

The role of fibrinogen and haemostatic assessment in postpartum haemorrhage

- Preparations for a randomised controlled trial

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LIST OF PAPERS

I. Wikkelsø A, Lunde J, Johansen M, Stensballe J, Wetterslev J, Møller AM, Afshari A. Fibrinogen concentrate in bleeding patients. *Cochrane Database Syst Rev.* 2013 Aug 29;8:CD008864.

II. Wikkelsø AJ, Afshari A, Wetterslev J, Brok J and Møller AM. Monitoring patients at risk of massive transfusion with Thrombelastography or Thromboelastometry: a systematic review. *Acta Anaesthesiol Scand* 2011; 55: 1174–1189.

III. Wikkelsø AJ, Hjortøe S, Gerds TA, Møller AM and Langhoff-Roos J. Prediction of postpartum blood transfusion – risk factors and recurrence. *J. Matern. Fetal. Neonatal Med.* 2014;27:1661-7.

IV. Kozek-Langenecker SA, Afshari A, Albaladejo P, Santullano CA, De Robertis E, Filipescu DC, Fries D, Görlinger K, Haas T, Imberger G, Jacob M, Lancé M, Llau J, Mallett S, Meier J, Rahe-Meyer N, Samama CM, Smith A, Solomon C, Van der Linden P, Wikkelsø AJ, Wouters P, Wyffels P. Management of severe perioperative bleeding: guidelines from the European Society of Anaesthesiology. *Eur J Anaesthesiol.* 2013 Jun;30(6):316-319 (270-382). Chapter 8.2.2 Obstetrical bleeding.

V. Wikkelseo AJ, Afshari A, Stensballe J, Langhoff-Roos J, Albrechtsen C, Ekelund K, Hanke G, Sharif HF, Mitchell AU, Svare J, Troelstrup A, Pedersen LM, Lauenborg J, Madsen MG, Bødker B, Møller AM. The FIB-PPH trial: fibrinogen concentrate as initial treatment for postpartum haemorrhage: study protocol for a randomised controlled trial. *Trials.* 2012 Jul 17;13:110.

ABBREVIATIONS

PPH Postpartum Haemorrhage

VHA Viscoelastic haemostatic assays

TEG Thrombelastography, TEG®, Haemonetics Corp., Braintree, MA, United States

ROTEM Thrombelastometry, ROTEM®, Tem Innovations GmbH, Munich, Germany

RBC Red Blood Cells

FFP Fresh frozen plasma

HES Hydroxyethyl starch

RCT Randomised controlled trial

FIB-PPH Trial acronym: "FIBrinogen as initial treatment for PostPartum Haemorrhage"

GRADE Grading of Recommendations Assessment, Development and Evaluation

INTRODUCTION

Postpartum haemorrhage

The epidemiological perspective

Postpartum haemorrhage (PPH) remains one of the leading causes of maternal mortality and morbidity throughout the world [1] accountable for nearly ¼ of maternal deaths worldwide and directly causing approximately 132,000 deaths each year [2]. The international definition of PPH is bleeding exceeding 500 ml from the birth canal following delivery, and "severe PPH" is defined by the limit of at least 1000 ml [3]. If bleeding arises within 24 hours postpartum it is termed "Primary PPH" [2]. In Europe approximately 6% of deliveries are complicated by PPH and 1.75% by severe PPH4. The diagnosis is based on estimated blood loss, but this varies with the applied method of measurement [4] and the actual blood loss present (small volumes tends to be exaggerated and large volumes to be underestimated) [5]. Incidence of PPH seems to be increasing during the last decades throughout the developed world, and this is only partly explained by an increased use of caesarean section, induction of labour and increased maternal age [6,7].

Maternal deaths caused by major obstetric haemorrhage was 0.39 per 100.000 maternities in the United Kingdom (UK) during the period of 2006-2008 [3]. Overall, maternal mortality in the developed countries remains a very rare event. Definitions of maternal morbidity and "near-miss" have therefore been introduced in order to evaluate developments in care: Transfusion of several units of blood, hysterectomy, Sheehan's syndrome (postpartum pituitary necrosis) [8] and complica-

tions related to procedures instituted to achieve haemostasis such as pelvic arterial embolization or uterine arterial ligation [9]. In addition one should not neglect the long term psychological impact of this condition [10].

Postpartum blood transfusions

Approximately 0.3-2.7% of deliveries are complicated by allogenic (RBC) blood transfusion [11–16], and obstetrical transfusion account for 3-4% of the annual use of blood products in the United Kingdom [17]. Extensive screening of blood donors has significantly reduced transfusion associated infectious risks during the past decades. Human immunodeficiency virus (HIV) transmission from donor blood is at present one in 2.3 million [18]. The emerging complications in focus are a broad group of “non-infectious serious hazards of transfusion (NISHOT)”: Problems related to immunization and haemolytic transfusion reactions are well described, but transfusion related acute lung injury (TRALI) and microchimerism are new issues of transfusion-related immune modulation [19,20]. Blood transfusion saves lives in cases of severe exsanguination, but the need of transfusion is also associated with increased mortality and morbidity despite adjustment for confounders [21,22]. It is thus of utmost importance to secure a restrictive and evidence based use of blood products [23].

Aetiology and risk factors

In order to simplify a huge field of epidemiological research regarding causes and especially risk factors of PPH, “the 4 Ts” were introduced by the American Academy of Family Physicians in the Advanced Life Support in Obstetrics (ALSO) courses. “The 4Ts” represent a summary of causes: Tone (uterine atony), Trauma (of the birth canal: lacerations, haematomas, inversion and rupture), Tissue (retained placental tissue including placental pathologies) and Thrombin (impairment of haemostasis) (Table 1). In PPH the incidence of uterine atony is estimated to be approximately 70% [24], trauma (20%), tissue related causes (10%) and impairment of haemostasis (1%). These incidences remain estimates despite being widely cited and may vary with mode of delivery and management of third stage of labour [2]. Table 1 summarises the most important investigated risk factors of PPH. The prediction of PPH remains difficult even considering the long list of associated risk factors. Furthermore, severe cases of PPH may present even in apparently low risk patients [25].

Table 1
Risk factors for primary PPH in association with PPH aetiology [2].

Aetiology	Risk factor of primary PPH
Tone	Macrosomia Multiple gestations Polyhydramnios Augmented labour Induced labour Prolonged stages of labour especially the third Increased placental weight Chorioamnionitis Uterine inversion Fibroids (Retained placental tissue)
Trauma	Instrumental delivery Episiotomy

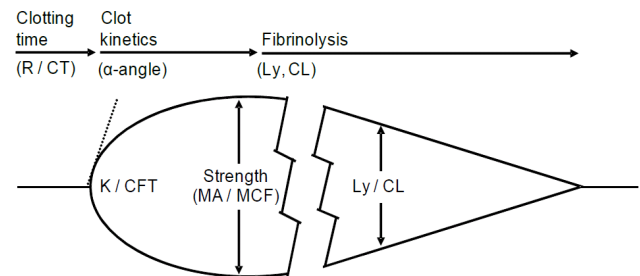
	Caesarean section Lower genital lacerations Uterine rupture
Tissue	Retained placental tissue Abnormal placentation (placenta previa, placenta accrete, placenta increta, placenta percreta) Previous caesarean section
Thrombin	Inherited or acquired haemostatic deficiencies Placental abruption Intrauterine foetal death Preeclampsia HELLP
Others	Previous PPH Maternal age above 35 years Obesity Ethnicity Parity

Coagulation

Measurement of the haemostatic functionality

Traditionally, haemostasis has been measured by levels of coagulation factors in the plasma phase. These plasmatic tests were developed for adjusting anticoagulant treatment and not for the assessment of bleeding patients [26,27]. Viscoelastic haemostatic assays (VHAs) measure the haemostatic function of whole blood reflecting the initiation, propagation and degradation of the clot [28,29]: A pin is dispensed in a small cup with the whole blood sample. An initiator (usually a form of tissue factor) is added and clot formation begins [29]. The pin or cup is rotated gently and when clot strings form between pin and cup the motion is reduced and the tracing is pictured as a graph (Figure 1). The level and functionality of fibrinogen and platelets are reflected mainly in the strength of the clot (measured as MA (maximum amplitude, TEG®) or MCF (maximum clot firmness, ROTEM®)). The contribution of fibrinogen to clot strength is isolated in assays such as Functional Fibrinogen (TEG®) and FIBTEM (ROTEM®), and this is achieved by blocking of the platelet function [30]. The VHAs reflect hypo- as well as hypercoagulation and are being used to assess haemostasis in cases of bleeding and to guide treatment [31,32]. The use of VHAs to predict risk of bleeding is less well documented [33,34].

Figure 1



Schematic picture of a TEG/ROTEM trace [35] depicting clot initiation (R-time or Clotting Time), clot propagation (alpha angle and K-time or Clot Formation Time), clot strength (Maximum Amplitude or Maximum Clot Firmness) and clot dissolution (Lysis or Clot Lysis).

Table 2

Causes of coagulopathy in relation to PPH

Inherited/ congenital deficiencies [44]	Pregnancy related - pregnancy specific [44]	Pregnancy related - not pregnancy specific [44]	Haemorrhage related [45, 46]
<ul style="list-style-type: none"> ▪ Congenital platelet disorders ▪ von Willebrand disease ▪ Haemophilia A and B ▪ Fibrinogen deficiency (hypofibrinogenaemia and dysfibrinogenaemia) ▪ Factor VII deficiency ▪ Factor X deficiency ▪ Other vitamin K dependent factor deficiencies ▪ Factor XI deficiency ▪ Factor XIII deficiency 	<ul style="list-style-type: none"> ▪ Gestational (incidental) thrombocytopenia ▪ Pre-eclampsia ▪ HELLP syndrome (haemolysis, elevated liver enzymes and low platelets) ▪ Acute fatty liver of pregnancy (APLP) 	<ul style="list-style-type: none"> ▪ Idiopathic thrombocytopenic purpura (ITP) ▪ Thrombotic thrombocytopenic purpura (TTP) ▪ Haemolytic uremic syndrome (HUS) ▪ Systemic lupus erythematosus ▪ Viral infection (HIV, CMV, EBV) ▪ Antiphospholipid antibodies ▪ Type 2B von Willebrand disease 	<ul style="list-style-type: none"> ▪ Coagulopathy of dilution (<i>substituting blood loss only with i.v. fluids and blood products not containing haemostatic parts</i>) ▪ Coagulopathy of impaired functionality (<i>Use of colloids, drug induced thrombocytopenia, hypothermia, acidosis and hypocalcaemia induced</i>) ▪ Coagulopathy of Consumption (<i>Placental abruption, ongoing haemorrhage and Disseminated intravascular coagulation</i>)

Changes due to pregnancy

Pregnancy is a state of hypercoagulability, but seems to be somewhat balanced [36]. A normal pregnancy is characterised by physiological anaemia, neutrophilia, mild thrombocytopenia, increased pro coagulant factors and diminished fibrinolysis [37]. The haemostatic ability related to these changes has been described using VHAs: A faster clot initiation, increase in clot strength and decreased fibrinolysis has been reported [38–41]. Fibrinogen levels increase steadily throughout pregnancy with the highest level present during the third trimester. The level reaches normality within the following six to eight weeks postpartum [42,43]. Coagulopathy in relation to postpartum haemorrhage falls into three major categories: Inherited deficiencies, pregnancy related and haemorrhage related (Table 2) [44–46]. Only 1% of PPH cases were estimated to be caused by impaired haemostasis by Anderson et al. and taught throughout the world on the ALSO courses [24]. This approximation may be correct if only inherited deficiencies are taken into account: The most frequent inherited bleeding disorder is von Willebrand disease characterised by easy tendency of bleeding and bruising, and with a prevalence of 1% (although only 1:10.000 seem to be clinically significant cases) [47]. The aetiology of a severe case of PPH is most often multifactorial and acquired deficiencies tend to be somewhat more frequent than expected previously [48], thus reports of 21% have been published [12].

Changes due to bleeding

The coagulation cascade is activated when bleeding occurs. The end-product of this process is the clot formation with activated platelets, cross-linked fibrin strands and captured red blood cells. The ability to achieve haemostasis depends on availability and function of coagulation substrates and most importantly the surgical treatment.

Dilution

The circulating blood becomes diluted when treatment with intravenous (i.v.) fluids are commenced [46]. Dilution with i.v. fluids decreases the concentration of red blood cells, platelets and coagulation factors. A decreasing level of red blood cells causes anaemia with impaired oxygen carrying capacity, but with an indirect effect on coagulation: Red blood cells tend to remain in the centre of the vessel and thus push the platelets into the periphery and closer to the endothelium [49]. Primary activation of coagulation is caused by the interaction between platelets and the endothelium [50], and a low hematocrit/relative anaemia may cause a functional state of low platelets. Fibrinogen is the first haemostatic factor to decrease to a critical level followed by prothrombin and finally platelets [51]. Dilution will also occur when blood products are given without a balance in the ratio between Red Blood Cells units (RBCs) and haemostatic blood products [46,52]. The concept of “damage control surgery” in trauma emphasizes normalisation of physiology and a rigorous use of protocols of massive blood transfusion in a fixed ratio [53] (in American literature the 1:1:1 ratio between RBCs, Fresh Frozen Plasma (FFP) and platelet units is advocated, but in a Danish scenario this would respond to 1:1:0.25 due to our pooled platelet products). The American approach is thus to avoid dilution, but other treatment strategies are also being used. The Austrian thrombelastometry-based [54] method is focused on monitoring the level of coagulation impairment due to dilution and treatment is instituted when coagulopathy becomes critical using concentrated products such as fibrinogen concentrate and prothrombin complex concentrate. The Copenhagen Concept of Hemostatic Control Resuscitation combines these two principles providing a balanced transfusion therapy with a blood product transfusion ratio equal to 1:1:1 and adjustments according to a goal-directed VHA-based algorithm [52]. The three treatment remain to be compared in an RCT [55].

Impaired function

Synthetic colloids such as hydroxyethyl starch (HES) impair coagulation beyond the effect of dilution [56–58]. This is a

result of a HES induced reduction in plasma concentration of factor VIII and von Willebrand factor, and a reduction in the availability of activated GPIIb-IIIa causing an impairment of fibrinogen function [59]. This impairment may be corrected by the administration of fibrinogen concentrate as shown in a randomised controlled trial (RCT) by Fenger-Eriksen et al. [60]. Hypothermia, hypocalcaemia and acidosis caused by the state of haemorrhagic shock also seriously impairs haemostasis including reduction in fibrinogen synthesis (hypothermia) and increased breakdown (acidosis) [61]. Anticoagulant treatment and other forms of drug induced thrombocytopenia may also play a significant role.

Consumption

Since patients bleed whole blood, the haemostatic components of blood will inevitably be reduced during ongoing bleeding. The coagulation system consumes haemostatic components in order to arrest bleeding. As a consequence, the haemostatic ability is reduced in cases of severe bleeding due to consumption. This is especially true in cases of multiple injuries (as in trauma) [46] or with an open placental bed at the endometrial surface, which in the post-delivery phase will be covered in a mesh-work of cross-linked extra vascular fibrin [62]. Pathological excessive consumption occurs in clinical conditions such as disseminated intravascular coagulation, placental abruption, retained dead foetus, amnion embolus [45] and post haemorrhagic shock. These conditions are usually complicated by excessive hyperfibrinolysis [63]: During hyperfibrinolysis the clot is dissolved prematurely, and in worst cases it dissolves before proper formation (e.g. primary hyperfibrinolysis) [64].

The role of fibrinogen

Fibrinogen (coagulation factor I) is present in the blood with a normal non-pregnant level of 2.0 – 4.5 g/dL [65]. During activation of the coagulation cascade thrombin converts fibrinogen to fibrin polymers forming the strings of the blood clot [50]. During pregnancy the fibrinogen level increases to an average

of 5 g/L [39] (3.7-6.1 g/L) [66]. A low level of fibrinogen or reduced function seriously impairs haemostasis, but the optimal fibrinogen level remains disputed [67–69]. During ongoing bleeding fibrinogen levels may become low due to consumption or dilution. In 2007 a French group investigated [70] 128 women suffering from PPH and found a strong association between an initial low level of fibrinogen (< 2 g/L) and the development of a severe course of bleeding reporting a positive predictive value of 100% (71-100%). Severe PPH was defined by a decrease in haemoglobin of at least 4 g/dL (2.5 mmol/L), transfusion of at least 4 units of red blood cells, need of haemostatic interventions (angiographic embolization, arterial ligation or hysterectomy) or maternal death. Other publications have reported similar findings [71] with a low fibrinogen level being associated with treatment failure of embolization [72], increased need of surgical treatment of PPH [73], estimated blood loss [74] and advanced interventional procedures to stop genital tract bleeding [75] (Appendix 1 summarises observational studies associating fibrinogen level and PPH). Fibrinogen may be substituted using either FFP, cryoprecipitate or fibrinogen concentrate (Table 3). All products are based on human plasma and no recombinant products are yet commercially available (advantages and disadvantages are summarised in Table 3). Four different fibrinogen concentrate products are available on the market, but only RiaSTAP®/ Haemocomplettan® (by CSL Behring, Marburg, Germany) is available in Europe (except for France) and the United States [69]. Fibrinogen concentrate (RiaSTAP®) can be stored at room temperature (2-25 °C) for 5 years [68,76] and may therefore be a treatment option in very basic clinical settings. However, one gram of fibrinogen concentrate is sold in Denmark at the price of 4898.60 DKr (906.5 USD) [77] (price assessed 25-10-2013), and since the planning of the FIB-PPH trial began in 2009 the price has gone up with 109% (The price of 1 gram of fibrinogen concentrate (RiaSTAP®, CSL Behring) was in our fund-applications from 2009 2340 DKr. (426.8 USD)).

The need for a randomised trial

Table 3

Comparison of fibrinogen sources: FFP, cryoprecipitate and fibrinogen concentrate, based on Levy et al. 2013 [69] and Rahe-Meyer & Sørensen 2011 [78].

Therapeutic option	Preparation	Constituents [78]	Storage	Administration	Reported fibrinogen concentration	Viral inactivation/removal
Frozen Plasma	FFP placed at -18 °C within 8 hours of phlebotomy; FP24 placed at -8 °C within 24 hours of phlebotomy	Contains all clotting factors and numerous other proteins	At -18 °C up to 12 months; once thawed (at 30-37 °C), then must be stored at 1-6 °C and used within 24 hours	Must be thawed and typed for compatibility with the recipient	1-3 g/L	Can be made from virally inactivated or reduced-liquid plasma
Cryoprecipitate	After thawing at 1-6 °C plasma is centrifuged at 5000 x g for 6 min. Precipitated proteins are resuspended in 10-15 mL of plasma and are refrozen	Contains clotting factor VIII and XIII as well as fibrinogen. Also contains von Willebrand factor	At -18 °C for up to 12 months	Must be thawed and typed for compatibility with the recipient	Approx. 15 g/L	Can be made from virally inactivated or reduced-liquid plasma
Fibrinogen concentrate	Manufactured from human plasma into a lyophilized powder	Mainly fibrinogen, only a few other protein constituents	Some concentrates can be stored at room temperature (2-25 °C)	Must be reconstituted in sterile water	15-20 g/L	Made from plasma that has undergone viral inactivation/removal steps

In most countries the fibrinogen concentrate is only approved for treatment of inherited fibrinogen deficiencies [69,76,79], thus substitution with fibrinogen concentrate in cases of acquired hypofibrinogenaemia represents “off-label” prescription. However, the growing awareness of haemostatic challenges in bleeding patients and the prospective study by Charbit et al. [70], together with perhaps the reminiscence of the optimistic stories of recombinant factor seven-A [80], have led to an increased use of early treatment with fibrinogen concentrate in many European countries [81] - We as clinicians seem to be urging for a “magic haemostatic bullet”! “Off-label” prescription is defined by the prescription of medication contrary to approved indications (e.g. for different indications, patient age-range, dose or route) [82]. This has several implications since the driving forces convincing physicians are not always evidence based, and the rationale for each clinician prescribing off-label drugs might instead be psychological and social reasons, especially in situations with on-going severe bleedings [83]. In a general perspective, arguments for off-label drug prescription includes a potential of promoting clinical innovation and to provide treatment options for patients for whom there are no other alternatives [80]. Arguments against points out that it undermines regulatory systems, might imply unjustified costs and puts patients at increased risk of harm [80]. In addition, the pharmaceutical companies might have conflicting interests promoting off-label use instead of proper evidence testing: More controlled studies might negate the efficacy claims that smaller and less formalised studies may make [80]. Therefore trials investigating drugs which are extensively being used off-label should be investigated in an independent set-up. Highest level of evidence should be sought: An RCT in a sponsor-investigator driven independent set-up with double/triple blinding ensuring lowest possible bias and enabling a comparison with reduced selection-bias [84–86].

OBJECTIVES

This thesis aimed to assess the current evidence for the use of fibrinogen concentrate and haemostatic assessment in bleeding patients with a special attention to the obstetrical population. As a cornerstone for this project a randomised placebo-controlled trial in women with severe postpartum haemorrhage was planned.

The thesis includes the following five studies:

- A Cochrane review assessing the current level of evidence (RCTs) evaluating the use of fibrinogen concentrate in a general population of bleeding patients, thus not strictly focusing on obstetrical patients (Paper I) [87].
- The current evidence for the use of viscoelastic haemostatic assays to guide treatment with haemostatic blood products was systematically assessed with a meta-analysis in severely bleeding patients in general (Paper II) [88].
- In a register based study we searched for clinically useful risk factors for postpartum transfusion and assessed the joint predictive value for the selection of patients with severe postpartum haemorrhage to be used as inclusion criteria in a future RCT (Paper III) [89].

- Recommendations for the use of haemostatic screening and evaluation, and the use of blood products and factor concentrates in obstetrics were systematically assessed as part of the guideline by the European Society of Anaesthesiology (Paper IV) [90].

- The published protocol of our RCT describes the objectives together with methodological and haemostatic considerations in order to state our trial aims and plans prior to data analysis (Paper V) [91].

METHODS AND METHODOLOGICAL CONSIDERATIONS

Evaluating treatment efficacy - the evidence hierarchy

The evidence hierarchy (Figure 2) is based on the possibility of reducing bias, defined as deviation from the truth that encourages one intervention over others [92]. Randomisation seeks to eliminate selection bias by balancing known and unknown prognostic factors, it permits the use of probability theory to express the likelihood that any difference in outcome between groups merely reflects chance, and it allows for blinding of patients and staff [86]. Methodologists dispute on whether an RCT or the systematic review of RCTs offers the best evidence [93], but the importance of randomisation is hardly questioned when evaluating the effect of treatments [84,94].

Figure 2



An example of the evidence based hierarchy of studies evaluating treatment efficacy [85]. Reprinted with permission from John Wiley & Sons, Inc.

Challenges related to trials involving bleeding or delivery

The Declaration of Helsinki by the World Medical Association in 1962 states that “the doctor should obtain the patient’s freely given consent after the patient has been given a full explanation” [95]. The patient needs to be well informed, competent enough and not coerced. Patients with legal or physical incapacity require permission of a legal guardian [96]. Different rules and regulations exist from country to country and with considerable evolution through time. In the Nordic countries a formal ethical committee system was developed in 1980 [97]. Written informed consent is now a keystone to biomedical research with human subjects.

Bleeding is a condition of a rapid and severe nature. A patient’s ability to mentally cooperate is directly linked to the amount of blood loss [98], and in cases of severe bleeding these patients will not be physiologically able to give an informed consent. The level of blood loss and the corresponding state of incapacity

Table 4

RCOG recommendations on timing of consent in trials related to labour. Modified from Royal College of Obstetricians and Gynaecologists Clinical Governance Advice No. 6A, August 2010 [102].

	Very common condition 1:1 – 1:10	Common 1:10 – 1:100	Uncommon or rarer <1:100
Antenatal period	<ul style="list-style-type: none"> • Full antenatal study information • Consider written informed consent before labour 	<ul style="list-style-type: none"> • Short initial outline information • Access to additional information • No antenatal information 	* If pre labour risk assessment indicates that the particular woman's risk exceeds the background of 1:100, then follow "Common"
When condition is confirmed during Labour/ Intrapartum/ Immediate postpartum	<ul style="list-style-type: none"> • Consent should be confirmed 	<ul style="list-style-type: none"> • Full trial information • Seek written informed consent 	<ul style="list-style-type: none"> • Information and consent if condition is confirmed
Example of condition	Caesarean section or first- and second-degree perineal trauma	Obstetric sphincter injuries and postpartum haemorrhage	Shoulder dystocia or uterine inversion

ity may in addition be influenced by trauma, pain, anxiety and anaesthetic treatment. The complexity of the intervention, comparison treatment and additional measurements should be kept at a minimum, especially if patients are enrolled in a pragmatic trial setup by the attending physician who is also in charge of the non-experimental treatment.

All trials involving labour and partum related conditions are challenging in regards to obtaining informed consent. Women giving birth are generally considered vulnerable due to their concern for the child, presumably leaving them at greater risk of coercion than other study participants [99,100]. The concerns of an interventional impact on the un-born child is not in question at the postpartum period, but conditions such as primary postpartum haemorrhage arise in close proximity of the delivery, and therefore ethical challenges of consent are similar to those of intra partum conditions [101]. Among different strategies applied [101], informed consent from all labourers during early labour was the most frequently used (46%) in trials involving third stage of labour. None of the studies sought consent from all pregnant women during the antenatal period, but 38% sought consent at time of complication with no other information provided. Finally, 15% gave antenatal information in groups, leaflets or individually with formal consent sought at the time of complication. Some advocate for early extensive information during pregnancy even if the objective of the trial is a rare complication only becoming relevant to very few women, while others argue that this could potentially cause anxiety and medicalise normal pregnancies [100,102]. However, it is crucial to achieve a balance between relevant timely information and avoiding unnecessary stress caused by detailed information about potential fatal, but rare scenarios. The Royal College of Obstetricians and Gynaecologists (RCOG) has a policy on the timing of consent based on how common the investigated labour-associated condition is [102] (Table 4).

In the FIB-PPH trial [91] we planned for written antenatal information given to all pregnant women by the midwives at planned visits. Consent was sought prior to caesarean section and in cases of manual placental removal (MPR) with a blood loss exceeding 500 ml or exploration of the uterine cavity (EUC) with a blood loss exceeding 1000 ml (inclusion criteria) [91]. In practice this led to a great number of consents being

given prior to caesarean sections or when performing epidurals with insufficient per operative blood loss (<1000 ml) to prompt inclusion. In cases of MPR and EUCs clinicians responded that it was difficult to obtain consent prior to the intervention with ongoing bleeding and preparations for transfer to the operation theatre. This was especially true if the midwives had failed to give the required information, if the women did not recall having read about the study, or if the situation was tense with a rapid ongoing haemorrhage and deterioration of their physical condition. In attempt to improve antenatal information we provided the midwife centres with posters and patient information on their web-sites, brochures at the delivery ward and approached women in labour following epidural placement. In further attempt to address this problem, a group of pregnant women were personally contacted by letter using the gestational data from the nuchal translucency scan at 11-12th gestational week to identify a group of women in their third trimester. One month in advance we sent letters to all women expected to give birth at Rigshospitalet during May 2013. However, this information on gestational age was not supplemented by that of misconceptions, so the immediate response from women with terminated pregnancies due to severe malformations or other unwilling conditions led to an immediate abandoning of this approach.

Initially we approached the ethical committee for two inclusion procedures: One with women giving their consent as outlined above and one with surrogate consent from two independent doctors in accordance with Danish regulations on drug trials in emergency situations [96]. The local ethical committee of the Capital Region of Copenhagen rejected this with regards to the option of testing the risks and efficacy first in women who gave their consent and not allowing for two parallel methods of consent in one trial. In addition they wanted us to have written antenatal consent from all pregnant women (38.000 in two years), but this decision was appealed and changed by the Danish Central Ethics Committee. One may argue that there could be a consequence of being unable to include the most severe patients; most likely the eventual effect of a haemostatic agent like fibrinogen concentrate will be correlated to the level of blood loss and probably also severity of the haemorrhagic condition (e.g. state of shock). Thus, by including only the non-severe cases the intervention may not seem to work. Being unable to include those at high-

est risk of transfusion (e.g. primary outcome) may also reduce the power of the study.

Clinical prediction models

Trials with bleeding patients and labour associated conditions carry the same challenge of a very late recognised eligibility. In the screening process we were able to rule out those with exclusion criteria [91], but consent must be given before the patient is anaesthetised for caesarean section or as early as possible in cases of MPR and EUCs before the blood loss becomes critical. Had we been able to predict the group of pregnant women with higher probability of being eligible, it could have reduced the time, resources and efforts used to give information and obtain consent from those eventually not eligible.

Postpartum haemorrhage is a clinical condition much feared by clinicians due to the rapid and unpredictable nature [25]. The general clinical view is that this condition should be expected in all labouring women. Several significant risk factors have been described, but reports of Odds Ratios (OR) of presumably independent risk factors do not assist the attending physician in determining the actual risk of the particular women giving birth. In Paper III [89] we assessed the joint predictive ability of having no risk factors compared with one or several and the subsequent need of RBC transfusion. Prediction is by definition related to a particular point in time aiming to assess a subsequent situation or state [103]. We chose five different time points: "During first pregnancy", "At first delivery", "Between pregnancies", "During second pregnancy" and "At second delivery". Thus, by selecting a population of women with a first and second delivery during the available time period of nine years, we were able to include relevant information of first delivery in the assessment of PPH risk at second delivery.

Sensitivity (describing the likelihood of true positive predictors among those who are truly ill) and specificity (describing the likelihood of true negative predictors among those who are truly not ill) are characteristics of predictive criteria/dichotome models. However, what the clinician really wants to know bedside is the positive and negative predictive values (e.g. if the test is positive what is the likelihood that the patient is ill, corresponding to the opposite if the test is negative what is the likelihood that the patient is not ill). Predictive values - positive and negative vary with the prevalence of the investigated outcome unlike sensitivity and specificity. Hence, even tests with optimal sensitivity and specificity will have poorer prediction if the investigated outcome/ illness is rare [103]. We chose a simple approach by characterising the predictive ability of having no risk factors versus one or several. This could be explored further by development of a risk score based on the described risk factors. Such a predictive score could be of clinical relevance when planning place of delivery, need for cross-match or as an inclusion criteria for an RCT or a stratification/ confounder variable [104]. It might also have a value in pre selecting the relevant high risk pregnancies with a probability of becoming eligible.

Practical trial challenges

Deliveries and postpartum haemorrhage happen during all hours of the day and week and this implies several challenges related to a trial set-up. Postpartum haemorrhage is a relatively rare occurring complication with an incidence in blood loss exceeding 1,000 ml of 1.75% [4]. Therefore we planned for

a multicentre setup. However, due to the planned haemostatic monitoring by thrombelastography (TEG®) as well as the need for uniform transfusion guidelines between centres, we were restricted to delivery centres/ hospitals within the Capital Region of Copenhagen. Due to practical reasons and to ensure higher risk of transfusion (primary outcome) only patients in contact with the operation theatre and anaesthetic personnel were included. It seemed most obvious to have the obstetricians to obtain consent; they are the first physicians to be present when bleeding tends to be critical in a postpartum scenario, and they may have been in contact with the labouring woman before this critical event occurs. Thus, the trust gained between woman and obstetrician would be beneficial during inclusion. The regulations of Good Clinical Practice imply, that curriculum vitae and extensive documentation of training apply to all personnel involved in trial related procedures [105]. This would mean that on top of training and obtaining curriculum vitas from all anaesthetic doctors (in charge of trial drug administration and data collection) and from all anaesthetic nurses (in charge of drug dispensation, randomisation and blood samples during the next 24 hours), we would have been obliged to address a large group of obstetricians. This resulted in a compromise in which the anaesthetist doctor obtained consent, but obstetricians and midwives were encouraged to give information prior to consent. The involvement of at least four different staff groups at four centres was a great administrative challenge.

Trials may attempt to answer different aspects of treatment effect. If the settings are optimal with every aspect of treatment being within the highest standards, then researchers are able to answer the question of absolute efficacy [106]. It is difficult or at least resource intensive to conduct an efficacy trial with a dedicated research team available during all hours ready to respond when the acute setting occurs. We encountered this challenge in a smaller scale due to the need of immediate handling of blood samples (haemostatic parameters would be lost if analysed the next day), and therefore we had to have a team of medical students to handle blood samples for the bio bank in a centre where the Blood Bank did not have the resources to assist.

If a trial is being run in an environment much like everyday life, we are able to investigate effectiveness in what is being called "a pragmatic trial" [107]. The main advantage of this setup is the higher external validity of the findings. The disadvantage is that several other factors of treatment regimen are being tested - not only the treatment itself [106]. Intervention and procedures need to be simple and easily applied in a pragmatic trial setup with the attending physician in charge of the enrolment. Previous reports of fibrinogen concentrate in obstetrics evaluate the efficacy in a population of patients with hypofibrinogenaemia (Appendix 2). The FIB-PPH trial aimed to address early pre-emptive treatment with fibrinogen concentrate, and we were afraid that the concept of "initial treatment" would be lost if haemostatic measurements were the final inclusion criteria to be met before randomisation. Randomisation seeks to eliminate selection bias, but in smaller trials baseline imbalance may occur, and stratification is a solid possibility of balancing known severe confounders⁸⁶. In the FIB-PPH trial we stratified by centre [91], but choose not to attempt stratification on parameters such as caesarean section versus vaginal delivery, amount of blood loss, use of tranex-

amic acid or vital signs due to the risk of confusing investigators and thus wasting important time during this essential trial stage.

Only 2 of 6 RCTs used blinding while assessing the efficacy of fibrinogen included in the Cochrane review [87]. In particular outcomes such as transfusion requirements may easily be prone to performance bias. In the FIB-PPH trial we blinded patients, clinicians and outcome assessors by using yellow syringes and dispensation by an allocated un-blinded nurse or doctor not involved in the treatment of the patient [91] or evaluation of outcome. We plan to evaluate this method and the completeness of blinding.

External validity

External validity is the description of to which extent the results of a study can be generalised to “real life” or to another setting or location [106]. In Paper III [89] we used national databases of deliveries (The Danish medical birth registry and the Danish transfusion database) hereby achieving the ability to assess risk factors in the entire national population. In this case external validity would be a more international perspective reflecting differences in health care or transfusion practice or a timely matter reflecting differences in transfusion strategies between the study period (2001-2009) and now. In relation to the FIB-PPH trial we deliberately chose few operational exclusion criteria based on the assumption of an increased risk of thrombosis: Related to the treatment regime of one dose administered to all (antithrombotic during pregnancy, inherited bleedings disorders or pre pregnancy weight below 45 kg) or the inability to assess the primary outcome (refusal to receive blood transfusion) [91]. In the Capital Region of Copenhagen only 1% of deliveries take place at home, so the study population reflects a general population of labouring women.

Meta-analysis and the challenge of few included studies

This thesis consists of two systematic reviews with meta-analyses (Paper I [108] and Paper II [88]); each consisting of six trials with 248 patients randomised and nine trials with a total of 776 patients, respectively. Compared to a single trial, a meta-analysis enables a more precise estimate of the treatment effect gained through more statistical power (larger sample size) [109]. The objective of a meta-analysis is generally to assess broader questions of treatment effect, and thus enable generalizability. This is the result of pooling different studies with the same intervention and outcomes irrespective of the clinical heterogeneity, differences in trial design and statistical dispersion of data. Small RCTs with narrow inclusion criteria (and often low external validity) tend to exaggerate the treatment effect, and when several of these are included for meta-analysis, the effect estimate tends to vary a lot from study to study [93]. The fixed effect model assumes the dispersion of the observed effects to reflect sampling error, so this model of analysis is best applied if the heterogeneity between studies is low. If heterogeneity is high we may apply the random effect model where the dispersion is assumed to be real. However, in cases of few trials/ randomised patients this assumption may not be true leading to errors in both effect estimate and confidence intervals [110].

We chose to carry out meta-analyses in both reviews; Fibrinogen for bleeding patients (Paper I [108]) and VHAs to guide

haemostatic treatment (Paper II [88]) despite few trials and randomised patients. This attempt was chosen in order to summarize the body of evidence in two rather new fields of research: In each case the review provides an overview of RCTs published in nine different databases with no language restrictions. Thus, in the case of Paper II [88] we had one of the included publications translated from Turkish. The reviews also provide a detailed summary of ongoing trials in the field. Each included trial was described systematically in detail (Tables/ characteristics of included trials) giving an overview of populations (with inclusion and exclusion criteria), the treatment and comparison regime, duration of intervention, follow up and an extensive risk of bias assessment. The reviews provide pooled estimates of treatment effect with indications of the dispersion of results. With the application of trial sequence analysis we provided an estimate of the statistical reliability of data in the cumulative meta-analysis by combining a cumulative sample size of all included trials with the threshold of statistical significance [111].

Extending research to “real life” clinical recommendations and guidelines

The role of systematic reviews with meta-analysis such as that of the Cochrane collaboration is not to give recommendations of clinical practice – they aim to give a summary of evidence [94]. Evidence based clinical guidelines use a similar systematic method, but they aim to give clinical recommendations based on the available evidence, also in cases where evidence is missing. The GRADE system (Grading of Recommendations Assessment, Development and Evaluation) was used in Paper IV. This system uses a scheme based on a systematic classification of recommendations (strong/ GRADE 1 or weak/ GRADE 2) and quality of evidence reflected in the confidence in estimates of effect (levelled as high (GRADE A), moderate (GRADE B), or low (GRADE C)) [112]. Paper IV is aimed to provide general recommendations regarding obstetrical bleeding, and thus it includes both aspects of anaemia and haemostasis giving recommendations on assessment/ diagnosis as well as treatments. It consists of chapter 8.2.2 “Obstetric bleeding” of a 112 paged guideline [90], and it supplements more general chapters by highlighting the specific subgroup challenges related to the obstetrical population. I deliberately separated the obstetric and the gynaecological part (even if these are often combined), due to the specific challenges of coagulation and thrombotic risk associated with pregnancy. I did not find it reasonable to support a pooling of gynaecological cancer patients and those with obstetrical bleedings – gynaecological cancer patients probably have more in common with other cancer patients regarding challenges of bleeding.

DISCUSSION

This thesis includes five studies aimed to support the investigation of the use of fibrinogen concentrate as initial treatment for PPH by assessment of the current evidence for the use of fibrinogen concentrate [87] and VHA [88] in bleeding patients and the exploration of postpartum transfusion predictability and current evidence-based recommendations [90]. In addition, the concepts, challenges and methodology were explained for an independent randomised trial investigating the efficacy of a haemostatic drug in a population of parturients with severe PPH [91].

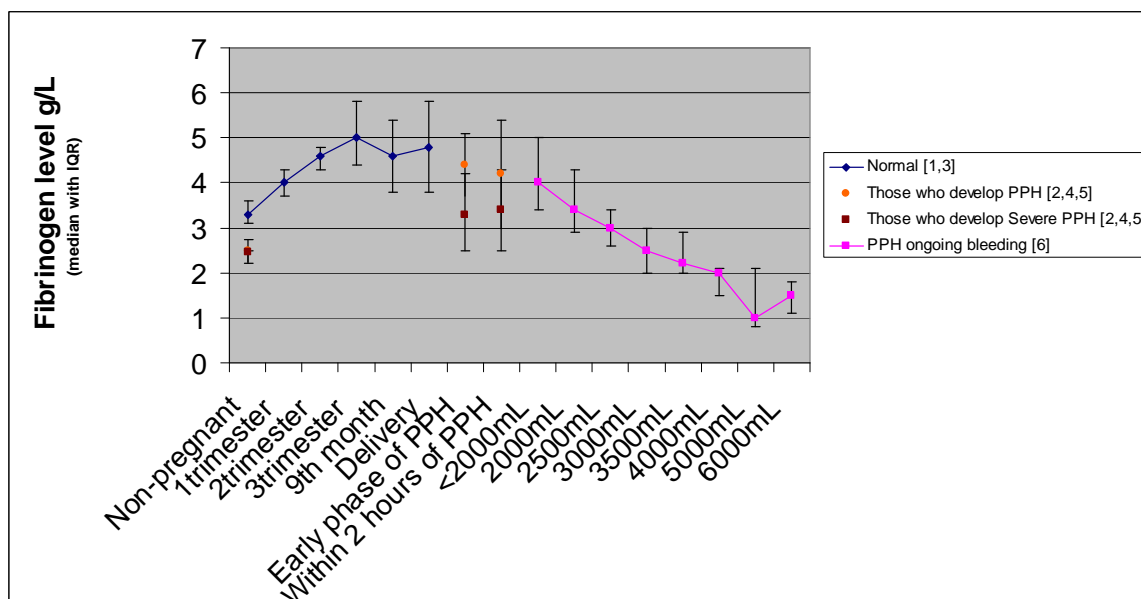


Figure 3

Fibrinogen level (median with IQR) at different clinical time points.

PPH=postpartum haemorrhage, [1]=Huisoud [39],[2]=Chauleur [115],[3]=Simon [116], [4]=Charbit [70], [5]=Cortet [71], [6]=De Lloyd (estimation based on reference Figure 1) [74].

Strengths and limitations

It is crucial to select a clinically relevant non-surrogate outcome when conducting a clinical trial. Mortality is perhaps the best outcome in all cases summarizing both benefits and harms. However, maternal mortality and morbidity in the developed countries are very low [2] and in our case this was not a feasible outcome measure for a randomised controlled trial. We chose "incidence of allogenic blood transfusion" as the primary outcome since it is an indicator of "near miss" [9] and increased awareness of immunological transfusion complications has motivated clinicians and patients to avoid blood transfusion when possible [18]. However, the incidence of postpartum haemorrhage and especially transfusion is also low in the group consisting of all women giving birth [16].

The relevance of an RCT addressing early pre-emptive treatment seems justified in the light of the low grade of evidence [90,113] to support an increased use of fibrinogen concentrate in relation to PPH [81]. Our decision to investigate a 33% risk reduction is justified by the results of our systematic review [87], which identified a 53% risk reduction in the need for allogenic transfusions - even if a smaller treatment effect is anticipated due to early pre-emptive treatment and no impaired haemostasis from cardiopulmonary by-pass. There seems to be a benefit from the use of VHA to guide treatment in terms of a reduced need of haemostatic transfusions (FFP and platelets) and blood loss [88]. However, in order to evaluate the early pre-emptive treatment regimen we abandoned plans for guiding the administration of fibrinogen concentrate by VHAs in the RCT.

We found that 1.6-2.1% of Danish vaginal deliveries were complicated by retained placental tissue (Paper III [89]) and 23.8-25.0% (This data is not shown in Paper III [89]) of these needed RBC transfusion translating to an anticipated incidence of blood transfusion of approximately 25% in the potentially

eligible vaginal deliveries. This proportion should be compared with the estimates of our sample size from the early preparation phase of the RCT: it was based on published obstetrical data mainly from other countries anticipating an incidence of 57% (Details of the original sample size calculation (only outlined in Paper V [91]): Incidence of postpartum transfusion was reported to be 0.17-2.7% [12,13,15,139], and we estimated the incidence to be 1% among Danish deliveries. This estimate refers to the incidence of PPH exceeding 1000 ml of 1.75% [4] which yielded an estimate of 57% incidence of transfusion in control/placebo group ($0.01/0.0175=0.57$). With a risk reduction of 33%, $\alpha=0.05$, power=80%: this translates to 107 patients in each group. With 15% margin for "drop-outs" a sample size of 245 was determined).

In addition the obstacle of getting an informed consent in a situation with ongoing bleeding may further influence the strength of our findings despite complete enrolments.

Initial low fibrinogen in postpartum haemorrhage: Causes and implications

The fibrinogen level increases during pregnancy in parallel with several other adaptations of haemostasis in a state of somewhat balanced hypercoagulability induced by oestrogens [36,43]. This seems to be a reasonable evolutionary adaptation since humans have an increased risk of PPH due to a more invasive placenta [114]. However, currently we do not know if PPH treatment ought to follow general haemostatic triggers or if the entire system is actually balanced differently implying higher pregnant-specific triggers: The first considers the excess coagulability of pregnancy to be an elevated baseline level allowing for larger blood loss' before depletion and the last may imply a complete re-evaluation of haemostatic PPH substitution strategies still keeping the increased puerperal thromboembolic risk in mind. Figure 3 summarises fibrinogen levels at different time points during pregnancy, at delivery

and after onset of PPH based on available published data [39,70,71,74,115,116].

The hypothesis of the FIB-PPH trial is based on the assumption that the relative acquired fibrinogen deficiency during the early phase of PPH described by Charbit et al. [70] play a causative role in the later development of a severe cause of bleeding. This is being caused by the relative impaired haemostasis resulting in an increased bleeding, even if the applied fibrinogen threshold of 2 g/L is within the low end of the non-pregnant normal-level and higher than the usual substitution trigger of 1-1.5 g/L [117]. If we can show a reduced risk of postpartum transfusion by increasing the fibrinogen level to at least the normal level of pregnancy-at-term, our results will support the use of a higher fibrinogen substitution threshold during PPH.

This assumption of a causative association might be questioned when looking closer at the Charbit results [70]: The difference between the initial fibrinogen level in the severe group and the non-severe group (time point: "early phase of PPH" in Figure 3) is suggested by the authors to be caused by an initial excess consumption of fibrinogen in the women who develop a severe course of bleeding. However, this may merely reflect a different amount of blood loss at inclusion: Inclusion criteria in the Charbit study [70] were based on the need for sulprostone (uterotonic) i.v. in accordance with the French guidelines from 2004 [118]: "Sulprostone infusion must be started without further delay if the first treatment (oxytocin, manual removal of the placenta, uterine revision, vaginal and cervical examinations) has been unsuccessful in the first 30 minutes after delivery." The authors favour this criteria due to the well described difficulties of estimating postpartum blood loss [5]. However, the gained precision of such inclusion criteria based on clinical treatment decisions remains unsubstantiated. This leads to a serious limitation of the Charbit study [70] caused by the fact that women who develop severe PPH might have bled larger amounts in this setting before inclusion. No data is provided on blood loss at the time of inclusion (The corresponding author of the Charbit study has been contacted for further information November 2013, but I received no reply), but the thought of an imbalance is supported by the authors reporting a trend towards a longer delay between delivery and the start of sulprostone infusion in the severe group. Charbit et al. [70] found no difference in the haemoglobin decrease between pre delivery and the inclusion and since no blood products were given before initial coagulation assessment they argue against such difference in the baseline blood loss between the groups. However, no data was reported regarding administered i.v. fluids prior to inclusion (e.g. dilution) and since fibrinogen seems to have a stronger correlation to blood loss [74] than haemoglobin and perhaps some heterogeneity in the pre delivery haemoglobin measurements, I believe such baseline imbalance may not be ruled out.

Most of the retrospective results of studies investigating the association between PPH and the fibrinogen level suffer the problem of missing data (see Appendix 1): Thus, when results are based on a subset of included patients with available coagulation screening, they are likely influenced by selection bias (confounding-by-indication). This is caused by clinicians ordering coagulation screening only when impairment is suspected, thus increasing the likelihood that fibrinogen is low compared to those not having a coagulation screening. Charbit et al. [70]

based prediction and adjusted analysis on 113/128 women and did not account for the 15 patients (11%) that was left out of the analysis [70].

The association between a large blood loss depicted in a low fibrinogen level and the development of severe PPH is further supported by Charbit et al. using a composite definition of "Severe PPH" [70]: Most (47/50) patients fulfilled the criteria of a haemoglobin decrease of ≥ 4 g/dL or transfusion of at least 4 RBCs (9/50), and these measures are closely related to the amount of blood loss. Charbit et al. [70] reported a 24 hour incidence of RBC transfusion of 13% (17/128), incidence of FFP transfusion of 11% (14/128) and 0.7% (1/128) for platelets and fibrinogen concentrate. Comparing this to our assumed RBC transfusion incidence of 25% our choice of inclusion criteria seems to be justified, but some inconsistency between the French and our setting exists especially in the light of the 11% receiving FFP in the Charbit study. This may be due to a more restrictive French RBC transfusion practice with a generally more proactive haemostatic transfusion practice [81]. Altogether it may indicate that the association between an initial low level of fibrinogen and the later development of severe PPH is in fact to some extent just a depiction of a rapid initial large blood loss especially in the group later developing severe PPH. This reduces the fibrinogen level to a "surrogate" measure of blood loss. Hereby the hypothesised consumption of fibrinogen in the early phase of PPH may be an overestimation, and maybe this mechanism is to be expected only in the very serious cases with severe hypovolemia, acidosis with hyperfibrinolysis and massive blood loss as reported in traumatology (e.g. acute coagulopathy of trauma shock) [119,120].

Fibrinogen concentration decreases with ongoing blood loss [51,74,121](Figure 3), and therefore a hypofibrinogaemic level will be reached earlier and at a lower amount of blood loss, if the fibrinogen-level is at the low end to begin with [121]. Maybe women, who have a pre-pregnant fibrinogen-level at the low end of the normal-level, are more prone to develop PPH following delivery? Such a theory was investigated, but not really confirmed by one study using 6-9 month post delivery haemostatic variables as indication of the pre pregnant state [115]. Thus if they applied the 2 g/L threshold of fibrinogen, the authors found an OR of 4 (95% CI 2-9) for development of severe PPH, but no increased risk of developing non-severe PPH (Appendix 1). The predictive ability was improved by the inclusion of other haemostatic variables and Group O blood type [115].

Women who fail to increase fibrinogen levels or hypercoagulability sufficiently during pregnancy may also be prone to increased risk of PPH? Assessing the risk of subsequent PPH at 9 month of pregnancy using fibrinogen levels < 2.9 g/L (limit represents lower 95% confidence interval of the fibrinogen level in the entire group) gives a positive predictive value of 17% for the prediction of PPH, and if assessed at delivery it increases to 39% (see Appendix 3). This poor prediction was confirmed by another study of pre partum fibrinogen predicting PPH with a blood loss exceeding 500ml [122].

Efficacy of treatment or diagnostic precision

While reviewing VHAs to guide haemostatic treatment [88], we actually evaluated a diagnostic test, not by its diagnostic ability to reproduce results of a "gold standard"-test, but instead we assessed the clinical and patient relevant efficacy of treatment

guided by VHAs. Critics may argue that the pooling of studies with such a broad definition of the intervention is not justified when the considerable heterogeneity of intervention is present [109]. However, we are not comparing the benefits or precision of one assay with another – we are merely comparing the use of them with the generally accepted “standard care” which is equally heterogenic. Therefore in my opinion the results are to be considered “a proof of concept” – similar to when we evaluate treatment protocols or fast-track regimens. The clinical relevance of such treatment evaluations depends on the choice of outcomes (e.g. mortality or reduction in transfusion requirements), and not on surrogate outcome measures like improvement in clot strength at some specific point of time.

Addressing the ethical and practical challenges of clinical trials

The informed consent is an ethical imperative in modern medicine and the prospective evaluation of treatment efficacy. However, it raises several challenges in relation to severe bleeding and intrapartum conditions as well as postpartum haemorrhage [100]. Ultimately it may be considered overwhelmingly problematic, e.g. when ethical research committees demand informed written consent from all pregnant women even if only few will be eligible, and the direct implication will inevitably be a more widespread off-label use with an uncontrolled risk of harm, inefficacy or increased cost. Pharmaceutical companies may have market driven agendas promoting off-label use [80], why advice on the evidence should be sought preferably by independent specialists. As a clinical researcher one is overwhelmed by many practical challenges when aiming to enrol patients in a multicentre randomised setup: A considerable amount of time and effort often goes into trial management and fund-raising. In my opinion, the daily management of the FIB-PPH trial would have benefitted from a research setup with more assisting personnel available at each site; with dedicated work time for trial assignments; increased focus on education of local investigators and possibly also a health system where research is considered a prioritized part of the production. Ultimately we as a community should decide if the pharmaceutical companies are to be the only ones evaluating the efficacy of their own investments.

Are we prepared for an RCT?

The increased clinical coagulation “awareness”, together with the experience gained from the enthusiastic case-report-driven off-label use of recombinant-factor seven-A of the past decade [80], have probably fuelled the widespread implementation of fibrinogen concentrate in clinical obstetric practice. In that sense we as clinicians are more than ready for an RCT aimed to assess the efficacy of fibrinogen concentrate in relation to PPH.

However, there are various objectives regarding the efficacy and these can not all be addressed in one trial: a) fibrinogen concentrate as replacement for FFP or cryoprecipitate, b) the ability of fibrinogen concentrate to reverse the haemostatic impairment of synthetic colloids, c) the role of fibrinogen concentrate in a goal directed treatment of hypofibrinogenaemia using algorithms based on VHAs, d) fibrinogen concentrate as an early pre-emptive treatment and e) the optimal fibrinogen level used to trigger haemostatic therapy during ongoing PPH.

The RCT is considered gold standard for assessing drug treatment efficacy, but expenses and efforts are much higher compared to other study types [84]. However, this should not be an argument for not carrying out proper RCTs - instead it commits trialists to prepare properly and assess feasibility beforehand. In my opinion the optimal preparation in cases of no previously published RCT would be to conduct a minor prospective observational trial in which the planned inclusion criteria, challenges of consent and the incidence of the planned outcome are being tested. These results should guide sample size estimates instead of estimates derived from foreign studies or databases. A prospective clinical observational assessment of the impact of consent, the baseline fibrinogen level and transfusion requirements of the potential eligible patients would most likely have increased the precision and strength of the FIB-PPH trial results since no previous obstetric RCT experiences were available during the planning of the study.

CONCLUSION

Fibrinogen concentrate seems to reduce the need for allogenic transfusion at least in cases of elective surgery and especially in relation to cardiac surgery involving cardiopulmonary bypass. A VHA directed haemostatic treatment significantly reduces blood loss and incidence of haemostatic transfusions, but the impact on mortality is unclear. It is difficult to implement when early pre-emptive treatment is in focus. Increased use of fibrinogen concentrate despite low grade of evidence emphasises the need for an RCT. Prediction of postpartum blood transfusion is difficult especially during pregnancy even if known risk factors are taken into account, and this may increase the challenge of pre-informing eligible patients and thereby improving the likelihood of obtaining consent during ongoing bleeding in the postpartum setting. Another on-going randomised trial investigating fibrinogen substitution during PPH (FFP versus cryoprecipitate) [123] has just been registered at clinicaltrials.gov (assessed 17-12-13) and we are aware of the plans for several others. Hopefully, we will reach another level of evidence regarding fibrinogen and haemostatic assessment in PPH in the decades to come.

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The present thesis includes the publication of the FIB-PPH trial protocol, but was written after the completion of enrolments and in parallel with the data validation and preparations for the FIB-PPH trial publication published in January 2015 [140].

SUMMARY

Pregnancy is a state of hypercoagulability that might be an evolutionary way of protecting parturients from exsanguination following child birth. Observational studies suggest an association between a low level of fibrinogen (coagulation factor I) at the start of postpartum haemorrhage (PPH) and subsequent severity of bleeding. Fibrinogen concentrate may be prescribed to correct acquired hypofibrinogenaemia, but evidence is lacking regarding the treatment efficacy. This thesis assesses the current evidence for the use of fibrinogen concentrate and haemostatic assessment in bleeding patients with special attention to the obstetrical population.

It includes five papers: In Paper I the benefits or harms of fibrinogen concentrate in bleeding patients in general was evaluated using a systematic Cochrane review methodology with metaanalysis of all published randomized controlled trials (RCTs). Six trials with high risk of bias were included (248 patients). Fibrinogen appeared to reduce the need of allogenic transfusions by 53 %. However, the included trials were conducted only in an elective surgical setting with a population of mainly cardiac surgical patients. Paper II was also a systematic review based on Cochrane methodology evaluating the use of viscoelastic haemostatic assays to guide haemostatic transfusion in bleeding patients. Nine RCTs (776 patients) with high risk of bias were included primarily in elective cardiac surgical patients and none were specific for the obstetric subpopulation. Viscoelastic haemostatic assay guided transfusion algorithm reduced blood loss and the proportion of patients exposed to fresh frozen plasma (FFP) or platelets. In both studies, we were unable to make firm conclusion on our primary outcome, "all cause mortality" due to lack of adequate data. Paper III was based on two national Danish registries evaluating the predictability of postpartum blood transfusion. Prediction was found difficult. However, retained placental parts seemed to be the strongest predictor. Since this diagnosis is made very late and often in association with the onset of bleeding, tools to perform an early diagnosis is highly warranted. Paper IV includes recommendations of the European Society of Anaesthesiology regarding the use of fibrinogen concentrate in PPH, and is based on very weak (GRADE 2) evidence and low confidence in estimates of effect (GRADE C). Paper V describes the protocol for a randomised controlled trial of early fibrinogen supplementation in women with severe postpartum haemorrhage.

Several practical, ethical and trial management challenges need to be addressed when conducting independent clinical research involving parturients with severe bleeding, placebo-controlled and blinded administration of a drug in a multicenter set-up with enrolments during the entire day and with many personnel involved.

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Appendix 1

Observational studies associating fibrinogen level and postpartum haemorrhage

Author /Year	N=, study design	Population	Outcome	Results
Simon 1997 [116]	N=797, prospective	Inclusion: Women with pre-anesthetic clinical assessment during the ninth month of pregnancy, physical status ASA I or II, and term >36 and <42 weeks at the time of delivery. Exclusions: medical history of haemostasis disorder (von Willebrand disease, diabetes mellitus, idiopathic thrombo-cytopenic purpura or lupus erythematosus), in anticoagulant treatment and planned elective caesarean sections. They seem also to have excluded the following conditions: Placenta praevia, placental abruption, Fever > 38.5, gestational hypertension with and without pre-eclampsia.	Severe clinical PPH: Postpartum blood loss requiring volume expansion and manual uterine exploration associated with either blood transfusion or a significant decrease (= 2 g/dL) in haemoglobin concentration, or both, within 24 h postpartum.	- Fibrinogen level measured before labour significant lower in women with vaginal delivery and PPH than those not developing PPH: Positive predictive value of fibrinogen < 2.9 g/L at 9th month pregnancy: 16.6% and at labour 39.1% (sensitivity 7.3 and 19.6, respectively). - Abnormal fibrinogen and platelet count measured at 9th month pregnancy did not predict the same abnormality at labour. NB! The objective of this trial was to assess changes in haemostatic parameters between 9 month pregnancy and delivery in estimation of the risk associated with epidural catheter insertion.
Charbit 2007 [70]	N=128, prospective	Inclusion: (PPH) Parturients with uterine bleeding, occurring in the first 24 h after delivery, persisting after manual exploration of the uterine cavity, and requiring i.v. prostaglandin administration according to French guidelines.	Development of Severe PPH within 24 hours: peripartum decrease of Hb = 4 g/dL (last Hb value before delivery considered as the reference); transfusion of at least 4 RBC units; haemostatic intervention (angiographic embolization, surgical arterial ligation or hysterectomy); or death. (39% of the included)	- The positive predictive value of an initial fibrinogen concentration <2 g/L was 100% (71-100%) for the development of severe pph. - Women who developed severe PPH had a significantly lower fibrinogen level during the whole course of bleeding. NB! Women in severe group might have bled more at initial stage explaining the association. Only 113/128 had data on initial fibrinogen (no explanation given)
Chaleur 2008 [115]	N=951, case control study	Inclusion: First intended pregnancies giving birth. Investigated with several tests of haemostatic variables and DNA 6-9 months after delivery.	PPH: (uterine bleeding occurring within 24 hours post delivery persisting after manual exploration of the uterine cavity and requiring i.v. prostaglandin): - Severe PPH (defined by decrease in haemoglobin > 40 g/L, transfusion of at least 4 RBCs or haemostatic intervention (surgical uterine sutures, artery ligation, artery embolization, hysterectomy) or death). - Non-severe PPH (the rest).	- Fibrinogen level was not significantly different between no PPH/PPH/severe PPH groups. - Fibrinogen < 2 g/L measured 6-9 months AFTER delivery is independently associated with severe PPH (but not non-severe PPH). - Authors conclusion "Women with some hemostasis-related variables at the low or high end of the population distributions are prone to the severe forms of PPH". NB! Haemostatic variables were taken 6-9 months AFTER delivery. These are interpreted as if they were taken BEFORE delivery.
Huissoud 2009 [14]	N=37, prospectively collected, with a case control like design (control group is from previously published work (n=54)39)	Control group: Normal pregnancies without bleeding.	Women with PPH: Estimated blood loss > 1000 ml during caesarean section or >500 ml after vaginal delivery.	- Objective was to correlate fibrinogen assay with ROTEM/ FIBTEM assay. - Fibrinogen level was significantly lower in haemorrhage group, but no indication of when the samples were taken (e.g. blood loss at the time of sampling). - Report corresponding level of fibrinogen < 2g/L being FIBTEM level CA after 5 min = 6 mm and CA after 15 min = 8 mm. NB! More than one sample was collected from each patient, but apparently they were statistically treated as independent.
Tempfer 2009 [124]	N=104, retrospective cohort study, monocenter, ten years period	Inclusion: Intrauterin foetal death (IUFD) > 24 weeks of gestation or a birth weight of > 500 g.	Outcome: Delivery complication associated with coagulopathy determined by need for blood transfusion (11.5% of included).	- Regarding fibrinogen: Significant lower fibrinogen level prior to delivery in women with outcome 2.5 g/L vs 4.7 g/L. - "Although all 4 women with IUFD who later developed a coagulopathy had coagulation parameters outside the laboratory thresholds at the time of presentation, this was also the case for 34% of women with IUFD who did not develop any clinical coagulation problem. Thus based on our data, it is not justified to recommend coagulation monitoring after a diagnosis of IUFD."
de Lloyd 2011[74]	N=456, retrospective chart	Inclusion: PPH: estimated blood loss = 1500 ml.	Outcome: Total estimated blood loss. Variables included lowest value of fibrinogen.	- The lowest recorded fibrinogen level fell progressively as estimated blood loss increased. NB! Coagulation screen was only available on 78% of patients. No timing was stated in regards to when "lowest" fibrinogen was measured during the course of bleeding.
Gayat 2011[75]	Two phase study: I: N=257, retrospective monocentre, II: N= 239, prospective multicentre – validation study	Inclusion: I: PPH defined by diagnosis in chart, most were transferred from other delivery centres. II: PPH (no definition) admitted to seven French referral centres.	Outcome: The need for an advanced interventional procedure (AIP) to stop genital tract bleeding defined as uterine artery embolization, intraabdominal packing, arterial ligation or hysterectomy.	- Fibrinogen < 2 g/L at admission is significantly associated with AIP compared to "medical managed-group" (1.8 vs 2.65 g/L). OR of 2.75. - Prediction score of AIP includes abnormality of placental implantation, Prothrombin time <50%, heart rate > 115 bpm, fibrinogen < 2 g/L and troponin I detectable.
Butwick 2011 [125]	N=52, prospective	Inclusion: Elective caesarean section with Pfannenstiel incision and uncomplicated singleton pregnancy, ASA I-II, age 18-40, Spinal anaesthesia. Exclusion: hypertension, preclampsia, liver disease, diabetes mellitus, inherited or acquired coagulation deficiencies, platelet count< 100x10 ⁹ , antithrombotic treatment or NSAID and if in active labour.	Outcome: Estimated blood loss.	- Estimated blood loss was 772 +/- 305 ml. Fibrinogen decreased significantly from 5.3 g/L to 4.1 g/L before and after caesarean section. - All patients were "primed" with 500 ml HES and given a mean of 1600 ml +/- 400 crystalloids. A weak correlation between MA in kaolin TEG/dot strength and estimated blood loss. A modest reduction in maternal hypercoagulability following caesarean section. NB! Findings are probably due to HES infusion; this is not discussed by authors.
Cortet 2012 [71]	N=738, secondary analysis of prospectively collected data, population based	Inclusion: Primary PPH following vaginal delivery (PPH defined by blood loss =500 ml or decrease in haemoglobin = 20 g/L) and with fibrinogen measured within 2 hours of PPH diagnosis. Exclusion: surgical cause of bleeding (uterine rupture, wound of birth canal, placenta accrete and placenta praevia).	Outcome: Severe PPH: peripartum haemoglobin decrease =40 g/L, transfusion of concentrated red cells, arterial embolization or emergency surgery, admission to intensive care, or death (44% of included).	- Mean fib in severe group was 3.4 g/L +/- 0.9 and 4.2 g/L +/- 1.2 in non-severe. - Initial level below 2 with OR 12 (2.6-56.1) and below 3 with OR 1.9 (1.2-3.1). NB! Only sensitivity and specificity was reported – not predictive values. High risk of selection bias by excluding 88% due to not having fibrinogen measured They also exclude 1013/7337=14% due to surgical cause of bleeding.
Poujade 2012 [72]	N=98, retrospective monocentre, but includes also referred patients	Inclusion: PPH with uterine artery embolization.	Outcome: Success of pelvic embolization: cessation of the haemorrhage with hemodynamic stability and the absence of any subsequent surgical procedure.	- Low Fibrinogen level (and other haemostatic measures) was associated with treatment failure. But embolization seemed overall successful.
Frimigacci 2012 [73]	N=310, Retrospective cohort (in French)	Inclusion: Severe PPH, transferred from primary care centre due to need of further treatment than sulprostone i.v.	Outcome: Need for treatment. i) Just medical treatment and blood products, ii) surgical interventions on top, iii) additional need of explorative laparotomy due to bleeding.	- A low level of fibrinogen (2 g/L versus 3 g/L) is associated with increased need of surgical treatment. NB! Few included had fibrinogen measured (no numbers given).
Peyvandi 2012 [122]	N=4461, secondary analysis of prospectively collected data (letter to editor)	Inclusion: Vaginal deliveries Exclusion: Age below 18, delivery before 37th gestational week, twin pregnancies, no comprehension of Italian language, refusal to sign written consent, deliveries occurring Friday afternoon, with no information on blood loss during delivery and those with no information on prepartum fibrinogen levels (=18%).	Outcome: PPH with blood loss =500 ml (24% of included).	- Prepartum fib was a poor predictor of PPH: Prepartum level of fibrinogen was equal in women who developed PPH and those who did not. AUC=0.51 [0.49-0.53]. NB! No information on prepartum fibrinogen levels in 18%. Prepartum fibrinogen was measured median 16 days prepartum.

Appendix 2

Published reports and interventional studies with fibrinogen concentrate and obstetrical bleeding

Name/Year	N=, study design	Population	Intervention	Comparison	Outcome and follow-up	Author Conclusion
PROSPECTIVE STUDIES						
Ducloy-Bouthors 2008 [126]	N=16, prospective, uncontrolled (Abstract from Symposium)	PPH: No inclusion or exclusion criteria given, but baseline blood loss of median 1700 ml.	Fibrinogen concentrate (FGT1, LFB) 30 mg/Kg	None	Failure of treatment defined as when ultimate resources were required to stop haemorrhage* or in case of massive transfusion or death. *Defined as invasive haemostatic intervention or treatment with Recombinant factor seven A. Adverse events	- Fibrinogen concentrate controlled bleeding in 75% of 12 cases NB! This small study has not been published (communication with author). But it will serve as basis for the plans of a future study. Only 12/16 patients were "clinically assessable" and evaluated in analysis
RETROSPECTIVE REPORTS						
Fenger-Erksen 2008 [127]	N= 43, retrospective, monocentre	Mixed population of bleeding patients with acquired hypofibrinogenemia (< 2g/L) treated with fibrinogen concentrate off-label. 28% obstetrical patients	Fibrinogen concentrate 2 g (range 1-5 g) administered	None	Transfusion requirements before and after administration Coagulation parameters before and after administration	- Transfusion requirements were significantly reduced following administration - Significant increase in fibrinogen level
Danes 2008 [128]	N= 69, retrospective	Mixed population with acquired hypofibrinogenemia (<1g/L) caused by bleeding or reduced synthesis and treated with fibrinogen concentrate. 9% obstetrical patients	Fibrinogen concentrate 3.5 g (range 0.5-8 g)	None	Coagulation parameters before and after administration Correlation between survival and fibrinogen level	- Fibrinogen levels improved by administration of fibrinogen. - "Our data indicate a direct relationship between plasma fibrinogen levels and survival in acquired fibrinogen deficiency"
Weinkove 2008 [129]	N=30, retrospective	Mixed population with acquired hypofibrinogenemia (<1.5 g/L) caused by bleeding and treated with fibrinogen concentrate. 33% obstetrical patients	Fibrinogen concentrate 4 g (range 2-14 g)	None	Increase in fibrinogen level following administration	- "Purified, virally inactivated fibrinogen concentrate appears effective in the management of acquired hypofibrinogenemic states and enables rapid administration of a known fibrinogen dose in an emergency setting"
Chaleur 2008 [13]	N=317, secondary analysis of prospective cohort Selected from cohort of first intended pregnancies giving birth	Severe primary PPH persisting after manual exploration of the uterine cavity and requiring i.v. prostaglandin and having a peripartum decrease of haemoglobin >40 g/L, at least 4 RBC transfusions, haemostatic intervention (surgical uterine sutures, artery ligation, artery embolization, hysterectomy) or death. Excluding previous occurrence of superficial or deep vein thrombosis in patient or first degree relative, antithrombotic treatment during pregnancy	Standard transfusion treatment including fibrinogen concentrate given at plasma level <1g/L and dose calculated based on weight and plasma concentration Median quantity 3 g (range 1.5-7.5)	Those not receiving fibrinogen concentrate or FFP or platelets	Venous thromboembolism (2 cases of superficial and 0 cases of deep vein thrombosis and pulmonary embolism)	Regarding fibrinogen: - Transfusion with platelets, plasma or fibrinogen do not markedly modulate the risk of venous thrombosis NB! Only 9% of patients received fibrinogen concentrate
Guasch 2009 [130]	N=124, observational, monocentre (in Spanish)	Severe PPH (no definition)	Fibrinogen concentrate was part of routine PPH transfusion protocol. Mean dose of 3.6 +/-1.4 g	None	Increase in fibrinogen level results"	Regarding fibrinogen concentrate: - "Use of fibrinogen concentrate is common and provides good - Level of fibrinogen before haemorrhage was 6 g/L, before transfusion 3.4 g/L and post transfusion 4.7 g/L NB! 49% of patients received fibrinogen concentrate
Bell 2010 [131]	N=6, case reports	Obstetrical haemorrhage with hypofibrinogenemia (pre-treatment fibrinogen level: 0.5-1.2 g/L)	Fibrinogen concentrate 2-4 g	None	Narrative case description	- "Fibrinogen concentrate may rapidly correct hypofibrinogenemia and should be given with fib level < 2g/L and ongoing bleeding despite initial obstetrical treatment"
Glover 2010 [132]	N=1, case report	Major antenatal vaginal bleeding with DIC following placental abruption and foetal death, fibrinogen level of 1.9 g/L	Fibrinogen concentrate 4 g with 4 units of FFP was administered	-	Narrative case description	- Cessation of bleeding within 10 minutes was reported - Quick and easy administration was noted
Thorarinnsson 2010 [133]	N=37, single centre, retrospective chart	Mixed population receiving minimum 6 RBCs and fibrinogen concentrate. Mean blood loss of 5675 ml. 4 (11%) obstetrical cases. Excluding repeated doses of fibrinogen concentrate, those receiving factor VIIa	Fibrinogen concentrate 2 g (1-6g)	None	Haemostatic increments, transfusion requirements, and survival (in-hospital and 6 months)	"...a single dose of 2 g fibrinogen concentrate improves coagulation and reduces RBC transfusion in severe haemorrhage"
Bonnet 2011 [134]	N=38, retrospective audit	Maternal deaths from PPH	Fibrinogen concentrate was given to 47%.	None	Outcomes relates to several organisational measures such as delay and treatment failure	No specific conclusion regarding fibrinogen concentrate: "Hemodynamic monitoring, repeated laboratory assessments and the protocols for general anaesthesia as well as several aspects of transfusion procedures including FFP/RBC ratio may be improvable"
Weiss 2011 [135]	N=223, multicentre, chart based	Mixed population fibrinogen concentrate administration due to bleeding in a "polypragmatic therapeutic concept". Approx. 2 L blood loss and fibrinogen level of 1.5 g/L 9 (6%) obstetrical patients	Fibrinogen concentrate 4 g (2-4 g) and FFP with 8 g of fibrinogen content (5-12 g)	None	Haemostatic increments Survival during hospital stay	ΔFibrinogen was 0.045 g/L pr gram of fibrinogen concentrate Massive haemorrhage seems to necessitate the administration of large quantities of fibrinogen to at least maintain physiological plasma concentrations Higher levels seem to be associated with survival
Ahmed 2012 [136]	N=77, chart audit, Allocation to either fibrinogen or cryoprecipitate was decided by change in Blood Bank policy	Major obstetric haemorrhage (estimated blood loss =2.5 L, transfusion of =5 RBCs or treatment of coagulopathy in the acute event)	Fibrinogen concentrate replaced Cryoprecipitate as standard treatment from July 2009, N=20	Cryoprecipitate was standard treatment until July 2009, N=14	Several outcomes Estimated blood loss, mean hematocrit, minimum platelet count and fibrinogen level, transfusion of RBCs, Plasma transfusion, platelet transfusion, clinical outcomes and adverse reactions, fibrinogen level post-treatment, high dependency unit stay and hospital stay	- 44% received cryoprecipitate or fibrinogen concentrate, All treated had fibrinogen level below 2g/L - No significant change in any outcome comparing fibrinogen group with cryoprecipitate group - "We have shown that a purified virally inactive fibrinogen concentrate is as efficacious as cryoprecipitate in correcting hypofibrinogenemia" NB! Authors call it "prospective", but they describe retrospective data collection
Gollop 2012 [137]	N=63, retrospective review of reported cases	Mixed population with hypofibrinogenemia (no explicit criteria) 14% obstetric (9 patients)	Fibrinogen concentrate off-label use: 49 (26-61) mg/kg, Received by 49 (77%)	No treatment with fibrinogen concentrate Received by 14 (13%)	Rate of bleeding and transfusion requirements, thrombotic events	- Clinicians reported that fibrinogen contributed to arrest of bleeding. Reduction in amount of RBC transfusions after infusion - Three venous and one arterial non-fatal thrombotic event were reported in fibrinogen group. One was in an obstetrical patient NB! High risk of underreporting of hypofibrinogenemic patients not treated with fibrinogen concentrate, e.g. selection bias
Kikuchi 2013 [138]	N= 18, retrospective audit	Fibrinogen concentrate administered to continuous massive haemorrhage (=1000 ml) with coagulopathy and acquired hypofibrinogenemia (<= 1.5 g/L)	Dilutional coagulopathy: atonic bleeding, uterine inversion, placenta previa or birth canal trauma. (total bleeding 3900 +/-1600 ml)	Consumptive coagulopathy: Placental abruption, amnion fluid embolism (total bleeding 2900 +/-1800 ml)	Cessation of bleeding ("good" if bleeding <=1000 ml following administration, "moderate" if bleeding was >1000 ml and "poor" if bleeding necessitated subsequent selective arterial embolization or surgical intervention (laparotomy or arterial ligation)). Adverse events until 28 days post administration	- Overall response to fibrinogen concentrate was considered good in 12 cases, moderate in four and poor in two - Response rate was equal in the two groups (consumptive/dilutional) and fibrinogen levels significantly increased following administration in both groups - No serious adverse events were causally associated with fibrinogen substitution therapy
Bonnet 2013 [81]	N=426, secondary analysis of prospectively collected data, population based	PPH: Clinical PPH (blood loss >500 or excessive blood loss prompting manual removal of the placenta or examination of the uterine cavity) requiring red blood cell transfusion within 12 hours after diagnosis	Fibrinogen concentrate was given routinely as part of transfusion management. 20% received fibrinogen concentrate	None	Compliance with 2002 national French transfusion guidelines and 2004 French guidelines on PPH management	Regarding the use of fibrinogen concentrate: - The authors reflect that the use is frequent despite lack of efficacy evidence DANISH MEDICAL JOURNAL 18

Appendix 3

The ability of the fibrinogen level to predict PPH or severe PPH

*PPH (Simon – Outcome definition): Bleeding requiring volume expansion, manual uterine exploration with either blood transfusion or Hb decrease of more than 2 g/dL within 24 hour.

• Initial phase of PPH (Charbit – inclusion criteria): Parturients with uterine bleeding, occurring in the first 24 h after delivery, persisting after manual exploration of the uterine cavity, and requiring i.v. prostaglandin administration according to French guidelines.

**Severe PPH (Charbit – outcome definition): peripartum decrease of Hb = 4 g/dL (last Hb value before delivery considered as the reference); transfusion of at least 4 RBC units; haemostatic intervention (angiographic embolization, surgical arterial ligation or hysterectomy); or death.

***Severe PPH (Cortet- outcome definition): peripartum haemoglobin decrease =40 g/L, transfusion of RBCs, arterial embolization or emergency surgery, admission to intensive care, or death.

Study	Time of prediction	Fibrinogen level threshold used as a predictor	Prediction of outcome	Sensitivity % (95% CI)	Specificity (%) (95% CI)	Positive Predictive value % (95% CI)	Negative predictive value % (95% CI)
Simon [116]	9th month of pregnancy	< 2.9 g/L	PPH*	4.3	98.4	16.6	93.4
	Delivery	< 2.9 g/L	PPH*	19.6	97.8	39.1	94.3
Charbit [70]	Initial phase of PPH* (blood loss is not reported)	<2 g/L	Severe PPH**	-	-	100 (71-100)	-
		<4 g/L	Severe PPH**	74	65	-	-
Cortet [71]	Primary PPH following vaginal delivery with blood loss of at least 500 ml or decrease in haemoglobin of at least 20 g/L (fibrinogen measured within 2 hours of PPH diagnosis e.g. blood loss at sampling is not reported)	<2 g/L	Severe PPH***	12.4 (8.8-16.0)	99.3 (98.4-100)	-	-
		<3 g/L	Severe PPH***	35.5 (30.7-41.1)	89.9 (85.9-91.9)	-	-