Chapter 1

General introduction and outline of this thesis

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Background

Due to the high prevalence of coagulopathy among patients at the intensive care unit (ICU) [1], administration of Fresh Frozen Plasma (FFP) to critically ill patients is common practice. Thirteen per cent of ICU patients receives FFP during their stay at the ICU [2] and this can be up to 60% in specific ICU patient populations [3]. However, knowledge regarding identifying coagulopathy as well as efficacy and adverse effects of FFP in the critically ill is scarce.

This thesis aims to study the risk benefit balance of FFP transfusion. In order to optimize the risk benefit decision to transfuse, we aimed to assess whether identification of patients with a coagulopathy and increased bleeding risk could be improved. In addition, we studied the effectiveness of FFP transfusion to correct coagulopathy in critically ill patients and thereby mitigate the risk of bleeding. To further improve risk benefit assessment, we studied pathophysiology, treatment and prevention of transfusion related acute lung injury, which is the main adverse outcome of FFP transfusion.

Coagulopathy in critically ill patients

Pathophysiology, prevalence and outcome

Coagulopathy is often referred to as “a condition in which the blood’s ability to clot is impaired” [4], however this description is a simplification of reality. The coagulation system consists of three main components. The pro-coagulant elements include the endothelium, thrombocytes, individual coagulation factors and fibrinogen. The anticoagulant system includes proteins C and S and antithrombin. The third component of coagulation is the fibrinolytic system (figure 1). In critical illness, these different components can be deranged in various ways, often resulting in an imbalance due to enhanced activation of coagulation and impaired inhibition of coagulation and fibrinolysis (figure 2). The result is a variable clinical picture with patients with an increased bleeding tendency (“consumption coagulopathy”) and those having disseminated intravascular coagulation (DIC) with the formation of (micro) vascular thrombi, associated with multiple organ failure.
**Figure 1**: Schematic and simplified overview of coagulation cascade, including the anticoagulant and fibrinolysis pathways.

The coagulation cascade (indicated in black) starts with endothelial injury, resulting in the formation of the tissue factor (TF) - factor VII (FVIIa) complex. Subsequently the conversion of prothrombin to thrombin results from the activation of factors X (FXa) and V (FVa). The whole process is enhanced via an amplification loop, thrombin is the key activator of this loop. Ultimately thrombin converts fibrinogen into fibrin resulting in a formation of a stable clot. The anticoagulant pathways are indicated in dashed lines. Activated protein C (APC) inactivates factors Va, while antithrombin (AT) blocks the action of multiple coagulation factors (including Xa and thrombin). Tissue factor pathway inhibitor (TFPI) inhibits the TF-FVIIa complex and hereby the stepwise activation of the coagulation cascade. The fibrinolytic system, responsible for clot degradation with the formation of fibrin degradation products (FDP), is indicated in grey. Thrombin activatable fibrinolysis inhibitor (TAFI) and plasminogen activator inhibitor (PAI-1) are the main inhibitors of fibrinolysis.
Figure 2: Factors influencing the hemostatic balance in critically ill patients.

<table>
<thead>
<tr>
<th>Thrombosis</th>
<th>Bleeding</th>
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<tr>
<td>Increased levels of VWF</td>
<td>Thrombocytopenia</td>
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<tr>
<td>Decreased levels of ADAMTS-13</td>
<td>Vitamin K deficiency</td>
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<tr>
<td>Increased levels of factor VIII</td>
<td>Reduced levels of factors II, V, VII, IX, X, XI</td>
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<td>Reduced proteins C and S and antithrombin</td>
<td>Reduced fibrinogen</td>
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<td>Downregulation of thrombomodulin</td>
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<td>Reduced capacity of TFPI</td>
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<td>Impaired fibrinolysis (increased PAI-1 and TAFI)</td>
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VWF = von Willebrand factor
ADAMTS-13 = a disintegrin and metalloproteinase with thrombospondin type 1, motif 13
TFPI = tissue factor pathway inhibitor
PAI-1 = plasminogen activator inhibitor type I
TAFI = thrombin activatable fibrinolysis inhibitor

Table 1: Main causes of coagulopathy in critically ill patients

<table>
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<tr>
<th>Causes of coagulopathy in critically ill patients</th>
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<td>Sepsis</td>
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<td>Multiple trauma</td>
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<td>Brain injury</td>
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<td>Major blood loss (e.g. gastro-intestinal, obstetric)</td>
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<td>Liver disease</td>
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<td>Disseminated intravascular coagulation</td>
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<td>Use of vitamin K antagonists before ICU admission</td>
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<td>Vitamin K deficiency</td>
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<td>Renal failure</td>
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<td>Cardiac surgery</td>
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<td>Thrombotic micro-angiopathies</td>
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Studies using conventional coagulation tests indicate that the prevalence of coagulation abnormalities in critically ill patients is high. Up to 30-66% of patients have an International Normalized Ratio (INR) of >1.5 or a prothrombin (PT) ratio of >1.5 [1,5] and 8 to 45% of patients develops thrombocytopenia during their intensive care unit (ICU) stay [6-9]. The most common causes of deranged coagulation are summarized in table 1 [4]. Of note, irrespective of the underlying disease, the occurrence of both prolonged PT/INR [1] and thrombocytopenia [7,9] are independently associated with increased mortality in critically ill patients. Furthermore, in addition to a hypocoagulable state, up to 20% of critically ill patients develops DIC and the resulting hypercoagulable state is associated with multiple organ failure and high mortality (45-78%) [10].

**Assessing coagulation status in critically ill patients**

Most commonly used tests to assess coagulation status include assessment of platelet count, coagulation times (activated partial thromboplastin time (aPTT), PT and INR) and levels of fibrinogen and d-dimers. Results from these tests only reflect a limited part of the haemostatic process. Therefore they fail to reflect *in vivo* haemostatic potential and hereby cannot reliably predict potential bleeding risk [11]. In addition, there is a lack of available tests for clinical use to detect a defective natural anticoagulant system and the same applies to markers of fibrinolysis [12]. Moreover, conventional coagulation assays lack the ability to detect a hypercoagulable state. To date, the ISTH DIC score [13], a composite of platelet count, PT prolongation, D-dimer and fibrinogen levels, is the only clinical way to diagnose DIC accompanied by a hypercoagulable state.

In contrast to conventional coagulation tests, rotational thromboelastography assesses the whole process of clot formation and degradation. The resulting thromboelastogram represents initiation of clot formation, fibrin formation and clot degradation [14]. In the past decade, evidence has shown that thromboelastography has additional value to rapidly detect depletion of coagulation factors and fibrinogen in massive bleeding [15-17]. In critically ill patients with a suspected coagulopathy, thromboelastography could improve identification of patients who have an increased bleeding risk and are in need of FFP transfusion.
Also, enhanced fibrinolysis or hyperfibrinolysis, in particular after trauma, can be diagnosed using thromboelastography [18-20]. Moreover, it has well been shown that this bedside test is capable of detecting a hypercoagulable state [21-23]. Of note, there is no uniform definition for hypercoagulability, which renders the interpretation of data on clinical relevance of thromboelastography detected hypercoagulability challenging. In addition, data from critically ill patients are scarce and reference standards for this population, with a high prevalence of coagulation abnormalities, are lacking. Hereby, despite its potential advantages, the additional value of this test in critically ill patients remains to be established.

**Plasma transfusion in the critically ill**

*Current indications and use of plasma in critically ill patients*

Fresh frozen plasma (FFP) effectively corrects multiple clotting factor deficiencies and guidelines recommend its use in severe bleeding [24,25]. However, a number of audits have shown that a substantial amount of FFP is administered prophylactically to patients who have a coagulopathy, but lack signs of active bleeding [26-28]. Of note, evidence that prophylactic administration prevents bleeding complications, in particular after interventions in patients with a coagulopathy, is absent [29,30]. Despite the reported lack of evidence on effectiveness, inappropriate use of FFP is widespread. In the Netherlands, 80,000 FFP units are issued annually (www.sanquin.nl). Of note, critical care physicians exert the majority of these requests [26,27]. The following paradigm responsible for inappropriate use of FFP has been postulated: clinicians assume that (1) elevated PT/INR predicts an enhanced bleeding risk in the setting of a procedure, (2) pre-procedural FFP administration improves PT/INR values and (3) prophylactic FFP transfusion indeed results in fewer bleeding complications (figure 3) [11,31-33].

*Efficacy of FFP to correct coagulopathy in critically ill patients*

A few small clinical trials have studied the efficacy of FFP in critically ill patients with a coagulopathy. These studies are hampered by different doses used and no assessment of the effect of FFP administration on occurrence of bleeding complica-
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Figure 3: Paradigm on the use of fresh frozen plasma to treat or prevent bleeding [11,33].

PT = prothrombin time
INR = international normalized ratio

tions [34,35]. Judgement of results is further hampered by the fact that both actively bleeding and patients without bleeding were included.

In assessment of efficacy of FFP, the dose is of importance. Interestingly, for prophylactic FFP transfusion the reported doses used in clinical practice are often less than 10 ml/kg [27,36]. These doses of FFP fail to correct marginally elevated INR values [37]. Despite these findings, physicians consider a small dose of FFP effective to reduce risk of bleeding in coagulopathic patients (figure 3). Altogether, evidence supporting the efficacy of FFP to correct coagulopathy in critically ill patients is scarce.

In addition to a lack of evidence on effectiveness, some reports suggest detrimental effects of FFP. In critically ill patients, FFP transfusion was shown to be an independent risk factor for the occurrence of lung injury [38-41] and was associated with an increased ICU length of stay [40-42]. Also, transfusion of FFP has been linked to occurrence of infectious complications [43,44]. Taken together, the widespread use of FFP together with a lack of knowledge on effectiveness of FFP in the critically ill and the potential detrimental effects of FFP transfusion in these patients resulted in an expressed need for more and better evidence supporting the use of FFP in ICU patients [32,45].
Chapter 1

Risks of plasma transfusion in the critically ill: transfusion related acute lung injury

Background

Despite obvious clinical benefit in bleeding, FFP is repeatedly associated with the occurrence of lung injury in trauma, postoperative and critically ill patients [46-48]. We investigated the problem of lung injury following FFP transfusion in more detail in the last section of this thesis.

Transfusion related acute lung injury (TRALI) is the leading cause of transfusion related morbidity and mortality [49,50] and is defined as the onset of acute lung injury (ALI) occurring within six hours after a blood transfusion. The ensuing pulmonary edema is characterized by worsening oxygenation and bilateral infiltrates on a chest radiograph [49]. TRALI most frequently occurs in at risk populations, such as critically ill and cardiac surgery patients, with incidences of 5-8% [39,48,51]. Attributable morbidity and mortality is considerable. Up to 70% of TRALI patients requires mechanical ventilation [52] and up to a fifth of patients die [53]. In addition, in critically ill patients, TRALI prolongs duration of mechanical ventilation, ICU length of stay and increases hospital and long-term mortality [39,51,54].

Pathophysiology

Pathophysiology of TRALI has not been fully elucidated yet. However, data acquired in the past decade have resulted in the concept of a ‘two-event’ model [55]. This model might explain the relatively high incidence of TRALI in the critically ill, as the first event composes of the patients’ underlying condition. An underlying inflammatory condition, such as sepsis, induces priming of pulmonary neutrophils and vascular endothelium. The second event is the transfusion of a blood product containing antibodies or other substances. Subsequently, primed neutrophils are activated with the release of inflammatory cytokines, resulting in endothelial damage and the development of lung injury [55]. The second event can be immune mediated or non-immune mediated. Immune mediated TRALI results from blood products containing human anti-neutrophil (HNA) or anti-leukocyte antibodies (HLA) that cross-react with recipients’ cognate antigens. Up to two-thirds of TRALI cases is thought to be immune mediated. In particular in plasma rich products, antibodies are frequently
Figure 4: The occurrence and severity of a TRALI reaction depends on the degree of predisposition of the recipient (grey box) and the strength of the neutrophil priming activity of the transfused product (white box). Edited from Bux et al. [60] and Vlaar [69].

present. A non-immune mediated TRALI reaction results from bioactive substances, which accumulate during storage of cell containing blood products. Implicated substances include lysophosphatidylcholines (LysoPCs), non-polar lipids, inflammatory cytokines and soluble CD40 [56-59]. However, the precise pathways that lead to activation of primed neutrophils and subsequent endothelial damage are still largely unknown.

In addition to the ‘two-event’ model, a threshold model has been postulated [60]. According to this hypothesis, the severity of patients’ underlying condition (‘first event’), determines the threshold for the second event [60,61]. This means that smaller amounts of antibodies or bioactive substances can induce a TRALI reaction in critically ill patients compared to more healthy individuals needing a transfusion (figure 4). Thereby, ICU patients may be at increased risk of acquiring TRALI. This underlines the need of a careful risk benefit assessment of the need of transfusion of FFP or any blood product in this population.

**Prevention and treatment**

As about two-thirds of TRALI cases are immune-mediated, a substantial amount of reactions can be prevented if donors harbouring HLA or HNA antibodies are excluded from donation of plasma containing blood products. The most important
reasons for occurrence of HLA or HNA antibodies within the donor population are previous pregnancies and a history of transfusion [62]. In particular multiple pregnancies induces antibody formation and up to a third of multipara women develops HLA antibodies [62,63]. Based on this knowledge, various donor risk reduction strategies have been implemented by different blood suppliers. Strategies range from active screening of allo-exposed donors and deferral of antibody positive donors [64], deferral of all allo-exposed donors [65-67] and deferral of all female donors [53,68]. The effect of the abovementioned strategies on the onset of TRALI varies, while they also differ in impact on the loss of potential donors and workload for blood suppliers.

Although preventive strategies have contributed to a reduction in occurrence of TRALI, cases still occur, in particular in the most severely ill patients, as they have the lowest threshold to develop a reaction. Despite the reported attributable morbidity and mortality, a pharmacological treatment is not available. Therefore, research into therapeutic strategies is highly warranted.

Outline of this thesis

This thesis focuses on the risk benefit balance of FFP transfusion. Improving identification of patients with a coagulopathy and increased bleeding risk may aid in an improved risk benefit decision to transfuse. Therefore, we studied in depth the assessment of coagulopathy in critically ill patients. The second part focuses on the ability of FFP transfusion to correct coagulopathy in critically ill patients and thereby mitigate the risk of bleeding. To further improve risk benefit assessment, the occurrence of TRALI as the main adverse outcome of FFP transfusion was investigated in detail using clinical and experimental studies on the pathophysiology, treatment and prevention of TRALI.

Part 1 investigates the ability of thromboelastometry to detect coagulopathic derangements in critically ill patient populations. Chapter 2 gives an overview of available literature on the use of thromboelastometry in the intensive care setting. Chapter 3 is a systematic review of literature on the diagnostic and prognostic value of thromboelastometry in patients with sepsis. These chapters are followed by large
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cohort study on the value of coagulopathic profiles as detected by thromboelastometry to assess outcome after major trauma (chapter 4). Other hemostatic tests, which assess coagulopathy, in addition to INR, are studied in part II. The efficacy of FFP in non-bleeding critically ill patients with a coagulopathy is another focus of part II. First the results of a multicentre randomized clinical trial on the ability of FFP to prevent bleeding complications in critically ill patients with a coagulopathy who need to undergo an intervention (TOPIC trial) are discussed (chapter 5). Chapters 6 and 7 describe the ability of hemostatic tests, including thromboelastometry, on assessment of coagulopathy and the in vivo effects of FFP transfusion on the coagulation system of critically ill patients. Effect of FFP on levels of individual coagulation factors, natural anticoagulants, thrombin generation and the fibrinolysis system are also described. In addition the effect of FFP transfusion on thromboelastometry is discussed. Furthermore, the effect of FFP transfusion on the inflammatory response and endothelial function in critically ill patients is discussed in chapter 8. Chapter 9 contains an evaluation of the TOPIC trial, as inclusion targets were not achieved in this trial despite a reported need for more evidence on the use of FFP in patients with a coagulopathy. The aim of this chapter was to identify constraints in conducting intervention trials on the effectiveness of FFP in critically ill patients and to make recommendations for further research in this field.

In part III, several aspects on the pathophysiology, treatment and prevention of TRALI are discussed. Chapter 10 reviews the risk factors for TRALI in critically ill patients. Chapter 11 is an observational study on the contribution of damage-associated molecular pattern molecules (DAMPs), which are thought to mediate host response to both infectious and non-infectious stimuli, to the development of TRALI after cardiac surgery. A general overview of potential strategies to reduce the risk of TRALI is given in chapter 12, while measures to specifically prevent immune-mediated TRALI are discussed in chapter 13. In addition, we performed a meta-analysis on the impact of low risk TRALI donor strategies for plasma containing blood products on the onset of TRALI (chapter 14).

Finally, chapters 15 and 16 describe the effects of administration of methylprednisolone and C1 inhibitor in a murine model of TRALI.

The results from all parts are summarized and discussed in chapter 17 (English) and chapter 18 (Dutch).
References


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