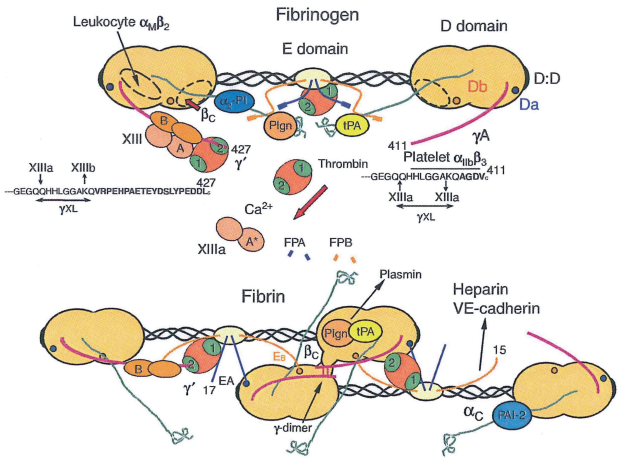

Figure 4. Fibrinogen, with permission from Journal of Thrombosis and Haemostasis.

There are several assays for measuring fibrinogen levels in plasma. Most laboratories use and recommend the Clauss method [26], with a non-pregnant reference range of 2.0-4.5 g/L and coefficients of variation of 7% at 2 g/L and of 5% at 3 g/L. The Clauss method is a functional assay based upon the time of fibrin clot formation. The immunological assay measures fibrinogen antigen rather than functional fibrinogen. In patients with dysfibrinogenaemias, there is a discrepancy between functional and antigen levels [27]. Studies with thromboelastography and thromboelastometry have shown associations between fibrinogen concentrations with their clot strength variables [28].

Fibrinogen has been studied since the mid-nineteenth century. During the twentieth century, it has been used to treat bleeding, but interest in the substance declined due to the product sometimes was contaminated [29]. Newer drugs have shown good efficacy [30, 31]. During pregnancy, the fibrinogen concentration increases progressively and remains high for approximately two weeks after delivery.

Later studies have reported associations between low fibrinogen postpartum and severe PPH [32-35]. Further on, low preoperative plasma levels of fibrinogen have been associated with increased bleeding in both cardiac and spinal surgery [36, 37]. However, it is unclear whether the reduced fibrinogen concentration after the onset of PPH is the result of a low endogenous fibrinogen concentration, existing already before labour, or of consumption, bleeding and/or haemodilution. Studies of non-obstetric bleeding have shown that fibrinogen is the first clotting factor to decrease to critically low levels [38, 39]. While congenital fibrinogen deficiency is very rare, there are individuals with fibrinogen concentrations <0.01 g/L, usually identified shortly after birth due to severe bleeding complications [40].

1.1.4 Factor XIII

FXIII has several functions, including stabilizing the fibrin clot, wound healing, maintaining pregnancy and interactions with inflammatory cells and the complement system [15, 21, 22, 41-44]. FXIII is a tetramer consisting of two A and two B subunits [22]. Subunit A, produced in cells of bone marrow origin, is the active part, whereas subunit B, synthesized in the liver, is the carrier for subunit A. The subunits form tetrameric complexes in circulating blood. The plasma FXIII complex concentration is 14-28 mg/L [22]. Mean half live about 9-10 days [45]. In plasma, all FXIII molecules are bound to fibrinogen. Thrombin initiates FXIII activation. Activated FXIII, cross-links fibrin chains into a three-dimensional insoluble fibrin network and incorporates anti-fibrinolytic proteins, which protect the clot from premature degradation by the fibrinolytic system [21, 42].

A number of different assays are available. In our studies, FXIII activity was determined with a chromogenic assay (non-pregnant reference range 0.70-1.40 kIU/L, coefficients of variation 8% at 0.4 kIU/L and 6% at 1.0 kIU/L). There are conflicting data about FXIII changes during normal pregnancy in healthy women; both increased and decreased levels have been reported [46-50]. Studies have also shown that non-pregnant patients with

unexplained intra-operative bleeding have lower FXIII activity and that trauma patients with haemorrhages consume FXIII [51, 52]. FXIII activity during PPH is unclear. Hereditary FXIII deficiency increases the risk of PPH, placental abruption and spontaneous abortion [42, 53, 54]. A FXIII activity of about 5% is sufficient to control bleeding in hereditary FXIII deficiency. In newborns with hereditary FXIII deficiency, the symptoms often start with bleeding from the umbilical stump.

1.1.5 Inhibition of coagulation

Several factors support haemostasis by inhibition, limiting blood coagulation to the injured vessels and preventing thromboembolic complications [15]. The most important inhibitors are antithrombin and protein C, including its cofactor protein S.

Antithrombin has several important properties, including inhibition of coagulation and anti-inflammatory activity [55-57]. Together with heparan sulphate and other glucosaminoglycans in vivo, and with heparin during treatment, antithrombin blocks thrombin and activated factors that circulate freely in blood vessels. This prevents coagulation in non-injured blood vessels and limits thrombin activation to the location of the injury.

Protein C activates when free thrombin binds to thrombomodulin, an endothelial cell receptor. Activated protein C (APC) and its cofactor protein S inactivate FVa and FVIIIa, which subsequently inhibit the production of thrombin. In addition to anticoagulation, APC also has other properties, including anti-inflammatory and barrier-protective effects [58].

1.1.6 Fibrinolysis

The final step of fibrinolysis is to dissolve the fibrin clot (Figure 5). Tissue plasminogen activator (t-PA) and urokinase plasminogen activator (u-PA) activate plasminogen to form plasmin [15]. Plasminogen circulates freely in plasma, binding to fibrin on the clot. Activation results in local fibrinolytic activity adjacent to the clot. When fibrin dissolves, a number of different fragments form (e.g. D-dimers).

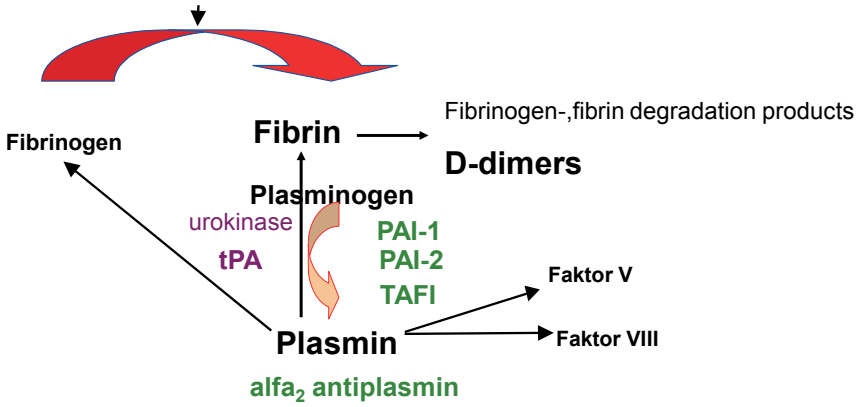

Figure 5. Fibrinolysis, t-Pa - tissue plasminogen activator, PAI-1 – plasminogen activator inhibitor 1, PAI-2 – plasminogen activator inhibitor 2, TAFI – thrombin activatable fibrinolysis inhibitor.

Fibrinolysis inhibitors, e.g. plasminogen activator inhibitor 1 (PAI-1), plasminogen activator inhibitor 2 (PAI-2) and antiplasmin, provide protection from uncontrolled fibrinolysis. During pregnancy, trophoblasts in the placenta produce PAI-2. PAI-1 and PAI-2 inhibit conversion of plasminogen to plasmin and antiplasmin inhibits plasmin [59]. Tranexamic acid, a pro-haemostatic drug, inhibits fibrinolysis by preventing the activation of plasminogen to plasmin.

1.2 Haemostasis during pregnancy

Haemostasis becomes significantly altered during pregnancy; estrogen causes most of the factors to increase [1-4, 60-62]. Nature thus reduces the risk of bleeding during childbirth, unfortunately also increasing the risk of thromboembolic complications.

Some investigators have noted a decrease in platelet count, whereas others have noted no change. Seven percent of pregnant women develop gestational thrombocytopenia ranging from 70 to 150 x 10^9/L [63]. Postpartum, there is a reactive increase in platelet count and normalization occurs within two months postpartum. Most coagulation factors increase,

including fibrinogen, FVII, FVIII, FIX, FX and Factor XII (FXII). FII and FV remain unchanged, while FXI and FXIII decrease. The coagulation inhibition factors change; antithrombin declines slightly but remains within the non-pregnant reference range, protein C will be unchanged while protein S decreases by about 50%.

Plasminogen increases, but PAI-1 and PAI-2 increase more, resulting in decreased fibrinolysis [64]. Together, the changes in haemostasis increase coagulation and decrease fibrinolysis, resulting in a hypercoagulable state, most likely entailing decreased risk of bleeding but increased risk of thromboembolic complications.

1.3 Postpartum haemorrhage

International studies, report an increasing trend in PPH [65]. Unfortunately, there are heterogeneous definitions of PPH; the frequency varies between 2% and 12%, depending on the study and definition. Studies suggest that some reports underestimate the frequency of PPH, which may be a fatal complication and which is one of the most common causes of maternal mortality worldwide [8]. In Europe, PPH is the most common cause of severe maternal morbidity and a common reason for intensive care [8, 9, 66].

During pregnancy, blood and plasma volume increase by about 40-50%. The erythrocyte amount rises less (20%), resulting in physiological anaemia. At the end of pregnancy, blood flow to the uterus is about 600-700 mL/min. If it fails to contract postpartum, blood loss may amount to 3500 mL within five minutes. Normal blood loss is <600 mL during labour and <1000 mL during caesarean section. A pregnant woman can bleed about 1000 mL during labour without any effect on her circulation.

PPH is usually caused by uterine atony and placental retention. Other causes are cervical and vaginal lacerations, uterine rupture, placental abruption, placenta previa, placenta accreta and congenital or acquired haemostatic disorders [67-69]. Immediate care, consisting of several simultaneous interventions (Figure 6), is fundamental in order to reduce the risk of maternal morbidity and mortality [70-77].

Immediate care for major obstetric haemorrhage (MOH) includes:

- ABCDE resuscitation, including compression of the aorta
- Pharmacological treatment of uterine atony, including oxytocin, methylergometrine, carboprost and misoprostol
- Obstetric surgical treatment, e.g. manual exploration of the uterus and vagina, insertion of a balloon tamponade, explorative laparotomy, hysterectomy and, if possible, angiographic embolization
- Haemostatic monitoring and treatment
- For optimal haemostasis during ongoing MOH, treatment goals should be: haemoglobin (Hb) >90 g/L, platelet count >100 x 10^9/L, prothrombin time PT(INR) <1.5, activated partial thromboplastin time (APTT) normal, fibrinogen >2.0-2.5, body temperature >36.5C, ionized Ca^{2+} >1.0 and pH >7.2.

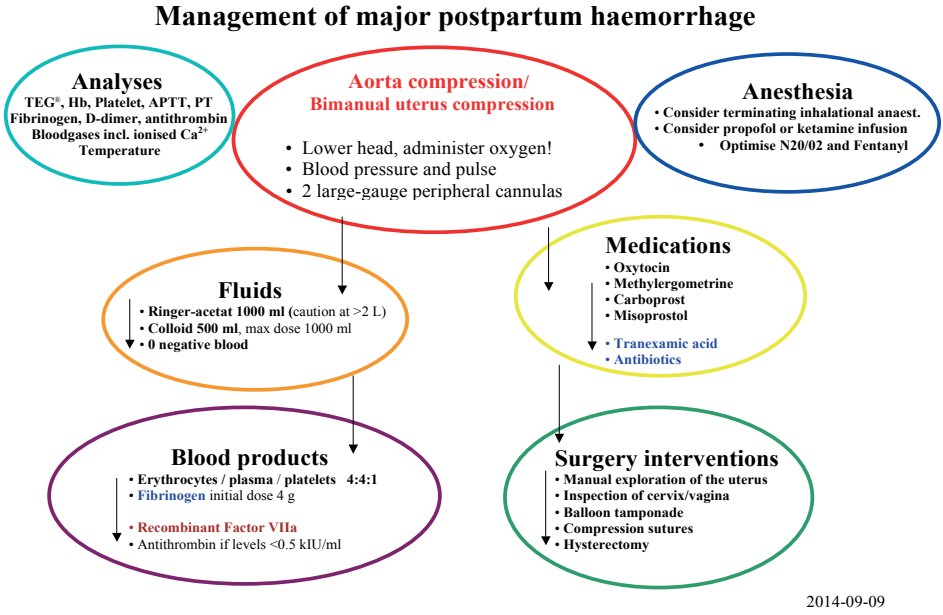

Figure 6. Algorithm care during postpartum haemorrhage

1.4 Monitoring of haemostasis

Haemostatic status can be assessed by laboratory analyses and/or by point-of-care devices [16, 78, 79]. Blood samples should be taken immediately when coagulopathy is suspected or when the woman has lost half her blood volume, at the latest. Sampling must be repeated regularly, due to rapid changes in haemostasis, due to bleeding per se, consumption or haemodilution [80-82].

Laboratory analyses are the most common assessment method but they do, however, usually require time for transportation, analysis and reporting back results. Haemostasis assessment by point-of-care devices is especially suitable in perioperative settings (cardiac surgery, liver surgery and obstetrics) and in intensive care units [83]. No time is required for transportation and, depending on the device, the results are available faster. Nevertheless, point-of-care devices require training of the staff who perform the analyses and interpret results. The devices must also be checked regularly, according to the manufacturer's instructions.

1.4.1 Laboratory analyses

Platelet count, APTT, PT(INR), fibrinogen, D-dimer and antithrombin are frequently used to assess haemostatic function [16].

- **Platelet count**: Reference range 165-387 x 10^9/L. An automatic method that measures the number of platelets. Increased risk of bleeding is rare at platelet count above 50 x 10^9/L and becomes more common particularly below 20 x 10^9/L, and especially below 10 x 10^9/L.
- **APTT**: Reference range 30-42 s. Coagulation time in plasma with reagents without TF. Measures the overall activity of fibrinogen and Factors II, V, VIII, IX, XI and XII. APTT is prolonged when the activity (< 25-30%) of any of these coagulation factors is significantly reduced.
- **PT(INR):** Reference range <1.2. Coagulation time in plasma with reagents, including the activator thromboplastin, and plasma, including FV and fibrinogen. Measures the overall activity of Factors II, VII and X (vitamin K-dependent factors). In congenital deficiency of Factors II, VII or X, PT(INR) is often around 1.3-1.5. Although FIX is vitamin K-dependent, PT(INR) does not measure FIX activity.

- **Fibrinogen**: Reference range 2.0-4.5 g/L. The method can yield false high levels if colloid is given. APTT can be normal or near the upper reference range despite fibrinogen < 1g/L. Since fibrinogen is an acute phase protein, levels > 5 g/L are common after surgery and during infection, as well as in pregnancy, especially in cases of preeclampsia.
- **D-dimer:** Reference range 0-0.5 mg/L. D-dimer represents fibrinolytic degradation products of fibrin. Although D-dimer is a result of fibrinolytic activity, it is nonetheless a primary marker of increased coagulation as crosslinked fibrin is necessary in order to develop D-dimers. D-dimer is increased during pregnancy [84].
- **Antithrombin**: Reference range 0.8 – 1.2 kIU/L. The method is based on reagents, including cofactor heparin and Factors X or II. Individuals with low levels do not have increased bleeding tendency but are at increased risk of thromboembolism.

1.4.2 Point-of-care devices

Different instruments can be located near the patient to analyse different aspects of haemostasis and give the ability to detect deterioration of haemostasis early [85-88]. The most common are thromboelastography (Figure 6), thromboelastometry and other viscoelastic instrument (e.g. ReoRox®) [89] and several instruments for assessing PT(INR).

Figure 7. Thromboelastography TEG®, with permission from Gothia Medical.

1.4.3 Thromboelastography (TEG®)

The method follows the viscoelastic changes during clot formation, providing data about clot formation, physical strength and stability and fibrinolysis [86]. There are limitations of TEG® analyses, including absence of flow dynamics and pharmaceutical platelet inhibition. One mL of native whole blood is gently mixed with kaolin (activator) and 360 µL of this mixture is pipetted into a pre-warmed cup (37°C). In the cup, a pin is connected to a detector system, and as fibrin forms between the cup and the pin, the cup's movements are transmitted to the pin and a trace is generated (Figure 8).

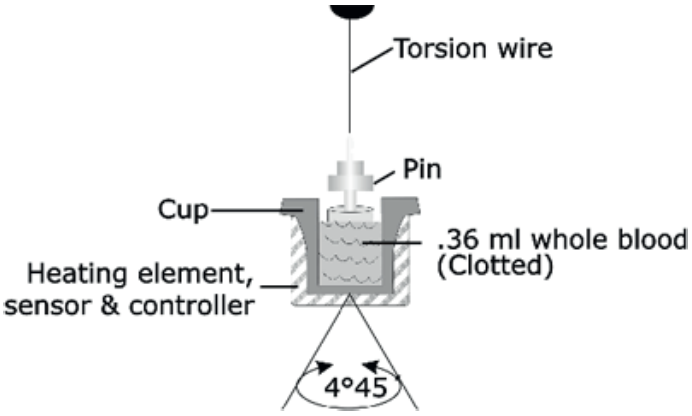

Figure 8. Thromboelastography principle, with permission from Gothia Medical.

Native whole blood samples should be assessed four minutes after being drawn. Kaolin speeds up the analysis and reduces running time by as much as half. Citrated samples are used if it is difficult to transport native whole blood to the instrument within four to six minutes. Citrated samples should be analyzed within two hours, but the recommendation is to standardize the analysis at a fixed time, e.g. 15 minutes.

TEG® variables (Figure 9)

- R (TEG®-R): Reaction time, time from the start of a sample run until the first significant level of detectable clot formation (2 mm amplitude)
- K (TEG®-K): K-time, time from R until a fixed level of clot firmness (20 mm amplitude)
- α (TEG®-α): Alpha angle, measures the kinetics of clot formation
- MA (TEG®-MA): Maximum amplitude, represents the ultimate strength of the fibrin clot
- LY30 (TEG®-LY30): Lysis at 30 minutes after MA, represents clot lysis

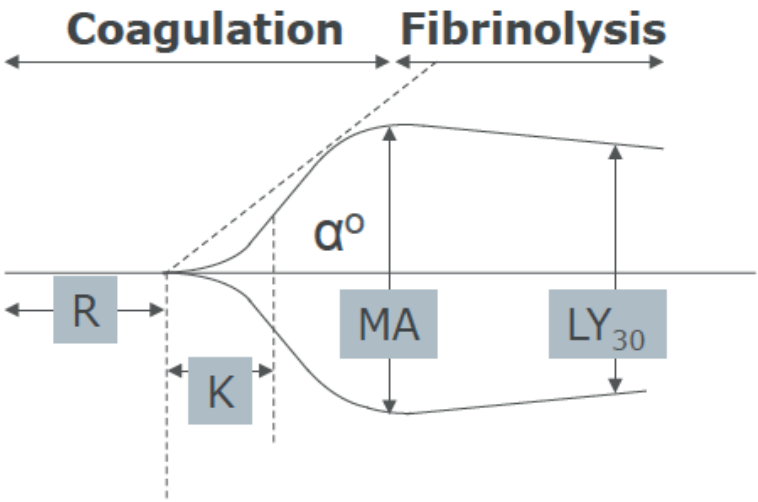

Figure 9. TEG® variables: R (TEG®-R) = Reaction time, K (TEG®-K) = K-time, α (TEG®- α) = Alpha angle, MA (TEG®-MA) = Maximum amplitude, LY30 (TEG®-LY30) = Lysis at 30 minutes, with permission from Gothia Medical.

Laboratory test	Value
INR	1.1
Platelets (× 10^9/L)	137
Fibrinogen (mg/dL)	396
Reaction time (min)	4.2
Kinetic time (min)	1.4
α-angle (degrees)	70.5
Maximum amplitude (mm)	60.4
Ly-30 (%)	0.8

Laboratory test	Value
INR	1.3
Platelets (× 10^9/L)	21
Fibrinogen (mg/dL)	174
Reaction time (min)	3.5
Kinetic time (min)	3.8
α-angle (degrees)	57.0
Maximum amplitude (mm)	36.8
Ly-30 (%)	0.6

Laboratory test	Value
INR	1.8
Platelets (× 10^9/L)	36
Fibrinogen (mg/dL)	118
Reaction time (min)	4.7
Kinetic time (min)	12.2
α-angle (degrees)	38.1
Maximum amplitude (mm)	25.2
Ly-30 (%)	0.0

Laboratory test	Value
INR	1.9
Platelets (× 10^9/L)	8
Fibrinogen (mg/dL)	88
Reaction time (min)	4.8
Kinetic time (min)	NA
α-angle (degrees)	23.6
Maximum amplitude (mm)	16.3
Ly-30 (%)	0.0

Figure 10. Different traces of TEG®, with permission from Gastroenterology & Hepatology.

1.4.4 Thromboelastometry (ROTEM®)

Like TEG®, this method also monitors the viscoelastic changes during clot formation. In the ROTEM® system, the pin rotates and the cup detects the viscoelastic changes during clot formation. The same variables are measured but have other names. Clot strength had the tightest correlation when TEG® and ROTEM® were compared [90]. An advantage of the ROTEM® system is that it is possible to operate with four channels simultaneously, allowing assessment of different coagulation process pathways at the same time, for example INTEM. EXTEM, HEPTEM and FIBTEM. FIBTEM assesses the functional level of fibrin. Recently, peri-partum reference ranges was published for ROTEM® variables [91].

ROTEM® variables

- CT: Clotting time
- CFT: Clot formation time
- α: Alpha angle
- MCF: Maximum clot firmness
- LI: Lysis index 30

2 AIM

The overall aim of this thesis were to improve our knowledge of haemostasis during pregnancy and postpartum, as well as in cases of obstetric complications, in order to improve obstetric care and reduce maternal morbidity and mortality.

The specific aims were:

- To describe changes in TEG® variables during normal pregnancy and 8 weeks postpartum (Paper I).
- To describe standard laboratory coagulation analyses during normal pregnancy and 8 weeks postpartum and to evaluate whether there are any correlations between these analyses and TEG® variables (Paper I).
- To describe FXIII activity during normal pregnancy and 8 weeks postpartum (Paper II).
- To describe the association between FXIII activity, fibrinogen concentration and platelet count during normal pregnancy and 8 weeks postpartum, and their relations to clot strength and bleeding volume during delivery (Paper II).
- To describe haemostasis during major obstetric haemorrhage, using TEG® variables and traditional laboratory analyses, in comparison with deliveries with normal postpartum blood loss (Paper III).
- To assess whether there are any correlations between TEG® variables, laboratory analyses and estimated blood loss during major obstetric haemorrhage (Paper III).
- To evaluate whether there is an association between fibrinogen concentration at admission to the labour ward and the severity of postpartum haemorrhage (Paper IV).
- To determine fibrinogen concentration before and after labour and to identify predictors for severe postpartum haemorrhage (Paper IV).

3 PATIENTS AND METHODS

3.1 Participants

The Regional Ethical Review Board in Gothenburg, Sweden, approved all studies. Written informed consent was obtained from all participants.

Paper I

Forty-five healthy Caucasian women with normal pregnancies were included in this prospective longitudinal study. Normal pregnancy was defined as the absence of obstetric complications, such as preeclampsia, other placental complications, gestational diabetes or bleeding complications. Demographic and obstetric data are shown in Table 1.

Table 1. Demographic and obstetric data in 45 healthy women during normal pregnancy and 8 weeks postpartum

Age (years)	Median	30.0
	Range	21-40
BMI (kg/m^2)	Median	23.5
(body mass index)	Range	17.9 - 32.3
Nulliparous, n		24
Parous, n		21
Vaginal delivery, n		39
Cesarean section, n	Emergency	3
	Planned	3
Bleeding at delivery, ml	Median	350
	Range	200-2600
Bleeding > 600 ml, n		8

Paper II

Forty-four healthy Caucasian women with normal pregnancies, from the study population reported in Paper I. Saved frozen plasma samples provided us with the opportunity to perform additional analyses.

Paper III

Forty-five women with major obstetric haemorrhage (MOH), estimated blood loss (EBL) ≥2000 mL, and 49 women with normal bleeding, EBL <600 mL, were included in this prospective observational study. MOH was secondary to placental retention (n=17), caesarean section (n=14), uterine atony (n=6), uterine rupture (n=2), placenta previa (n=2), cervical or vaginal lacerations (n=2), placental abruption (n=1) or placenta accreta (n=1). Patient characteristics are shown in Table 2.

Table 2. Patient characteristics

	Controls (n=49)	MOH all (n=45)	MOH 2-3L (n=35)	MOH >3L (n=10)
Age (years)	30.5 ± 5.0 [20-39]	32.2 ± 4.7 [21-46]	32.7 ± 5.1 [21-46]	30.1 ± 2.4 [26-33]
Body mass index (kg/m^2)	24.0 ± 3.6 [16-33]	25.3 ± 4.0 [18-37]	25.1 ± 4.2 [18-37]	26.0 ± 3.5 [20-31]
Nulliparous	24	17	13	4
Singleton pregnancy	49	42	33	9
Vaginal/Caesarean delivery	49/0	25/20	19/16	6/4
Hb at TEG® sampling (g/dL)	122 [97-145]	92 [68-120]	91 [68-117]	101.5 [72-120]
EBL at TEG® sampling(mL)	400 [200-600]	2500 [2000-3700]	2410 [2000-2900]	3300 [3000-3700]
EBL total (mL)	400 [200-600]	2650 [2000-7300]	2500 [2000-7300]	3450 [3050-4000]

Data are mean ± SD, median [range] or number, MOH: major obstetric haemorrhage; Hb: haemoglobin; EBL: estimated blood loss

Paper IV

Two thousand women were recruited at five delivery units in Västra Götaland, Sweden, in this prospective observational study. The final study group consisted of 1951 women, after exclusion of 49 women without documented social security numbers. Some fibrinogen concentration results were missing, due to haemolysis (n=23), coagulated sample (n=7) or missing data (n=78). The final analyses of fibrinogen concentration and its associations with PPH thus included 1843 women. Eighty of the women at one of the delivery units were included in a substudy with a second blood sample. The participants' characteristics are presented in Table 3.

Table 3. Participants' characteristics and outcome variables

Characteristic	All women n = 1951	Women with fibrinogen samples before and after delivery, n = 80
Age, years	30.6 (5.0)	31.3 (5.3)
Body mass index, kg/m^2	24.9 (4.7)	23.1 (3.4)**
Parity 0, n	948 (48.6)	49 (61.2)
1, n	529 (27.1)	21 (26.2)
2, n	199 (10.2)	7 (8.8)
3, n	53 (2.7)	1 (1.2)
≥ 4, n	26 (1.3)	0 (0)
Missing data, n	196 (10.0)	2 (2.5)
Gestational week at delivery	40.1 (27.7-43.3)	40.3 (34.4-42.3)
Fibrinogen, g/L	5.34 (0.83) 5.30 (2.90-8.80)	5.34 (0.82) 5.30 (2.90-8.80)
Preeclampsia, n	60 (3.1)	0 (0%)
Spontaneous labour, n	1530 (78.4)	73 (91.2)
Induction of labour, n	417 (21.4)	7 (8.8)*
Missing data, n	4 (0.2)	0 (0)
Oxytocin stimulation, n	1082 (55.6)	39 (51.3)
Epidural analgesia, n	864 (44.4)	42 (52.5)
Vaginal delivery, n	1605 (82.3)	67 (83.8)
Instrumental delivery, n	136 (7.0)	8 (10.0)
Caesarean delivery, n	206 (10.6)	5 (6.2)
Missing data, n	4 (0.2)	0 (0)
Postpartum exploration, n	93 (4.8)	3 (3.8)
Estimated blood loss, mL	450 (70-4400)	450 (150-2180)

Data are mean (SD), median (range) or number (%).
Group with two samples compared to the remaining group: * $p<0.01$, ** $p<0.001$

3.2 Methods

The TEG® method is described in the Introduction section. Laboratory analysis methods for all studies are shown in Table 4. Blood loss at delivery and postpartum was estimated by the delivery midwife by weighing surgical sponges and pads and measuring collected blood.

Table 4. Laboratory methods

Analysis	Non-pregnant reference interval	Laboratory method
Platelet count	165-387 x 10^9/L	CellDyn Sapphire (Abbott, Illinois, USA)
Activated partial thromboplastin time	30-42s	STA®-APTT/Automate 5 (Diagnostica Stago, Asnières sur Seine, France)
Prothrombin time	PT (INR) <1.2	Stago Prothrombin Complex Assay (Diagnostica Stago, Asnières sur Seine, France)
Fibrinogen	2.0-4.5 g/L	Clauss method, Fibri-Prest Automate (Diagnostica Stago, Asnières sur Seine, France)
Factor XIII	0.70-1.40 kIU/L	BCS® XP analyser, Siemens Healthcare Diagnostics, Skärholmen, Sweden
Antithrombin	0.80-1.20 kIU/L	Chromogenic substrate method, STAchrome ATIII (Diagnostica Stago, Asnières sur Seine, France)
Soluble fibrin	<9 mg/L	Automated latex-enhanced immunoturbidimetric assay, LPIA-Iatro (Mitsubishi Kagaku Iatron inc, Tokyo, Japan)
D-dimer	0-0.5 mg/L	Quantitative latex agglutination assay, STA-Liatest® D-DI (Diagnostica Stago, Asnières sur Seine, France)

Paper I

The women were monitored regularly by midwives during pregnancy. Blood was sampled at gestational weeks 10-15, 20-22, 28-30 and 38-40 and at 8 weeks postpartum. The results at 8 weeks postpartum were used as non-pregnant references. Blood was sampled between 8 and 12 AM, after a 15-minute rest. The first portion was discarded. The following TEG® variables were assessed: TEG®-R, TEG®-K, TEG®-Angle, TEG®-MA and TEG®-LY-30. The following laboratory analyses were assessed: platelet count, APTT, PT(INR), antithrombin, soluble fibrin and D-dimer.

The course of the pregnancy and EBL at delivery, were obtained from the electronic patient records (Obstetrix, Siemens AB, Healthcare Sector, Upplands Väsby, Sweden). The TEG® variables and laboratory analysis results during pregnancy were compared with those at 8 weeks postpartum and levels during later pregnancy were compared with those at gestational weeks 10-15. The TEG® variables were also correlated to laboratory analysis results.

Paper II

The women were monitored regularly and blood sampling was performed. The course of the pregnancy and EBL at delivery had already been recorded for Paper 1, as had platelet count and clot strength (TEG®-MA). FXIII activity and fibrinogen concentration were assessed in the saved frozen plasma samples.

FXIII activity, fibrinogen concentration and platelet count were compared with 8 weeks postpartum and levels during later pregnancy were compared with those at gestational weeks 10-15. Correlations between FXIII activity, fibrinogen concentration, platelet count, clot strength and bleeding during delivery were calculated.

Paper III

Women with MOH were brought to the operating theatre, if not already there because of caesarean section. Blood tests were taken when EBL ≥2000 mL. Patients were treated according to local guidelines. Depending on the amount of bleeding, treatment included crystalloids, colloids, blood products and tranexamic acid. The following TEG® variables were assessed: TEG®-R, TEG®-K, TEG®-Angle, TEG®-MA and TEG®-LY30. The following laboratory analyses were assessed: platelet count, APTT, PT(INR), fibrinogen, antithrombin, and D-dimer.

In women with uncomplicated delivery and EBL < 600 mL, blood was sampled within 2-6 hours postpartum at the labour ward. TEG® variables and laboratory analyses in this group without complications were compared with the corresponding results in the women with MOH. Correlations between TEG® variables, laboratory analyses and EBL were calculated.

Patient characteristics, such as age, body mass index (BMI), parity, diagnosis and EBL at delivery were obtained from the electronic patient

records (Obstetrix, Siemens AB, Healthcare Sector, Upplands Väsby, Sweden).

Paper IV

Venous blood was sampled after arrival at the labour ward. In the substudy of 80 women, the second blood sample was collected immediately after the placenta was delivered. All fibrinogen concentrations in plasma were analyzed with the same batches of reagents and the same instrument.

Patient characteristics, such as age, BMI, parity, gestational age at delivery, epidural analgesia, diagnosis and EBL at delivery were obtained from the electronic patient records (Obstetrix, Siemens AB, Healthcare Sector, Upplands Väsby, Sweden). Correlations between fibrinogen concentration and EBL were calculated. Predictors for severe PPH were identified.

3.3 Statistics

Paper I

The number of women to be included in the study was based on data from previous longitudinal studies of haemostatic laboratory variables, during different periods of pregnancy and postpartum.

The longitudinal analysis was performed with a mixed effects model, in which subject is considered to be a random effect. The mixed procedure was used to estimate effects over time. Because there was no a priori design indicating when to terminate the study, 99% confidence intervals were used and Dunnett's method was applied to control the family-wise error rate of the multiple comparisons of trimester measurements with the baseline assessments and the non-pregnant state, respectively. Correlations between TEG® variables and laboratory analyses were assessed by Pearson's correlation. All statistical analyses were performed with SAS, version 9.2 (SAS Institute Inc, Cary, NC).

Paper II

The longitudinal analysis of the data was performed with a mixed effects model, in which subject is considered to be a random effect. Dunnett's method was applied to control the family-wise error rate of the multiple comparisons of trimester measurements with the baseline assessments and the non-pregnant state, respectively.

Correlations between FXIII, fibrinogen concentration, platelet, clot strength and EBL were assessed by Pearson's correlation. All statistical analyses were performed with SPSS version 21 (IBM, New York, USA).

Paper III

The number of women to be included in each group was based on data from our previous studies of haemostatic laboratory variables during different periods of pregnancy.

The unpaired student's t-test was used for group comparisons. Variables were assumed to be normally distributed. For sub-analysis, the MOH group was further divided into MOH 2-3 L and MOH > 3 L. Correlations were assessed by Pearson's correlation. All statistical analyses were performed with SPSS version 19 (IBM, NY, USA).

Paper IV

Calculation showed that 2000 subjects were needed to achieve 80% power and a significance level of 0.05 with multiple logistic regression, to detect a significant interaction between low/high fibrinogen levels and vaginal/caesarean delivery regarding the probability of severe bleeding, as well as to control for dropouts [92].

For comparison between two groups, the Mann-Whitney U-test was used for continuous variables. All correlations were assessed by Spearman's correlation coefficient. Changes over time were compared using the Wilcoxon Signed Rank test. In order to select bivariate predictors of EBL > 1000 mL, binary logistic regression analysis was performed.

In order to select independent predictors of EBL > 1000 mL, all significant bivariate predictors were included into a stepwise multiple regression analysis. The results of the logistic regression analyses were given as odds ratios with 95% confidence intervals (CI) and p-values. All descriptive and statistical analyses were performed with Statistica 12 (StatSoft, OK, USA) or SAS Software version 9.3 (SAS Institute NC, USA).

4 RESULTS

Paper I

Prospective longitudinal study of thromboelastography and standard hemostatic laboratory tests in healthy women during normal pregnancy

The TEG® variables showed an increase in blood coagulation and a decrease in fibrinolysis during pregnancy, compared to 8 weeks postpartum. There were no or weak correlations between TEG® variables and laboratory analyses.

Changes in TEG® variables during pregnancy, compared to 8 weeks postpartum

TEG®-R was 23-26% shorter until gestational weeks 28-30, but not at gestational weeks 38-40. TEG®-K was 18-35% shorter, TEG®-Angle was 12-20% wider and TEG®-MA was 6-8% higher, throughout pregnancy in all three variables. TEG®-LY30 was 67-73% lower from gestational weeks 28-30 and onward. The TEG® variables are shown in Table 5 and Figure 11.

Changes in TEG® variables during later pregnancy, compared to gestational weeks 10-15

TEG®-R was 23% longer at gestational weeks 38-40, TEG®-K was 26% longer at gestational weeks 38-40 and TEG®-Angle was 7% decreased at gestational weeks 38-40. TEG®-MA was higher during early pregnancy and remained thus throughout pregnancy. TEG®-LY30 was decreased by 72% at gestational weeks 38-40, compared to early pregnancy.

Table 5. TEG® variables in healthy women during normal pregnancy and 8 weeks postpartum

TEG	Gestational weeks	10-15	20-22	28-30	38-40	8 weeks' post partum
	Women, n	45	43	42	38	44
R, min	Mean	6.8	7.2	7.1	8.4	9.2
	99%CI	6.1–7.6	6.5- 8.0	6.4- 7.9	7.6- 9.2	8.5- 10.0
	Median	6.9	7.3	7.2	8.0	8.4
	Range	3.7–13.2	2.8–11.1	3.2–12.4	5.7–13.7	4.1–16.2
K, min	Mean	1.8	1.9	2.0	2.2	2.7
	99 % CI	1.5-2.0	1.6-2.2	1.7-2.2	1.9-2.5	2.4-3.0
	Median	1.7	1.9	1.8	1.9	2.6
	Range	0.9–4.5	0.9–2.9	1.2–4.3	1.4–4.1	1.6–5.4
Angle, degree	Mean	65.9	63.6	63.9	61.6	54.7
	99% CI	63.2-68.7	60.8-66.4	61.1-66.8	58.6-64.5	51.9-57.5
	Median	66.2	63.1	65.2	62.2	54.8
	Range	41.8–79.3	50.9–78.1	42.7–73.3	45.9–72.2	34.5–68.0
MA, mm	Mean	67.5	66.9	68.2	68.5	63.6
	99% CI	65.9-69.2	65.2-68.6	66.5-70.0	66.7-70.3	62.0-65.3
	Median	66.7	66.6	68.2	68.0	63.3
	Range	61.3–82.8	46.3–76.3	58.0–77.7	62.5–76.2	54.3–77.0
LY30	Mean	1.0	0.7	0.4	0.3	1.2
	99% CI	0.5-1.5	0.2-1.2	-0.1- 0.9	-0.2- 0.9	0.8-1.7
	Median	0.5	0.1	0.1	0.0	0.6
	Range	0.0–3.3	0.0–5.6	0.0–3.5	0.0–3.1	0.0–9.5

R = time until fibrin formation, K = time until amplitude of 20 mm, Angle = angle of clotting, MA = maximum amplitude, LY30 = percent of lysis at 30 minutes

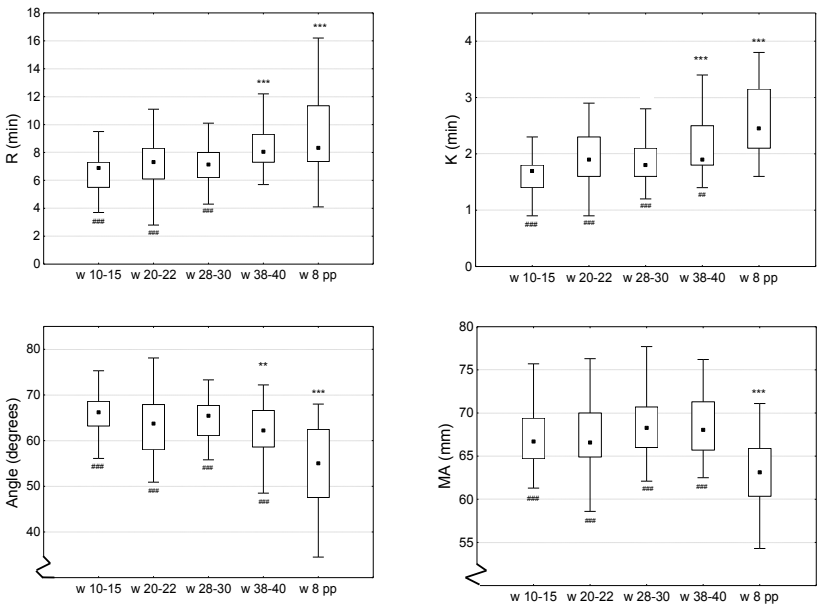

*Figure 11. Thromboelastographic variables during pregnancy (gestational weeks 10-15, 20-22, 28-30 and 39-40) and 8 weeks postpartum. Box-whisker plot with median, 25%-75% percentile, minimum and maximum. R=time until fibrin formation, K=time until amplitude of 20 mm, Angle=rate of clot growth, MA=maximum amplitude. * = p<0.05, ** = p<0.01, *** = p<0.001 versus 10-15 weeks. # = p<0.05, ## = p<0.01, ### = p<0.001 versus 8 weeks postpartum*

Changes in laboratory analysis results during pregnancy, compared to 8 weeks postpartum

Platelet count was 12-21% lower during pregnancy, except at gestational weeks 20-22. APTT was 6-9% shorter throughout pregnancy. PT(INR) was 18-31% lower from gestational weeks 20-22 and onward, but was unaltered at gestational weeks 10-15. Antithrombin was 6-9% lower throughout pregnancy. Results are shown in Table 6.

Changes in laboratory analysis results during later pregnancy, compared to gestational weeks 10-15

Platelet count, APTT and antithrombin did not change further, compared to early pregnancy. PT(INR) was additionally 17-31% lower during later pregnancy.

Table 6. Laboratory analyses in healthy women during normal pregnancy and 8 weeks postpartum,

Laboratory analyses	Gestational weeks	10-15	20-22	28-30	38-40	8 weeks' post partum
	Women, n	43	44	43	37	44
APTT, s	Mean	32.0	32.0	31.5	31.2	34.2
	99% CI	31.0-33.1	31.1-33.1	30.5-32.5	30.2-32.3	33.2-35.3
	Median	32.0	32.0	31.0	30.0	33.5
	Range	25–38	28–39	27–39	27–37	29–46
PT(INR)	Mean	1.0	0.8	0.8	0.7	1.0
	99% CI	0.9-1.0	0.7-0.9	0.7-0.8	0.6-0.8	0.9-1.1
	Median	1.0	0.9	0.9	0.9	1.0
	Range	0.5–1.3	0.5–1.1	0.5–1.1	0.5–1.0	0.5–1.2
Platelet count $x10^9$/L	Mean	265	271	263	237	300
	99% CI	241-289	246-295	239-288	212-262	276-325
	Median	272	272	260	233	290
	Range	170–417	154–554	158–438	152–390	208–512
AT kIU/L	Mean	0.97	0.98	1.01	0.99	1.07
	99% CI	0.93-1.01	0.95-1.02	0.97-1.05	0.95-1.03	1.03-1.11
	Median	0.96	1.00	1.01	0.98	1.08
	Range	0.81–1.21	0.82–1.22	0.84–1.23	0.84–1.34	0.90–1.27
D-dimer mg/L	Mean	0.4	0.7	0.8	1.4	0.4
	99% CI	0.2-0.7	0.4-0.9	0.6-1.1	1.1-1.7	0.1-0.6
	Median	0.3	0.5	0.7	1.0	0.2
	Range	0.1–3.0	0.1–3.2	0.2–3.2	0.3–5.6	0.1–3.2
Soluble fibrin mg/L	Mean	4.6	7.7	7.1	8.3	4.8
	99% CI	0.7-8.5	4.0-11.3	3.5-10.8	4.5-12.1	1.2-8.5
	Median	2.3	4.0	4.2	5.8	3.6
	Range	0.4–49.6	0.0–56.4	0.0–48.4	1.8–48.1	0.2–15.1

APTT = activated partial thromboplastin time, PT = prothrombin time, INR = International Normalized Ratio, AT = Antithrombin

Soluble fibrin, D-dimer and TEG®-LY30

Soluble fibrin exhibited marked inter-individual variation and the changes were not significant. D-dimer was 140-297% increased during gestational weeks 28-30 and 38-40, compared to 8 weeks postpartum. Soluble fibrin, D-dimer and TEG®-LY30 levels are shown in Figure 12.

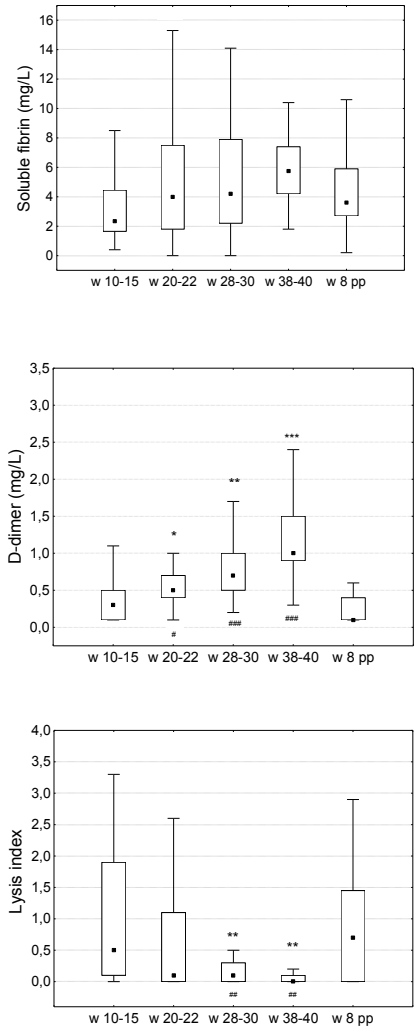

*Figure 12. Soluble fibrin, D-dimer and Lysis index (TEG®-LY30) during pregnancy (gestational weeks 10-15, 20-22, 28-30 and 38-40) and 8 weeks postpartum. Box-whisker plots with median (square), 25% - 75% percentile, minimum and maximum. * = p<0.05, ** = p<0.01, *** = p<0.001 versus 10-15 weeks, # = p<0.05, ## = p<0.01, ### = p<0.001 versus 8 weeks postpartum.*

Paper II

A longitudinal study of factor XIII activity, fibrinogen concentration, platelet count and clot strength during normal pregnancy

FXIII activity and platelet count were lower, while fibrinogen concentration was higher, during normal pregnancy, compared to 8 weeks postpartum. The resulting clot strength was nonetheless higher during pregnancy, compared to 8 weeks postpartum.

Changes in laboratory analysis results during pregnancy, compared to 8 weeks postpartum

FXIII activity was 6-18% lower from gestational weeks 20-22 and onward. Fibrinogen concentration was 27-58% higher and platelet count was 10-21% lower throughout pregnancy. Clot strength (TEG®-MA) was 5-8% higher throughout pregnancy. Changes and other data concerning FXIII activity, fibrinogen concentration, platelet count and clot strength are shown in Table 7 and Figure 13.

Table 7. Factor XIII, fibrinogen, platelets and TEG®-MA in healthy women during normal pregnancy and at 8 weeks postpartum

Analysis	GW 10-15	GW 20-22	GW 28-30	GW 38-40	8 weeks postpartum
Factor XIII (kIU/L)	1.17±0.20	1.06±0.19*$^{###}$	0.91±0.18***$^{###}$	0.91±0.23***$^{###}$	1.12±0.21
Fibrinogen (g/L)	4.1±0.8***	4.3±0.8***	4.6±1.0***$^{##}$	5.1±0.9***$^{###}$	3.2±0.5$^{###}$
Platelet (x 10^9/L)	266±57**	274±76**	265±62***	243±59***$^{##}$	301±64$^{##}$
TEG-MA (mm)	67.5±4.2***	66.9±5.2***	68.1±3.7***	68.6±3.9***	63.6±4.4$^{###}$

Data are mean ± SD. TEG®-MA: maximum amplitude, GW: gestational weeks
Mean compared to 8 weeks postpartum: * $p<0.05$, ** $p<0.01$, *** $p<0.001$
Mean compared to gestational weeks 10-15: ## $p<0.01$, ### $p<0.001$

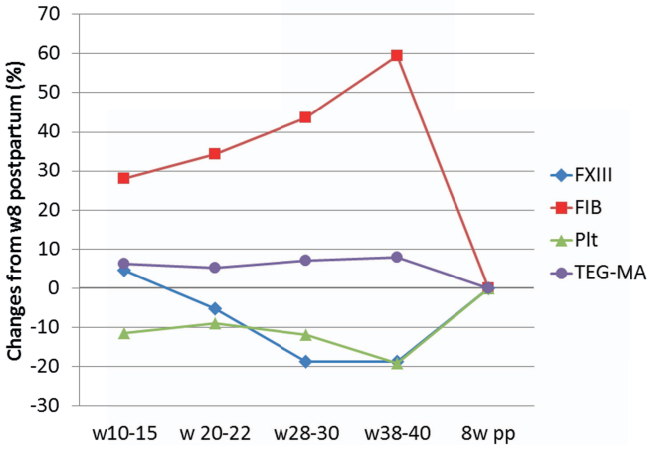

Figure 13. Changes during normal pregnancy in Factor XIII activity, fibrinogen concentration, platelet count and clot strength (TEG®-MA) compared to 8 weeks postpartum. FXIII = factor XIII, FIB = fibrinogen, Plt = platelet, TEG®-MA = maximum amplitude with thromboelastography.

Changes in laboratory analysis results during later pregnancy, compared to gestational weeks 10-15

FXIII activity was 11-22% additionally decreased and fibrinogen concentration was 12-25% additionally increased at gestational weeks 28-30 and 38-40. Platelet count was 11% additionally decreased at gestational weeks 38-40. Clot strength (TEG®-MA) was already higher at all testing occasions and did not change significantly during later pregnancy.

Correlations

There were several significant correlations between clot strength (TEG®-MA) and fibrinogen concentration and platelet count, but not between clot strength and FXIII activity. In addition, there were significant correlations between FXIII activity and fibrinogen concentration at all time-points, although FXIII activity decreased and fibrinogen concentration increased throughout pregnancy. Correlations are shown in Table 8.

Table 8. Correlations between Factor XIII, fibrinogen, platelets and TEG®-MA and between Factor XIII and fibrinogen in healthy women during normal pregnancy and at 8 weeks postpartum

Gestational weeks		Factor XIII/ TEG-MA	Fibrinogen/ TEG-MA	Platelet/ TEG-MA	Factor XIII/ Fibrinogen
10-15	r	0.17	0.32	0.38	0.42
	p	0.32	0.06	0.01	0.01
20-22	r	0.25	0.36	0.26	0.53
	p	0.15	0.03	0.10	0.001
28-30	r	0.20	0.12	0.16	0.43
	p	0.26	0.50	0.31	0.01
38-40	r	0.13	0.54	0.34	0.46
	p	0.48	0.001	0.04	0.007
8 weeks postpartum	r	0.21	0.41	0.38	0.44
	p	0.21	0.01	0.01	0.007

TEG®-MA = maximum amplitude

Paper III

Major obstetric haemorrhage: monitoring with thromboelastography, laboratory analyses or both?

In women with EBL ≥ 2000 mL, TEG® variables and laboratory analyses showed impaired haemostasis. Laboratory analyses showed greater differences in coagulation variables and correlated better with EBL.

TEG® variables in women with MOH, compared with controls

TEG® variables reflecting clot stability and fibrinolysis were decreased in women with MOH. TEG® profiles were narrower, indicating impaired haemostasis, compared to women with EBL <600mL (Figure 14). TEG®-R was 20% shorter (p=0.002) while TEG®-K was unchanged. TEG®-Angle was 6% narrower (p=0.028) and TEG®-MA was 11% lower (p<0.0001). TEG-LY30 was reduced by 75% (p=0.003). There were no significant differences in any of the TEG® variables between women with EBL >3 L and those with EBL 2-3 L. TEG® variables are shown in Table 9.

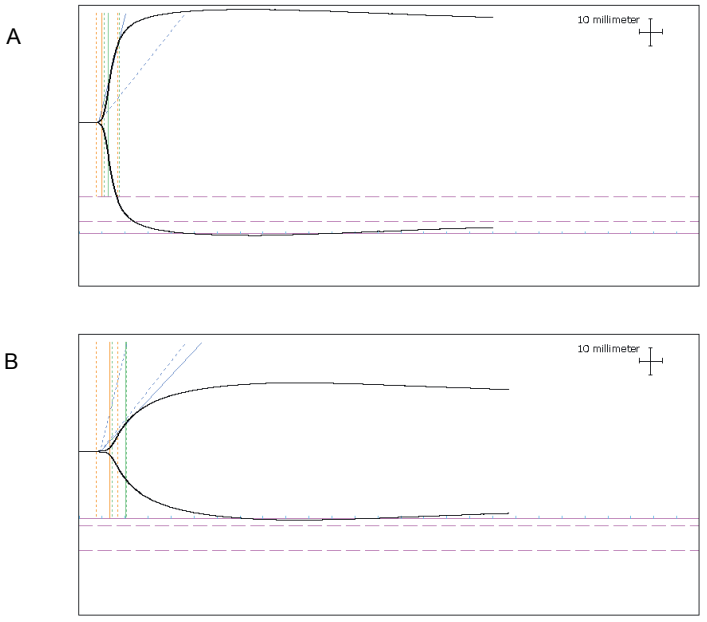

Figure 14. Two thromboelastography profiles A: TEG profile in a woman with normal bleeding postpartum. Estimated blood loss 250 mL, TEG-R 4.9 min, TEG-MA 81.4, platelets 239x109/L, fibrinogen 6.0 g/L and antithrombin 0.98kIU/L. B: TEG profile in a woman with major obstetric haemorrhage. Estimated blood loss 2500 mL. TEG-R 6.6 min, TEG.MA 48.9 mm, platelets 55x109/L, fibrinogen 1.7 g/L and antithrombin 0.37kIU/L.

Table 9. TEG® variables in women with major obstetric haemorrhage

	Controls (n=49)	MOH all (n=45)	MOH 2-3L (n=35)	MOH >3L (n=10)
R (min)	6.3 (5.8 to 6.9) [1.8–13.4]	5.1† (4.5 to 5.7) [1.3–9.6]	5.2 (4.5 to 5.9) [1.6–9.6]	4.5 (3.2 to 5.9) [1.3–6.7]
K (min)	1.8 (1.6 to 2.1) [1.0–5.3]	2.0 (1.8 to 2.2) [1.2–3.7]	2.0 (1.8 to 2.2) [1.2–3.7]	2.1 (1.6 to 2.6) [1.3–3.3]
Angle (degree)	65.2 (62.7 to 67.6) [38.6–77.1]	61.3* (58.7 to 63.8) [42.1–72.1]	61.4 (58.4 to 64.3) [42.1–72.1]	60.8 (55.0 to 66.7) [48.5–71.8]
MA (mm)	72.9 (71.4 to 74.4) [54.2–81.6]	64.8Σ (62.4 to 67.3) [38.0–79.4]	65.1 (62.2 to 68.1) [38.0–79.4]	63.8 (59.7 to 67.9) [55.4–74.6]
LY30 (%)	1.5 (0.9 to 2.2) [0–9.3]	0.4 † (0.1 to 0.7) [0–5.9]	0.4 (0.1 to 0.8) [0–5.9]	0.2 (-0.06 to 0.4) [0–0.9]

Data are mean (95% CI) and [range]. R: time start of clotting, K: time to 20 mm clot firmness, Angle: clot growth rate, MA: maximum clot amplitude, LY30: lysis 30 minutes after MA.
*P<0.05, †P<0.01, ǂP<0.001, ΣP<0.0001 compared to controls

Laboratory analysis results in women with MOH, compared to controls

Laboratory analyses showed impaired haemostasis; the most pronounced decreases were in platelet count, fibrinogen concentration and antithrombin activity. Platelet count was 36% lower ($p<0.0001$), APTT was 15% longer ($p<0.0001$), PT(INR) was increased by 12% ($p<0.0001$), fibrinogen concentration was 39% lower ($p<0.0001$), antithrombin activity was 38% lower ($p<0.0001$) and D-dimer was increased by 100% ($p<0.0001$). Fibrinogen level was below 2.5 g/L in 34%, platelet count was below 100 x 10^9/L in 16% and antithrombin was decreased to <0.5 kIU/L in 32% of the women with MOH. Analysis results are shown in Table 10 and Figure 15.

Table 10. Laboratory analyses in women with major obstetric haemorrhage

	Controls (n=49)	MOH all (n=45)	MOH 2-3L (n=35)	MOH >3L (n=10)
Platelets (x 10^9/L)	260 (242 to 278) [134–461]	168$^{\Sigma}$ (145 to 190) [55–343]	180 (154 to 205) [55–343]	128 (84 to 172) [68–285]
APTT (s)	31.4 (30.8 to 31.9) [28–36]	36.1$^{\Sigma}$ (34.7 to 37.4) [30–49]	35.5 (33.9 to 37.1) [30-49]	37.9 (35.0 to 40.8) [30-43]
INR	0.95 (0.9 to 1.0) [0.8–1.1]	1.07$^{\Sigma}$ (1.0 to 1.1) [0.8–1.4]	1.06 (1.0 to 1.1) [0.8–1.4]	1.10 (1.0 to 1.2) [0.9–1.2]
Fibrinogen (g/L)	4.8 (4.6 to 5.0) [3.6–6.7]	3.0$^{\Sigma}$ (2.7 to 3.3) [1.0–5.0]	3.1 (2.8 to 3.5) [1.0–5.0]	2.5 (1.9 to 3.0) [1.8–4.1]
Antithrombin (kIU/L)	0.96 (0.93 to 0.99) [0.73–1.19]	0.60$^{\Sigma}$ (0.54 to 0.65) [0.34–1.10]	0.62 (0.55 to 0.69) [0.34–1.10]	0.53 (0.45 to 0.61) [0.40–0.78]
D-dimer (mg/L)	4.1 (3.1 to 5.1) [1.3–21.0]	8.1$^{\Sigma}$ (6.1 to 10.2) [1.1–21.0]	8.3 (5.9 to 10.7) [1.1–21.0]	7.6 (2.8 to 12.4) [1.6–21.0]

Data are mean (95% CI) and [range] APTT: activated partial thromboplastin time; INR: international normalized ratio; $^{\Sigma}P<0.0001$ compared to controls

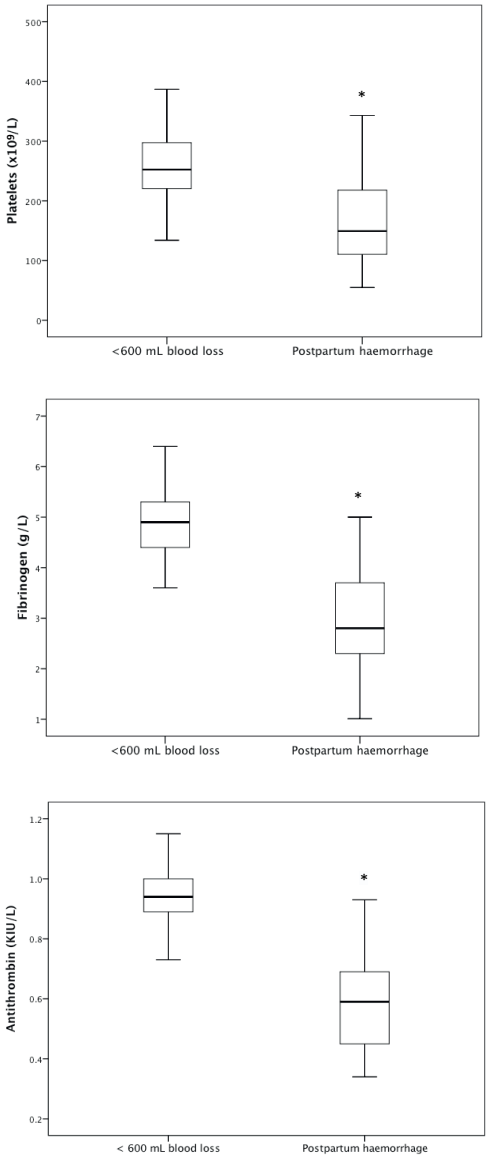

*Figure 15. Platelets, fibrinogen and antithrombin in women with normal bleeding postpartum and in women with major obstetric haemorrhage. Box-whisker pots with median, 25%-75% percentile, minimum and maximum. * p<0.0001*

Correlations

There were several correlations between TEG® variables and laboratory analyses in women with MOH, but these correlations were not found in women with EBL <600 mL. The strongest correlation between TEG® variables and laboratory analyses was found between TEG®-MA and fibrinogen concentration. EBL correlated with several TEG® variables and laboratory analyses. The strongest correlations were found between EBL and fibrinogen concentration and between EBL and antithrombin level. The correlation coefficients are shown in Table 11.

Table 11. Correlation between TEG variables, laboratory analyses and estimated blood loss in major obstetric haemorrhage

		Platelets	APTT	INR	Fibrinogen	Antithrombin	D-dimer	EBL
TEG®-R	r	-0.26	0.49	0.14	0.13	-0.08	0.20	-0.30
	P value	0.12	0.002	0.4	0.44	0.63	0.24	0.006
TEG®-K	r	-0.42	0.28	0.38	-0.39	-0.13	0.16	0.19
	P value	0.01	0.10	0.02	0.02	0.44	0.36	0.09
TEG®-Angle	r	0.53	-0.40	-0.51	0.47	0.25	-0.27	0.27
	P value	0.001	0.02	0.001	0.004	0.15	0.11	0.012
TEG®-MA	r	0.50	-0.36	-0.50	0.70	0.48	-0.34	-0.53
	P value	0.002	0.03	0.002	<0.0001	0.004	0.04	<0.0001
TEG®-LY30	r	-0.03	-0.05	-0.02	-0.15	-0.08	0.34	-0.29
	P value	0.85	0.75	0.91	0.39	0.62	0.04	0.008
EBL	r	-0.58	0.57	0.53	-0.77	-0.78	0.38	
	P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	

r: Pearson's correlation coefficient; APTT: activated partial thromboplastin time; INR: international normalized ratio; EBL: estimated blood loss

Paper IV

Fibrinogen plasma concentration before delivery is not associated with postpartum haemorrhage: a prospective observational study

Mean fibrinogen plasma concentration at admission before labour was not correlated with PPH in the entire cohort or in any subgroup. Fibrinogen concentration did not decrease significantly during normal labour. Oxytocin stimulation, instrumental delivery, caesarean section and manual exploration of the uterus postpartum were independent predictors of EBL > 1000 ml.

Fibrinogen concentration and estimated blood loss

Mean fibrinogen concentration at admission to the labour ward was 5.34 (SD 0.83, range 2.9 – 8.8) g/L. Median EBL was 450 (range 70-4400) mL. The distribution of EBL is shown in Figure 16. In the whole study group, there was no significant correlation between fibrinogen concentration before labour and EBL (r=0.003, p=0.90). The distributions of EBL as a function of fibrinogen concentrations are shown in Figure 17.

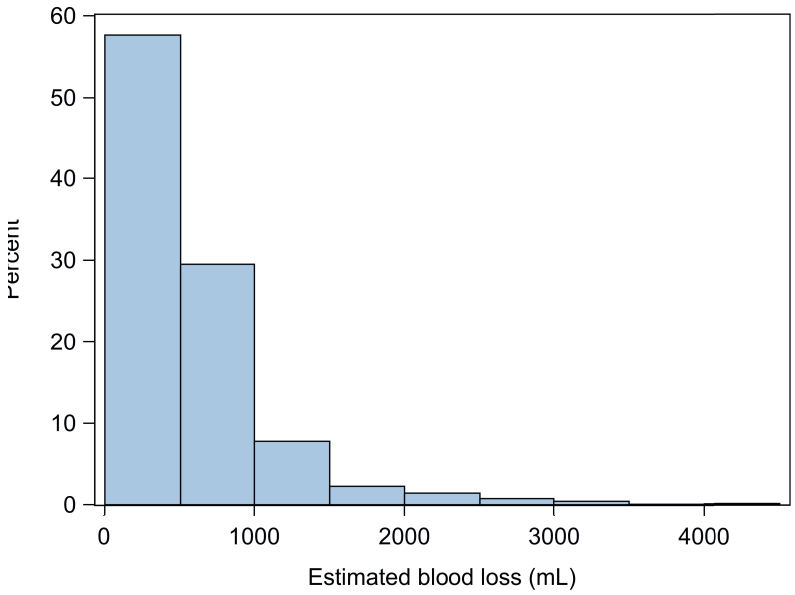

Figure 16. Distribution (%) of estimated blood loss during delivery in 1951 patients

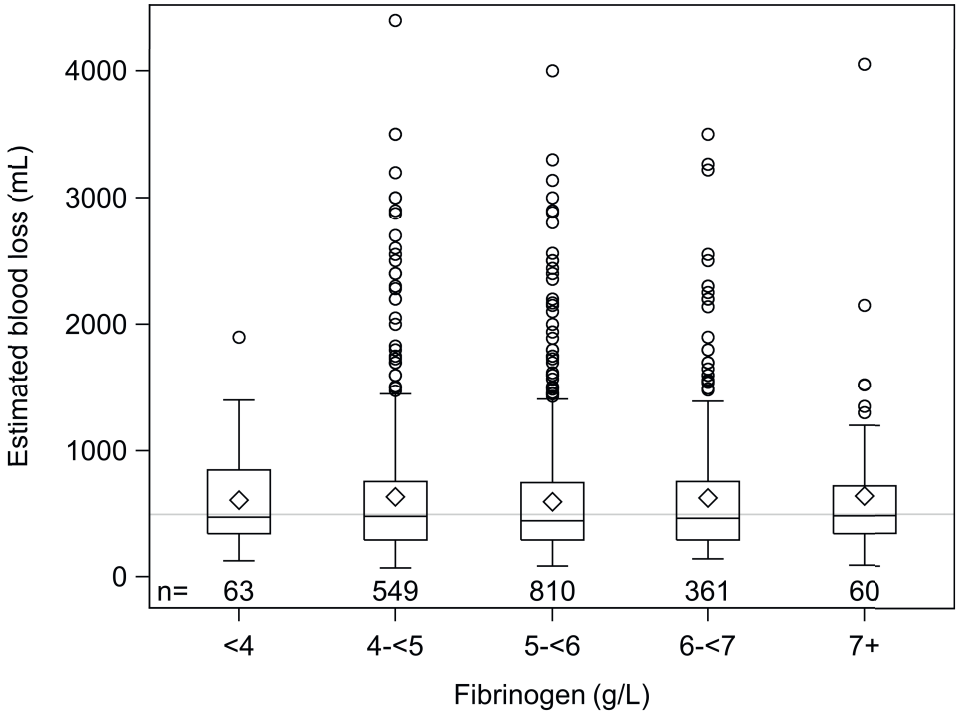

Figure 17. Fibrinogen concentration and estimated blood loss during delivery in 1951 women. Box whisker plots with median (line), mean (square) and 25%-75% percentile. Dots are outliers. The grey line shows estimated blood loss of 500 mL.

Fibrinogen concentration before and after labour

Fibrinogen concentration did not differ significantly before and after delivery (5.4 (standard deviation (SD) 0.9) g/L vs 5.3 (SD 0.9) g/L, p=0.25). There was a strong correlation between fibrinogen concentration at admission and after delivery of the placenta (r=0.90, p<0.0001). The median interval from blood sampling at admission until the placenta was delivered was 5.25 hours (range 0.67-35.25).

Prediction of estimated blood loss > 1000 mL

The total number of women with EBL >1000 mL was 250 (12.8%). The following factors were associated with EBL >1000 mL: higher age, increased BMI, later gestational age at delivery, induction of labour, oxytocin stimulation, instrumental delivery, caesarean section and manual exploration of the uterus. Odds ratios are presented in Table 12. Parity, fibrinogen

concentration, preeclampsia and epidural analgesia were not associated with increased risk of severe PPH.

In the multivariate logistic regression analysis, oxytocin stimulation, instrumental delivery, caesarean section and manual exploration of the uterus were independent predictors of EBL >1000 mL, Table 12. The area under the ROC curve for the model was 0.716. Fibrinogen concentrations and EBL for these subgroups are presented in Table 13.

Table 12. Bivariate and multivariate prediction of estimated blood loss >1000 mL in different subgroups of women.

Variable		Number (%) with bleeding >1000 mL	Odds Ratio (95% CI) Original scale	p-value	Adjusted Odds Ratio (95% CI) Multivariate	Adjusted p-value
Age, year	<25	22 (8.1)				
	25-<30	85 (13.1)				
	30-<35	80 (12.4)				
	≥35	61 (16.2)	1.03 (1.01-1.06)	0.016		
Body mass index, kg/m^2	<24.9	131 (12.5)				
	25-29.9	54 (12.2)				
	30-34.9	20 (12.2)				
	≥35	17 (25.8)	1.04 (1.01-1.07)	0.014		
Parity	0	138 (14.6)				
	1	56 (10.6)				
	2	24 (12.1)				
	≥3	6 (7.6)	0.86 (0.73-1.02)	0.078		
Gestational week at delivery	<37	4 (5.9)				
	37-42	219 (12.5)				
	>42	24 (21.4)	1.15 (1.04-1.27)	0.005		
Fibrinogen, g/L	<4	7 (11.1)				
	4-<5	72 (13.1)				
	5-<6	102 (12.6)				
	6-<7	49 (13.6)				
	≥7	8 (13.3)	1.01 (0.85-1.19)	0.93		
Preeclampsia		11 (18.3)	1.56 (0.80-3.04)	0.19		
Spontaneous labour		179 (11.7)				
Induction of labour		71 (17.0)	1.55 (1.15-2.09)	0.004		
Oxytocin stimulation		173 (16.0)	2.00 (1.50-2.67)	<0.001	1.7 (1.2-2.3)	0.002
Epidural analgesia		119 (13.8)	1.18 (0.90-1.54)	0.23		
Vaginal delivery		178 (11.1)				
Instrumental delivery		27 (19.9)	1.99 (1.27-3.11)	0.003	1.7 (1.02-2.8)	0.042
Caesarean section		43 (20.9)	2.11 (1.46-3.06)	<0.001	2.7 (1.8-3.9)	<0.001
Uterus exploration		64 (68.8)	20.0 (12.6-31.8)	<0.001	23.0 (14.3-37.1)	<0.001

Estimated blood loss was >1000 mL in 250 patients. Number and percent of women in the subgroup. Logistic regression analyses are bivariable and multivariable. Odds ratio (OR) describes the OR for estimated blood loss for an increase in one unit in the predictor variable. The analyses for Odds Ratio were performed on continuous values.

Table 13. Fibrinogen concentration and estimated blood loss in the subgroups that were independent predictors of estimated blood loss > 1000 mL.

Subgroup	n	Fibrinogen g/L	Estimated blood loss mL
Oxytocin stimulation	1082	5.38 (SD 0.85) 5.3 (2.9 – 8.8)	684 (SD 538) 500 (85 – 4400)
Instrumental delivery	136	5.53 (SD 0.89) 5.50 (3.6 – 8.8)	732 (SD 601) 500 (140 – 4400)
Caesarean section	206	5.47 (SD 0.94) 5.4 (3.3 – 8.2)	784 (SD 408) 700 (160 – 3000)
Postpartum exploration	93	5.34 (SD 0.83) 5.3 (2.9 – 8.8)	1741 (SD 1016) 1600 (100 – 4400)
Remaining population	771	5.27 (SD 0.79) 5.2 (3.1 – 8.3)	477 (SD 314) 400 (70 - 2440)

Data are; fibrinogen: mean (SD), blood loos: median (range) and n: number

5 DISCUSSION

Prospective longitudinal study of thromboelastography and standard hemostatic laboratory tests in healthy women during normal pregnancy

We have shown, in Paper I, that the TEG® method demonstrated increased coagulability and decreased fibrinolysis during normal pregnancy, compared to 8 weeks postpartum. Our findings of increased coagulability agree, to some extent, with a longitudinal study of another viscoelastic method, a cross-sectional study using ROTEM® and a study including only late pregnancy and the postpartum period [93-95].

TEG®-R and TEG®-K were shorter and TEG®-Angle and TEG®-MA were increased during pregnancy. The cross-sectional study with thromboelastometry did not find a shorter time to clotting start or an increased maximum clot firmness early in pregnancy; this difference in results is probably due to small numbers and a different study design [93].

In the control group with normal bleeding postpartum in Paper III, all TEG® variables demonstrated even further increased coagulability immediately (2-6 hours) after delivery. This control group had shorter TEG®-R and TEG®-K and increased TEG®-Angle and TEG®-MA, compared to the group in Paper I. The mean TEG-MA in the control group was 72.9, vs 68.5 in the group at gestational weeks 38-40 in Paper I. The results indicate that the coagulability increase peaks at delivery and some days postpartum.

We observed marked inter-individual changes in soluble fibrin levels, which may contribute to the absence of statistically significant difference between the different time points, because earlier reports have shown an increase during pregnancy [4, 96]. D-dimer levels increased as pregnancy progressed, consistent with other studies [1, 4, 97]. However, TEG®-LY30 decreased from gestational weeks 28-30. This decrease indicates declining fibrinolysis, due to lower t-PA activity, minor increase in thrombin activated fibrinolys inhibitor and a marked increase in PAI-1 and PAI-2 [4, 96, 98, 99]. The increased D-dimer level is more likely a result of increased coagulation activity and fibrin formation rather than fibrinolysis [100].

The strength of this study was the prospective and longitudinal design and the large number of women who participated throughout the study

period. One limitation was the absence of pre-pregnancy baseline TEG® variables and laboratory analyses. The results at 8 weeks postpartum cannot be regarded as absolute non-pregnant values, although most of the haemostatic variables are normalized at that time [1, 4, 95]. The absence of fibrinogen concentration determination is a major limitation. This is due to the fact that, during the planning stage of this study, fibrinogen concentration was not included in the standard emergency haemostatic analyses at our institution, which were to be compared with the TEG® method. Future larger studies are needed to calculate new reference ranges for all TEG® variables during pregnancy and to investigate whether the increase in clot strength (TEG®-MA) is of importance in connection with PPH.

A longitudinal study of Factor XIII activity, fibrinogen concentration, platelet count and clot strength during normal pregnancy

In Paper II, we showed that FXIII activity and platelet count were lower, while fibrinogen concentration was higher, during normal pregnancy, compared to 8 weeks postpartum. Nonetheless, clot strength was increased.

There are conflicting data about FXIII changes during normal pregnancy in healthy women; both increases and decreases in FXIII activity have been reported [46-50]. The previous reports are based on old studies that needed to be repeated. Nevertheless, the decrease in FXIII activity in this study was statistically significant but moderate and within the normal reference range for non-pregnant individuals. This study failed to determine whether the reduction in FXIII activity is an effect of reduced production, increased plasma volume or increased consumption in the utero-placental unit.

In this study, fibrinogen concentration increased and platelet count decreased during pregnancy, which is in accordance with previous studies [1-3]. As mentioned above, most coagulation factors increase during pregnancy due to hormonal changes, especially altered estrogen levels [1, 3, 60, 97]. The decrease in platelet count may be explained by increased platelet consumption, because of intravascular coagulation in the utero-placental circulation [1, 2].

We found that the mean fibrinogen concentration was 5.1 g/L in this study. In Paper IV, the mean fibrinogen concentration was 5.3 g/L at admission before labour and 5.3 g/L after the placenta was delivered. In

Paper III, the mean fibrinogen level was 4.8 g/L in the control group with normal bleeding, 2-6 hours after delivery. In the HIP study, the mean fibrinogen concentration peaked on day three after delivery [95]. We thus found that the fibrinogen concentration peaked at the time of, or a few days after, delivery; this occurs partly in order to reduce the risk of bleeding and partly as an acute phase reaction.

Fibrinogen and platelets have been suggested to be the main determinants of clot strength [93, 101-103]. The correlations between clot strength and fibrinogen concentration and between clot strength and platelet count in this study support these suggestions, despite the decrease in platelet count. FXIII activity did not correlate to clot strength, as earlier in vitro studies have shown, e.g. FXIII, together with fibrinogen, improved clot strength in blood diluted with Ringer's acetate solution [41, 52, 89, 104, 105]. There is no previous in vivo study on the association between FXIII and clot strength.

There was a persistent, significant positive correlation between fibrinogen concentration and FXIII activity, despite the fact that fibrinogen increased and FXIII decreased. Correlations were calculated for each gestational age period and FXIII activity and fibrinogen levels were positively correlated in each period. It can be speculated that the decrease in FXIII might be a result of consumption equal to the decrease in platelets.

The strength of this study is the prospective and longitudinal design. One limitation was the absence of pre-pregnancy baseline values. The results at 8 weeks postpartum cannot be regarded as absolute non-pregnant references, although most haemostatic variables are normalized at that time [1, 3, 95]. Furthermore, the study population was limited, making analysis of correlations with bleeding volume difficult. Future larger studies are needed to calculate new reference ranges for FXIII during pregnancy and its importance in obstetric complications, especially PPH.

Major obstetric haemorrhage: monitoring with thromboelastography, laboratory analyses or both?

Paper III showed that haemostasis is impaired when blood loss exceeds 2000 mL, demonstrated by both TEG® and laboratory analyses. TEG® can provide rapid and clinically important information about haemostatic changes in

connection with MOH, perhaps revealing indications for specific blood product therapy, earlier than traditional laboratory testing.

In Papers I and II, we demonstrated how TEG® variables and laboratory analyses change during normal pregnancy and 8 weeks postpartum. In this paper, we showed how TEG® variables and laboratory analyses change during bleeding complications. However, impaired haemostasis can result from bleeding itself and/or haemodilution by administration of crystalloids and colloids [80, 82, 105-107].

TEG® variables showed faster initiation of blood clotting and impaired clot strength. This faster initiation of clotting contrasts with a previous study of PPH using thromboelastometry, possibly due to different timing of the TEG® analyses in relation to bleeding [12]. Faster initiation of clot formation has been described during haemorrhage, but it may also be delayed due to consumption of coagulation factors and platelets [108]. Impaired clot strength was also found in the thromboelastometry study [12].

When it came to the laboratory analyses, the largest decreases were noted in platelet count, fibrinogen concentration and antithrombin activity, similar results have previously been reported [32-34]. Platelet count is normally lowered at the end of pregnancy, and further reduced in cases of MOH [32, 63, 95]. Studies of non-obstetric bleeding have shown that fibrinogen is the first clotting factor to decrease to critical levels [39, 109].

The decreased antithrombin activity must be considered when administering coagulation factors [110]. The combination of factor concentrate transfusion and low antithrombin activity with reduced fibrinolysis probably increases the risk of thrombotic complications and post-haemorrhage inflammatory processes [57, 111].

The variables that best correlated with EBL in our study were fibrinogen concentration and antithrombin activity, although platelet count and clot strength (TEG®-MA) also correlated well. Similar results have been reported previously [12, 112, 113]. If viscoelastic methods are not used, fibrinogen administration may be delayed [83]. Our study indicates that TEG® or other viscoelastic methods are probably important as point-of-care instruments for analysing the aetiology of bleeding and ongoing therapy.

One limitation of the study was that there was no standardized transfusion strategy and different approaches to transfusion and fluid management may have altered the results. Coagulopathies can result from

bleeding itself and/or dilution of clotting factors by administration of crystalloids and colloids [80]. Another limitation of our study was that 12 of 45 in the MOH group were given plasma and 14 of 45 were given tranexamic acid. Plasma and tranexamic acid may improve impaired haemostatic variables. Indeed, when the statistical analyses were repeated after exclusion of these individuals, the difference between the MOH group and the control group became less pronounced, indicating that haemostasis in the MOH group was actually more affected and that administration of plasma and tranexamic acid had alleviated these impairments.

Fibrinogen plasma concentration before delivery is not associated with postpartum haemorrhage: a prospective observational study

In Paper IV, we showed that plasma fibrinogen concentration, measured at admission just before labour, does not influence postpartum bleeding in a general obstetric population. Fibrinogen concentration was not reduced after delivery, in the absence of severe PPH. Our study also showed that oxytocin stimulation, instrumental delivery, caesarean section and postpartum manual exploration of the uterus increased the risk of severe PPH, concurring with previous studies.

During pregnancy, the fibrinogen concentration increases progressively and remains high for approximately two weeks after delivery [95]. This study was initiated after two publications reported an association between low fibrinogen concentration and severe PPH [33, 34]. In these studies, fibrinogen concentration was measured after the onset of bleeding and it could thus not be determined whether the low fibrinogen levels were caused by low endogenous fibrinogen levels before bleeding started or by the bleeding per se. In addition, treatment with crystalloid or colloid solutions in bleeding patients may reduce the fibrinogen concentration further [28, 80]. A post hoc analysis found that elevated prepartal fibrinogen levels were not associated with a reduced risk of severe PPH, fibrinogen concentration was measured 6-24 days before delivery [114]. Our study clearly showed that there is no association between prepartal fibrinogen concentration and postpartum bleeding volume.

Knight et others showed an increasing trend in PPH and that heterogeneous definitions result in different frequencies of PPH in different countries, concluding that there is a need for a common definition of PPH [65, 115, 116]. In this study, the frequency of severe PPH, defined as EBL

>1000 mL, was 12.8%. This frequency is markedly higher than the 2% reported in a recent WHO report, in which PPH was defined as bleeding >500 mL [117]. Bleeding volume was prospectively registered in our study, which may have yielded a more accurate estimation of the PPH frequency, which we suspect is underestimated in many reports [117, 118].

In the subgroup of patients in which fibrinogen concentration was analyzed both before and after delivery, concentrations did not differ. Studies have shown that fibrin is deposited in the placenta during labour [119, 120]. Our results suggest that this deposition only consumes a small amount of the circulating fibrinogen at normal delivery. However, fibrinogen is an acute phase protein; we do not know how this increases concentrations, nor do we know how much they decrease due to deposition.

The strengths of this study include the prospective design, fibrinogen analysis just before delivery and the large number of women included. The fact that the vast majority of women underwent a normal pregnancy is a limitation. Our results do not rule out that there might be subgroups of women in which the fibrinogen concentration may be more important. Future studies should therefore include women with abruptio placentae, placenta previa/accreta, HELLP syndrome and amniotic fluid embolism. Furthermore, although our study was meant to include consecutive women, inclusion was occasionally halted due to the workload at the maternity units, which may have resulted in a selection bias.

6 CONCLUSION

In summary, in Papers I and II, we have demonstrated increased coagulability and decreased fibrinolysis during normal pregnancy, both with TEG® variables and laboratory analyses. In Paper III, we have shown impaired haemostasis during severe PPH, demonstrated both with TEG® variables and laboratory analyses. In Paper IV, we have shown that fibrinogen concentration at admission, before labour, does not predict severe PPH. Severe PPH is mainly due to obstetric complications.

Specific conclusions

Paper 1

- The TEG® method shows increased coagulability and decreased fibrinolysis in healthy women during normal pregnancy, compared to 8 weeks postpartum.
- Initiation of haemostasis occurred faster, with a minor increase in clot strength, and fibrinolysis decreased during late pregnancy.
- There are no or weak correlations between TEG® and laboratory variables in healthy women during normal pregnancy.

Paper II

- FXIII activity and platelet count are lower, while fibrinogen concentration is higher, during normal pregnancy, compared to 8 weeks postpartum. Nonetheless, clot strength is higher during pregnancy, compared to 8 weeks postpartum.
- Clot strength is associated with fibrinogen concentration and platelet count, but not with FXIII activity.

Paper III

- When estimated blood loss exceeds 2000 mL, impaired haemostasis is shown by both TEG® variables and laboratory analyses.
- The TEG® method provides faster results than standard laboratory analyses, which is advantageous in the setting of ongoing major obstetric haemorrhage. TEG®-MA correlated with estimated blood loss.

- Laboratory analyses, especially fibrinogen concentration and antithrombin activity, exhibited greater differences than TEG® variables and correlated better with estimated blood loss.

Paper IV

- Plasma fibrinogen concentration at admission, before labour, does not predict severe postpartum haemorrhage in a general obstetric population.
- Fibrinogen concentration does not decrease significantly during normal delivery.
- Excessive bleeding postpartum is mainly due to obstetric causes, for example oxytocin stimulation, instrumental delivery, caesarean section or manual exploration of the uterus postpartum.
- Low fibrinogen concentration during postpartum haemorrhage is due to bleeding, consumption and haemodilution.

Knowledge of these changes in haemostasis during pregnancy and labour is important and monitoring haemostasis with viscoelastic methods during obstetric complications such as PPH is fundamental to providing good obstetric care.

7 FUTURE PERSPECTIVES

Studies of obstetric haemostasis are important in order to provide good care to women during pregnancy and labour, as well as in cases of obstetric complications. Sahlgrenska University Hospital, with 10,000-11,000 deliveries annually, is a appropriate site for research in this area, generating knowledge that can be disseminated to other obstetric units.

Studies of viscoelastic methods in women on anticoagulant therapy are important. At present, this group are often given general anaesthesia if surgery is required. Viscoelastic methods may clarify when it is possible to administer regional anaesthesia to women on anticoagulants, especially important if they are obese.

Future studies of women with PPH are needed, aimed at determining the best strategies for transfusion, factor concentrate therapy and other medications, in order to optimize care of parturients with bleeding complications.

Studies of viscoelastic methods in the obstetric patient with complications such as preeclampsia, abruptio placentae and placenta accreta are also needed. Viscoelastic methods can provide important information faster than most laboratory analyses, also increasing the possibility to provide the best obstetric care.

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