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# Pathophysiology and Treatment of Coagulopathy in Massive Hemorrhage and Hemodilution

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## ABSTRACT

Fluid resuscitation after massive hemorrhage in major surgery and trauma may result in extensive hemodilution and coagulopathy, which is of a multifactorial nature. Although coagulopathy is often perceived as hemorrhagic, extensive hemodilution affects procoagulants as well as anticoagulant, profibrinolytic, and antifibrinolytic elements, leading to a complex coagulation disorder. Reduced thrombin activation is partially compensated by lower inhibitory activities of antithrombin and other protease inhibitors, whereas plasma fibrinogen is rapidly decreased proportional to the extent of hemodilution. Adequate fibrinogen levels are essential in managing dilutional coagulopathy. After extensive hemodilution, fibrin clots are more prone to fibrinolysis because major antifibrinolytic proteins are decreased.

Fresh frozen plasma, platelet concentrate, and cryoprecipitate are considered the mainstay hemostatic therapies. Purified factor concentrates of plasma origin and from recombinant synthesis are increasingly used for a rapid restoration of targeted factors. Future clinical studies are necessary to establish the specific indication, dosing, and safety of novel hemostatic interventions.

**I**N patients with trauma and those who undergo major surgery, multiple breaches of vascular integrity result in bleeding, and in some cases, exsanguination. Fluid (volume) replacement with crystalloids or colloids is usually the initial

measure to stabilize systemic circulation by compensating for hypovolemia. When the blood loss is considered major (hemoglobin concentration below 6–10 g/dl),<sup>1</sup> erythrocyte (RBC) concentrates are transfused to sustain hemoglobin levels (*i.e.*, oxygen-carrying capacity). The transfusion of ten or more erythrocyte units (*i.e.*, replacement of one blood volume) within 24 h is generally considered as massive transfusion in adults.<sup>2</sup> Other arbitrary definitions include six or more erythrocyte units within 12 h and over 50 units of blood product use within 24 h, including erythrocytes, platelet concentrates, and fresh frozen plasma (FFP).<sup>3,4</sup> There are differences in the initial pathophysiology of coagulopathy between trauma and major surgery, which can be attributed in part to the mechanism of vascular injury, extent of hemorrhage, type of fluid resuscitation, and prophylactic use of antifibrinolytic therapy.<sup>5–8</sup> However, hemostatic defects based on conventional laboratory data are often indistinguishable between trauma and major surgery after massive transfusion. Unlike congenital bleeding disorders that are due mostly to a single factor deficiency (*e.g.*, hemophilia, afibrinogenemia), coagulopathy encountered in trauma and major surgery is of a multifactorial nature. All elements in coagulation, including procoagulant, anticoagulant, fibrinolytic, and antifibrinolytic proteins, exhibit various degrees of deficiency. Although this topic has been reviewed recently by others,<sup>5,8,9</sup> the mechanism of coagulopathy related to massive transfusion and hemodilution is not fully understood. In this review, we focus on the effects of hemodilution on thrombin generation, fibrin polymerization, and fibrinolysis, using experimental results as well as existing clinical data to shed light on the mechanisms of dilutional coagulopathy. In addition, we discuss various therapeutic approaches and their clinical implications.

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## Effects of Hemodilution on Coagulation Factors and Blood Components

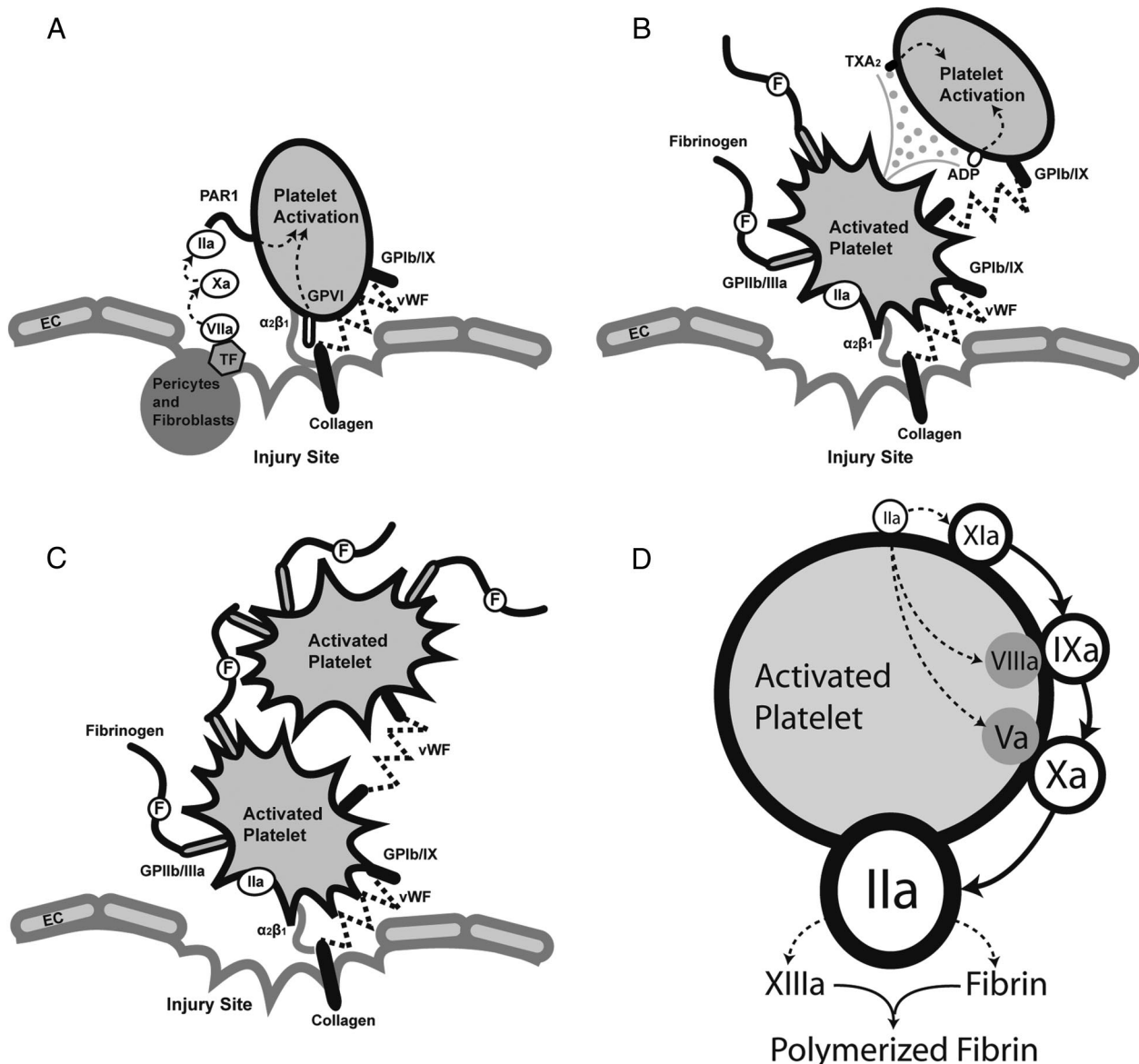
Volume resuscitation with crystalloids, colloids, or erythrocytes can lead to dilutional coagulopathy with reduced levels of most hemostatic elements, whereas FFP transfusion dilutes corpuscular elements in blood, but sustains soluble clotting factors at nearly normal levels.<sup>10</sup> According to *in vitro* experiments, the extent of dilution is proportional to the infused volume.<sup>10,11</sup> However, it is less clear whether this is true for *in vivo* situations; for example, plasma FVIII and von Willebrand factor can be acutely increased because of the release from endothelium by stress hormones, including epinephrine and vasopressin.<sup>12–14</sup> Further, platelet count is often higher than predicted by the extent of dilution, presumably because of the release of sequestered platelets from the spleen and lungs and from the bone marrow in premature forms.<sup>15</sup> In addition to the reserve of some hemostatic elements *in vivo*, it is also important to point out that the critical level of a hemostatic element occurs at a different time point during hemodilution. The threshold level of fibrinogen at 1 g/l is observed after a loss of about 150% of circulating blood volume, whereas critical concentrations of enzymatic coagulation factors and platelet count are reached after a loss of more than 200% of blood volume.<sup>16</sup> Besides changes in plasma and cellular elements, hypothermia and acidosis, commonly associated with trauma and massive transfusion, reduce thrombin generation by affecting enzyme kinetics.<sup>17–19</sup>

Although hemostatic defects are primarily attributed to decreased procoagulant factor levels, anticoagulant factor levels are decreased proportional to the extent of hemodilution. For example, antithrombin (formerly antithrombin III) activity decreases to below 30% after 1:6 dilution of whole blood with normal saline *in vitro*.<sup>11</sup> Decreased antithrombin activity prolongs the half-lives of thrombin and activated FX,<sup>20</sup> and thus it potentially contributes to improved hemostasis in the hypocoagulable state after hemodilution.<sup>10,11,21,22</sup> On the other hand, excess activity of thrombin and activated FX in circulation may contribute to the pathogenesis of trauma-induced coagulopathy and disseminated intravascular coagulation.<sup>23</sup>

Fibrinolytic and antifibrinolytic activities are also affected in massive hemorrhage. The plasma concentration of  $\alpha_2$ -antiplasmin is normally high (70  $\mu\text{g/ml}$ , 1  $\mu\text{M}$ ), and it rapidly neutralizes plasma free plasmin.<sup>24</sup> In addition,  $\alpha_2$ -antiplasmin is rapidly cross-linked to fibrin  $\alpha$ -chains by activated FXIII, conferring fibrin more resistant to fibrinolysis.<sup>25,26</sup> Progressive hemodilution of  $\alpha_2$ -antiplasmin and FXIII reduces fibrin cross-linking and prolongs the plasma half-life of plasmin.<sup>10,27</sup> Plasma levels of other antifibrinolytic proteins are also progressively lowered by hemodilution.<sup>10,11</sup> Thrombin-activatable fibrinolysis inhibitor circulates in plasma (5  $\mu\text{g/ml}$ , 75 nM), which, after being activated by high levels of thrombin, cleaves C-terminal lysine residues from fibrin, preventing plasminogen binding.<sup>28–30</sup> Plasma plasminogen activator inhibitor-1 (0.01  $\mu\text{g/ml}$ , 200 pM) as well as platelet ( $\alpha$ -granule)-derived plasminogen activator inhibitor-1 are decreased because of hemodilution and thrombocytopenia<sup>31,32</sup>; thus plasma tissue plasminogen activator (tPA) activity is prolonged. Plasma levels of tPA can be increased in acute stress because of release from Weibel–Palade bodies of endothelium. Thrombin, epinephrine, vasopressin, desmopressin, bradykinin, and other substances are known to trigger tPA release.<sup>33</sup> Taken together, in conjunction with high baseline levels of plasminogen (200  $\mu\text{g/ml}$ , 2  $\mu\text{M}$ ), the fibrinolytic pathway is relatively well preserved during major hemodilution. On the contrary, fibrin clot becomes more susceptible to plasmin digestion after hemodilution, and even systemic fibrinolytic states may be observed in about 20% of trauma patients when plasmin activity is no longer controlled by endogenous antifibrinolytic proteins.<sup>34,35</sup>

## Regulation of Thrombin Generation

Thrombin generation is a critical event in achieving hemostasis in a timely manner after vascular injury. Thrombin is a potent serine protease, and its activation involves a series of reactions among proteases and cellular components (fig. 1). Three key components of coagulation (substrate, enzyme, and cofactor) are concentrated on the activated platelet surface to support thrombin generation locally.<sup>36–38</sup> Notably, the initial hemostatic response is triggered by an “extrinsic pathway”; tissue factor expressed on subendothelial pericytes and fibroblasts forms a complex with trace amounts of circulating activated FVII during the initiation phase (fig. 1A). Rapidly generated small quantities of activated FX proceed to generate trace amounts of thrombin. In the amplification phase, thrombin generation distant from the vascular wall needs to be sustained without major contributions of tissue factor. Thrombin is capable of activating FXI, FVIII, and FV to maintain its own generation *via* the “intrinsic pathway.”<sup>36–38</sup> In particular, thrombin-activated FVIII and FV play key roles during the subsequent propagation phase because activated FVIII-FIX complex (tenase) and activated FV-FX complex (prothrombinase) exponentially increase the activation rate of FX and prothrombin, resulting in the generation of large amounts of thrombin on the platelet surface (fig. 1D).<sup>37,39</sup> Indeed, the minimal hemostatic level for FVII can be much less than for prothrombin and fibrinogen because the latter two are more rapidly consumed toward the end of cascade reactions (fig. 1D and table 1). During the propagation phase of coagulation, local thrombin concentration rapidly increases from less than 1 nM to as high as 500 nM.<sup>10,11,40</sup> One may simply speculate that thrombin generation would be reduced as the prothrombin level falls because of hemodilution, but the peak level of thrombin generation is less affected relative to the prothrombin level after hemodilution. Peak thrombin levels were reduced to 58% and 32% of baseline, respectively, when prothrombin levels were decreased to 43% and 17% of baseline by *in vitro* hemodilution with saline (fig. 2).<sup>10</sup> The discordance between prothrombin and thrombin generation can be partly explained by reduced antithrombin activity. Antithrombin is a major serine protease inhibitor that circulates at a high concentration (2.7



**Fig. 1.** Clot formation at injury site. (A) At the site of injured endothelial cells (EC), platelets adhere to subendothelial collagen via interactions between von Willebrand factor (vWF) and platelet-surface glycoprotein receptor (GP), GPIb/IX. The platelet integrin receptor ( $\alpha_2\beta_1$ ) reinforces the binding to collagen. Trace amounts of thrombin are generated during the initiation phase of coagulation by FXa via interactions between circulating FVIIa and tissue factor (TF) expressed on subendothelial pericytes and fibroblasts. (B) Platelets activated by collagen and thrombin release adenosine-diphosphate (ADP) and thromboxane (TXA<sub>2</sub>), which activate platelets in the vicinity. (C) Activated platelets express GPIIb/IIIa and capture fibrinogen (F). On the activated platelet surface, thrombin-mediated feedback activations of FXI, FVIII, and FV result in the propagation phase of thrombin generation. Sustained activation of prothrombin is feasible via formation of tenase (activated FIX-FVIII) and prothrombinase (activated FX-FV). (D) Polymerization of fibrin is achieved by thrombin-activated FXIII during the propagation phase.

$\mu\text{M}$ , 150  $\mu\text{g}/\text{ml}$ ) in plasma. Subthreshold levels of thrombin and activated FX that circulate downstream from the injury are rapidly neutralized by antithrombin bound to endothelial heparan sulfate (fig. 3).<sup>41</sup> Although thrombin is an essential enzyme for hemostasis and survival, uncontrolled thrombin activity can be harmful to the host. Multiple mechanisms are available to limit excessive thrombin generation and to scavenge free proteases (e.g., thrombin, activated FX) in circulation. Tissue factor pathway inhibitor is a key regulator of activated FX when it is in a complex with tissue factor-acti-

vated FVII.<sup>42</sup> In addition, it was recently shown that protein S facilitates the inhibitory interaction between tissue factor pathway inhibitor and activated FXa.<sup>43</sup>

Analogously, end-stage liver disease is associated with concomitant decreases in procoagulant factors (FII, FVII, FIX, and FX) and anticoagulant elements including antithrombin, protein C, and protein S. Endogenous thrombin generation may still be near normal despite abnormal clotting times in liver cirrhosis,<sup>44,45</sup> and similar data exist for dilutional coagulopathy.<sup>10,11,46</sup> When endogenous antico-

**Table 1.** Plasma Levels, Half-lives and Availability of Concentrates for Coagulation Factors and Inhibitors

Factor	Level ( $\mu\text{M}$ )	Half-life (h)	Available Concentrate(s) <sup>152</sup>
Fibrinogen	7.6	72–120	pd-Fibrinogen, Cryoprecipitate
Prothrombin	1.4	72	PCC, FEIBA
Factor V	0.03	36	None
Factor VII	0.01	3–6	pd-FVII, r-FVIIa, PCC*, FEIBA
Factor VIII	0.00003	12	pd-FVIII, r-FVIII
Factor IX	0.09	24	pd-FIX, r-FIX, FEIBA
FX	0.17	40	pd-FX, PCC, FEIBA
Factor XI	0.03	80	pd-FXI
Factor XIII	0.03	120–200	pd-FXIII, r-FXIII, Cryoprecipitate
vWF	0.03	10–24	pd-vWF, Cryoprecipitate
Protein C	0.08	10	pd-Protein C, PCC*
Protein S	0.14	42.5	PCC*
Antithrombin	2.6	48–72	pd-Antithrombin, r-Antithrombin

Fresh frozen plasma contains all the above coagulation factors at near-normal concentrations.

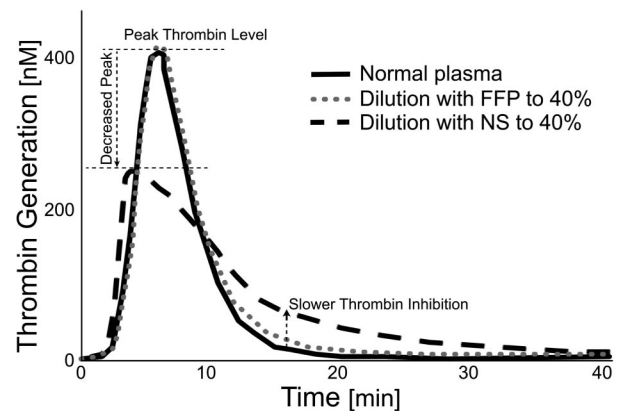
FEIBA = Factor eight inhibitor bypassing activity; PCC = prothrombin complex concentrate (\*certain PCC products contain minimal levels of FVII, protein C, and protein S); pd = plasma-derived; r = recombinant; vWF = von Willebrand factor.

agulants are deficient, thrombin activity is sustained at the injury site as well as in circulation. In severe hemodilution, thrombin and activated FX are more likely to be released into circulation because polymerized fibrin, which normally adsorbs and contains serine proteases, is reduced.<sup>47,48</sup> Further, systemic thrombin activity is associated with a release of tPA and thrombomodulin-mediated activation of protein C (fig. 3). In trauma patients with hemodilution, these pathologic responses are called early trauma-induced coagulopathy,<sup>6,49</sup> and they are mechanistically similar to disseminated intravascular coagulation with the hemorrhagic phenotype.<sup>23</sup>

In addition to hemodilution, thrombin generation can be directly affected by hypothermia and acidosis, which are commonly observed during resuscitation. Using the porcine model, Martini *et al.* demonstrated that hypothermia (32°C) and acidosis (pH 7.1) distinctly affect hemostasis.<sup>18,50</sup> Hypothermia mostly influences the initiation of clot formation, whereas acidosis disturbs the propagation of coagulation. In cases of hypothermia, thrombin generation reaches levels similar to those of normothermia, but the process is slower. In contrast, acidosis significantly impairs thrombin generation, resulting in a decreased hemostatic capacity.

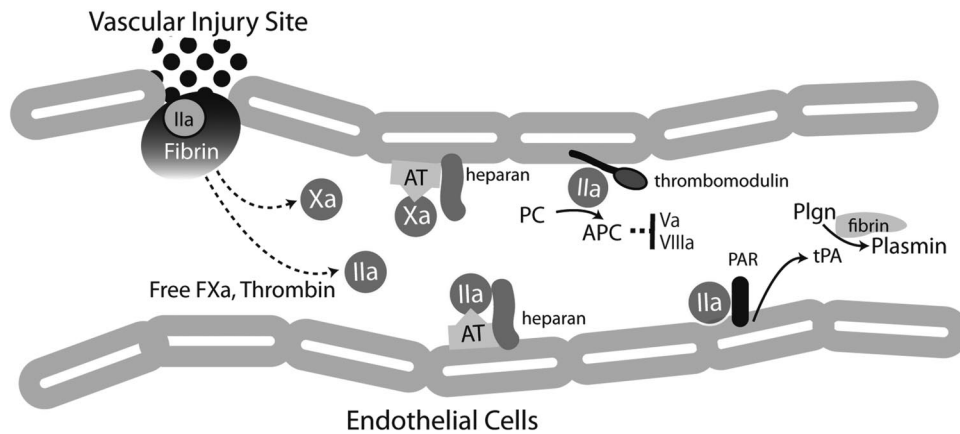
### Fibrin Polymerization and Fibrinolysis

The cleavage of fibrinogen bound to platelet glycoprotein IIb/IIIa receptors and subsequent polymerization of fibrin



**Fig. 2.** Thrombin generation after dilution. Thrombin generation patterns in platelet-poor plasma are shown before and after dilution to about 40% of baseline. The patterns are similar between baseline and dilution with fresh frozen plasma (FFP). The peak thrombin level decreases (downward arrow) after dilution with normal saline (NS) because of a reduced concentration of procoagulant clotting factor. A concomitant reduction in antithrombin activity results in sustained thrombin activity (upward arrow). Data are adapted from Bolliger D, Szlam F, Levy JH, Molinaro RJ, Tanaka KA: Haemodilution-induced profibrinolytic state is mitigated by fresh-frozen plasma: Implications for early haemostatic intervention in massive haemorrhage. *Br J Anaesth* 2010; 104: 318–25, used by permission of Oxford University Press.

are achieved by amplified generation of thrombin and thrombin-activated FXIII (fig. 1D). Plasma fibrinogen concentration is the highest (7.6  $\mu\text{M}$ , 2.5 g/l) among coagulation factors, and it is increased as an acute-phase reactant during inflammation and pregnancy.<sup>51,52</sup> Large amounts of fibrinogen are captured by activated platelets *via* abundant glycoprotein IIb/IIIa receptors (more than 12,000 copies per platelet) (fig. 1B).<sup>53,54</sup> Fibrinogen molecules are converted to fibrin monomers after thrombin removes N-terminal peptides (fibrinopeptides) from the fibrinogen A $\alpha$  and B $\beta$  chains.<sup>55</sup> Activated platelets release FXIII A subunits that are activated by thrombin, and activated FXIII polymerizes fibrin monomers into fibrin. Activated FXIII also cross-links  $\alpha_2$ -antiplasmin to fibrin, making fibrin more resistant to degradation.<sup>26,56</sup> Thus, local thrombin levels affect both the thickness and the fibrinolytic resistance of fibrin fibers.<sup>30,57</sup> In normal plasma, a high peak thrombin level (200–500 nM) can be achieved,<sup>10,11,40</sup> and a dense network of thin fibrin strands (firm clot) is produced to establish hemostasis.<sup>57,58</sup> Conversely, a lower thrombin level in bleeding disorders (*e.g.*, hemophilia) is associated with coarsely gathered thick fibrin strands (loose clot).<sup>58,59</sup> It can be easily speculated that the extent of thrombin generation is nonhomogeneous inside the clot (fig. 4). The maximal thrombin generation is expected to be near the vessel wall, where platelets release procoagulant microparticles<sup>60</sup> after being maximally activated by collagen and tissue factor-pathway derived thrombin. The pivotal role of thrombin in conferring antifibrinolytic activity is related to cross-linking of  $\alpha_2$ -antiplasmin to fibrin by activated FXIII and activation of thrombin-activat-



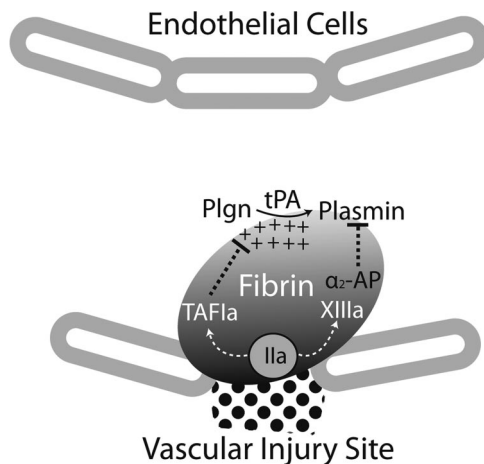
**Fig. 3.** Mechanism of intravascular clot formation and thrombin regulation. Thrombin is an essential enzyme for hemostasis and survival, but uncontrolled thrombin activity can be harmful to the host. Subthreshold levels of thrombin (FIIa) and activated factor X (FXa) that circulate downstream from the injury are rapidly neutralized by antithrombin (AT) bound to endothelial heparan sulfate. Thrombomodulin-mediated activation of protein C (PC) to activated protein C (APC) inhibits activities of FV and FVIII, thereby limiting thrombin generation. Systemic thrombin activity is also associated with binding to a protease-activated receptor (PAR) and with consecutive release of tissue plasminogen activator (tPA), leading to conversion of plasminogen (Plgn) to plasmin and finally fibrinolysis.

able fibrinolysis inhibitor.<sup>25,26,29</sup> Densely packed thin fibrin strands serve as a local container for activated proteases, thrombin and activated FX.<sup>48</sup> Indeed, high-affinity nonsubstrate binding site of fibrin for thrombin is known as antithrombin I.<sup>47</sup> Deficiency of both fibrinogen and antithrombin in severe hemodilution can be detrimental to the control of procoagulant activity. Without adequate fibrin polymerization, thrombin and activated FX generated at the injury site are re-

leased into systemic circulation (fig. 3).<sup>48,61</sup> These activated proteases exacerbate disseminated intravascular coagulation in conjunction with low levels of anticoagulant factors.<sup>10,62</sup>

It is not known what minimal levels of fibrinogen and FXIII should be kept to minimize perioperative bleeding. The international guidelines before 2009 recommended minimal fibrinogen levels between 0.8 and 1.0 g/l,<sup>1,63,64</sup> a level similar to the management of congenital afibrinogenemia (table 2).<sup>65</sup> However, more recent European guidelines recommend higher fibrinogen cutoffs (1.5–2.0 g/l) for perioperative coagulopathy.<sup>66,67</sup> These changes are in closer agreement with recent clinical data in postpartum hemorrhage,<sup>51</sup> replacement of the aorta,<sup>68</sup> coronary bypass grafting surgery,<sup>69–71</sup> cystectomy,<sup>72</sup> and *in vitro* hemodilution,<sup>11</sup> which indicated even higher fibrinogen levels of 2–3 g/l for adequate hemostasis (table 3). The overestimation of fibrinogen concentrations by the Clauss method after volume replacement with colloids is also an important consideration.<sup>73</sup> For the minimal FXIII level, recent clinical data suggest the maintenance of above 50–60% to reduce bleeding tendency after major surgery, particularly in the presence of low fibrinogen levels (less than 1.5 g/l).<sup>27,74</sup>

Fibrinolytic activation is an important process in preventing excess fibrin formation that occludes injured blood vessels. Plasmin activation is catalyzed by locally concentrated tPA and plasminogen, which bind to positively charged lysine residues expressed on fibrin (fig. 4).<sup>75</sup> Normally, endogenous antifibrinolytics, plasminogen activator inhibitor-1,  $\alpha_2$ -antiplasmin, and activated thrombin-activatable fibrinolysis inhibitor, are highly concentrated at the focal point of blood coagulation according to the gradient of activated platelets, thrombin, and activated FXIII.<sup>76,77</sup> Thus, fibrin near the vessel wall is highly resistant to fibrinolysis, whereas intraluminal fibrin is more accessible by fibrinolytic enzymes for recanalization of the injured blood vessel (fig. 4).<sup>78</sup> Reduced thrombin generation,<sup>30,79</sup> low



**Fig. 4.** Regulation of fibrin polymerization and fibrinolysis within the clot. The maximal thrombin generation is expected to be near the vessel wall where thrombin (IIa) generation is maximal over the highly catalytic phospholipids surface on platelets activated by collagen and tissue factor-pathway derived thrombin. Endogenous antifibrinolytics,  $\alpha_2$ -antiplasmin ( $\alpha_2$ -AP) and active thrombin-activatable fibrinolysis inhibitor (TAFIa), are also cross-linked to fibrin by thrombin-activated factor XIII (XIIIa) according to the extent of thrombin generation. Thus, fibrin near the vessel wall is highly resistant to fibrinolysis, whereas intraluminal fibrin is more accessible by tissue plasminogen activator (tPA) activation of plasminogen (Plgn) for recanalization of the injured blood vessel.

**Table 2.** Minimal Fibrinogen Levels in Different Studies

Study	Year	Fibrinogen Level (g/l)	Surgery/Conditions (Time Point)
Gerlach <i>et al.</i> <sup>74</sup>	2002	> 1.5	Neurosurgery (after surgery)
Charbit <i>et al.</i> <sup>51</sup>	2007	> 2.0	Postpartum hemorrhage
Bolliger <i>et al.</i> <sup>69</sup>	2009	> 2.0	CABG on-pump and off-pump (after surgery)
Bolliger <i>et al.</i> <sup>11</sup>	2009	2–3	<i>In vitro</i> hemodilution
Fenger-Eriksen <i>et al.</i> <sup>72</sup>	2010	2.4	Cystectomy (after surgery)
Blome <i>et al.</i> <sup>70</sup>	2005	2.7	CABG on-pump (after surgery)
Karlsson <i>et al.</i> <sup>71</sup>	2009	3.1	CABG on-pump (after surgery)
Rahe-Meyer <i>et al.</i> <sup>68</sup>	2009	3.6	Replacement of ascending aorta (after surgery)

Fibrinogen levels are the cutoff levels in retrospective studies,<sup>51,69–70,74</sup> the optimal level in the *in vitro* study,<sup>11</sup> and the levels in the interventional groups of placebo-controlled studies.<sup>68,71–72</sup>

CABG = coronary artery bypass grafting.

$\alpha_2$ -antiplasmin levels,<sup>24</sup> or low levels of thrombin-activatable fibrinolysis inhibitor<sup>80</sup> may be associated with a fibrin structure that is prone to fibrinolysis. Premature fibrinolysis associated with rebleeding may easily occur after extensive hemodilution with crystalloids, colloids, or erythrocytes because endogenous antifibrinolytic proteins are decreased and their interaction is diminished.<sup>26</sup> Prophylactic uses of antifibrinolytics have been shown effective in reducing fibrinolytic tendency after a progressive hemodilution in cardiac surgery.<sup>7</sup> It is possible that antifibrinolytic activity can be maintained by supplementing FFP<sup>10</sup> or FXIII.<sup>56</sup>

The effects of hypothermia and acidosis on fibrinogen synthesis, fibrin polymerization, and fibrinolysis have been experimentally evaluated in the porcine model and *in vitro*. In the porcine model, it was shown that hypothermia decreases fibrinogen synthesis, whereas acidosis increases fibrin degradation without affecting fibrinogen.<sup>81</sup> The rate of fibrin polymerization is reduced synergistically by hypothermia ( $\leq 33^\circ\text{C}$ ) and acidosis ( $\text{pH} \leq 7.1$ ).<sup>17</sup> The rate of fibrinolysis seems to remain constant in hypothermia ( $32^\circ\text{C}$ ), but acidosis increases fibrin degradation.<sup>81,82</sup>

## Hemostasis Monitoring for Massive Hemorrhage

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) represent the most common screening tests for

coagulation abnormalities in massive transfusion.<sup>83</sup> The prolongation in PT is presumably proportional to the extent of coagulation factor loss and hemodilution.<sup>84</sup> Using the cut-off value of international normalized ratio of more than 1.5 times normal, PT demonstrates a sensitivity of 88% and a specificity of 88% in detecting at least one nonhemostatic coagulation factor level after trauma.<sup>84</sup> On the other hand, aPTT (more than 1.5 times normal) demonstrates a sensitivity of only 50% and a specificity of 100%. This is because FVIII is often increased as an acute phase reactant in trauma and surgical patients.<sup>12</sup> Several important limitations should be considered when PT/aPTT are used to evaluate bleeding. First, perioperative bleeding is typically associated with multiple coagulation defects resulting from hemodilution, consumptive loss, fibrinolysis, anticoagulant use, hypothermia, and other mechanical and metabolic derangements. Second, PT and aPTT do not provide any information on *in vivo* interaction of platelets with coagulation factors. Third, PT and aPTT remain prolonged even if thrombin generation is improved because of antithrombin or protein C deficiency.<sup>22,45</sup> Further, it is not possible to estimate the overall stability of a hemostatic thrombus using PT/aPTT because both tests are terminated at very low thrombin levels of about 10 nM<sup>85</sup> and before fibrin is polymerized by activated FXIII. Finally, PT/aPTT remain normal when bleeding is caused by in-

**Table 3.** Minimal Fibrinogen Levels in Different International Guidelines

Study	Year	Fibrinogen Level (g/l)	Source
ASA <sup>1</sup>	2006	> 0.8–1	American Guideline
O'Shaughnessy <i>et al.</i> <sup>63</sup>	2004	1	British Guideline
American Red Cross	2007	1	American Guideline
Spahn <i>et al.</i> <sup>64</sup>	2007	1	European Guideline
Bundesärztekammer <sup>66</sup>	2009	1.5	German Guideline
ÖGARI	2010	1.5–2	Austrian recommendations
Rossaint <i>et al.</i> <sup>67</sup>	2010	1.5–2	European Guideline

The Red Cross guideline (Practice Guidelines for Blood Transfusion; via <http://www.redcross.org/www-files/Documents/WorkingWiththeRedCross/practiceguidelinesforbloodtrans.pdf>; accessed July 14, 2010) and ÖGARI guideline (Coagulation Management 2010; via <http://www.oegari.at/arbeitsgruppe.asp?id=116>; accessed July 14, 2010) are on-line publications.

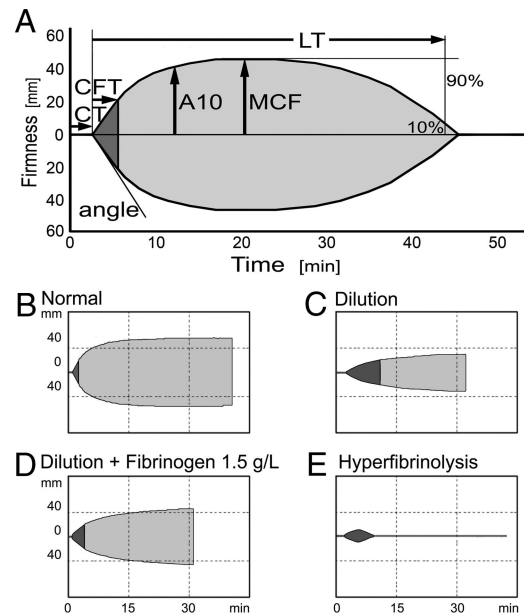
ASA = American Society of Anesthesiologists; ÖGARI = Austrian Society of Anesthesiology, Reanimation and Intensive Care Medicine.

creased fibrin breakdown (*i.e.*, hyperfibrinolytic state) such as occurs in congenital deficiency of  $\alpha_2$ -antiplasmin.<sup>24</sup>

There are some point-of-care devices available for determination of PT/aPTT, but the majority of PT/aPTT testing is still performed in the laboratory, which requires a substantial time delay. In this regard, thromboelastography (TEG®; Hemonetics Corporation, Braintree, MA) or thromboelastometry (ROTEM®; TEM International, Munich, Germany) are advantageous because they can be performed as point-of-care hemostasis monitoring when appropriately trained personnel are available.<sup>34,84</sup> Both TEG® and ROTEM® technologies are based on the original invention of H. Hartert (reported in 1948),<sup>86</sup> which predates the introduction of aPTT. The main endpoint of ROTEM®/TEG® is the polymerization of fibrin in the presence of activated platelets. Given some differences, both assays are particularly useful for the evaluation of fibrinogen deficiency, factor XIII deficiency, hemophilia, and fibrinolytic state.<sup>11,30,87–89</sup> In patients with major trauma, early diagnosis and treatment of coagulopathy may be feasible using ROTEM®-guided (goal-directed) hemostatic therapy (fig. 5).<sup>90</sup> The commonly used thromboelastometric variables include (fig. 5A): coagulation time (in seconds), clot formation time (in seconds), angle ( $\alpha$ ; in degrees), maximum clot firmness (in millimeters), and lysis time (in seconds). Coagulation time represents the onset of clotting, while clot formation time and angle both represent the initial rate of fibrin polymerization. Maximal clot firmness is a measure of the maximal viscoelastic strength of clot (fig. 5B–D). Lysis time is used for the diagnosis of premature lysis or hyperfibrinolysis (fig. 5E).<sup>34,35</sup>

It is of interest to know whether coagulation time values correspond to conventional screening tests (PT/aPTT). In a recent clinical study of trauma-induced coagulopathy, the correlations between coagulation time values and PT/aPTT were rather poor ( $r = 0.47–0.53$ ).<sup>84</sup> Nevertheless, other ROTEM® parameters related to fibrin polymerization (*e.g.*, amplitude after 15 min, clot formation time) seem to be useful for an early detection of coagulopathy represented by abnormal PT/aPTT (more than 1.5 times normal).<sup>83</sup> Maximal clot firmness is highly influenced by fibrinogen levels and platelet count (fig. 5C–D),<sup>11,91</sup> and maximal clot firmness in the presence of cytochalasin D (FIBTEM) correlates well with fibrinogen levels.<sup>84,92</sup> In trauma-induced coagulopathy, a FIBTEM amplitude after 10 min of less than 5 mm was reported to be a good predictor of low plasma fibrinogen (less than 1.0 g/l), with a sensitivity of 91% and a specificity of 85%.<sup>84</sup> In a recent retrospective analysis of 131 patients, FIBTEM- maximal clot firmness below 10 mm and EXTEM-clotting time more than 1.5 times normal were shown to be effective targets of administering fibrinogen concentrate and prothrombin complex concentrate, respectively.<sup>90</sup>

Other hemostatic monitoring, such as PT/aPTT and activated clotting time, can also be used at bedside. The measurement of thrombin generation and individual coagulation factor levels are used mostly for research purposes unless there is a high clinical suspicion because of preexisting con-



**Fig. 5.** Thromboelastometry after dilution. Thromboelastometry assesses the kinetics of clot formation and stability or lysis of the formed clot. (A) Thromboelastometric parameters are defined as follows: Initiation of coagulation measured as coagulation time (CT) shows initial thrombin and fibrin formation. Propagation of clot formation is a function of the interactions of fibrin(ogen) with platelets. It is measured as  $\alpha$  angle or clot formation time (CFT), which is defined as the time needed to achieve a clot firmness of 20 mm. Maximal clot firmness (MCF) represents the final clot strength and results from firm aggregation of platelets and formation of a stable fibrin network. A10 represents the amplitude 10 min after the onset of clot formation. Clinically relevant fibrinolysis can be diagnosed by shortened lysis time (LT), which is defined by the time to diminish the clot firmness to 10% of maximal clot firmness. (B–E) Thromboelastometric patterns in normal whole blood (B), after severe dilution (C), after severe dilution and supplementation with 1.5 g/l fibrinogen (D), and in hyperfibrinolysis (E). Data are adapted from Bolliger D, Szlam F, Molinaro RJ, Rahe-Meyer N, Levy JH, Tanaka KA: Finding the optimal concentration range for fibrinogen replacement after severe haemodilution: An *in vitro* model. *Br J Anaesth* 2009; 102:793–9, used by permission of Oxford University Press.

ditions (*e.g.*, hemophilia, antithrombin deficiency). The predictive value of novel impedance platelet aggregometry in trauma and surgical bleeding still needs to be determined.<sup>93</sup>

## Interventions for Coagulopathy

### Initial Resuscitation

In patients with traumatic hemorrhage, time between injury and admission to hospital should be minimized.<sup>67</sup> Permissive hypotension may be considered in patients who present with moderate bleeding, but massive volume resuscitation cannot be deferred if patients are in severe hypovolemic shock.<sup>94</sup> Major resuscitation efforts using blood products and other hemostatic interventions are initiated when patients are admitted to a tertiary care center.

### Initial Volume Resuscitation

Resuscitation of the hypovolemic patient after major blood loss usually involves an initial infusion of crystalloids and colloids to stabilize systemic circulation.<sup>1</sup> Both crystalloids and colloids dilute the coagulation factors, platelets, and hemoglobin. Although with clear advantages in sustaining intravascular volume and therefore normovolemia, colloids may have some disadvantages regarding hemostasis. Colloids such as hydroxyethyl starch solutions, gelatins, and dextrans impair platelet function, inhibit fibrin polymerization, and may induce an acquired von Willebrand syndrome.<sup>95–97</sup> The degree of such derangement depends on the amount and the physicochemical characteristics of the colloid solution.<sup>98</sup> They may also increase fibrinolytic tendency, probably because of interaction with fibrin polymerization and  $\alpha_2$ -antiplasmin–plasmin interactions.<sup>96,99</sup> Crystalloid solutions primarily induce dilution of the coagulation factors and platelets.<sup>10,11</sup> Interestingly, mild dilution has been associated with hypercoagulability on thromboelastography.<sup>100</sup> However, this finding has been questioned<sup>101</sup> and may reflect *in vitro* effects of decreased hematocrit.<sup>89,102,103</sup>

Transfusion of erythrocytes is performed to improve oxygen carrying capacity, but increased hematocrit may also be beneficial for hemostasis. In the arterial vessel, platelets are preferentially distributed near the vessel wall (margination) because of the red cell mass.<sup>102,103</sup> The platelet count measured in a static blood sample may therefore not correctly reflect the *in vivo* platelet concentration next to the injured vessel wall, and this may explain a relatively low incidence of spontaneous bleeds until platelet count is below 10,000 per  $\mu\text{L}$ .<sup>104</sup> Erythrocytes also facilitate platelet aggregation by releasing adenosine diphosphate under shear flows,<sup>105</sup> and they may function as a reactive surface for the coagulation cascade.<sup>106</sup> In summary, low red cell mass (anemia) seems to worsen bleeding tendencies.<sup>103</sup> In contrast, thromboelastometric measurement in anemic patients (mean hematocrit 28%) showed that angle and maximal clot firmness values were increased by 5° and 10 mm, respectively, compared with normal subjects (hematocrit 41%).<sup>89</sup> However, thromboelastometric measurements are conducted under low shear rates (0.1/s), and the red cell mass is “in the way” of spreading fibrin strands and their interaction with platelets glycoprotein IIb/IIIa.<sup>107</sup>

### Fresh Frozen Plasma

FFP contains all the components in donor plasma, including procoagulant, anticoagulant, and antifibrinolytic factors, albumin, and immunoglobulins. In thawed FFP kept at 1–6°C, residual levels of labile FV remain adequate for 5 days.<sup>108</sup> Such plasma may be useful when FFP is acutely needed for massive transfusion. Several retrospective analyses demonstrated the potential clinical benefit of aggressive hemostatic resuscitation using the empirical transfusion ratio of FFP:RBC over 1:1 in military and civilian trauma cases.<sup>2,109–111</sup> The survival rate was significantly worse with a low FFP:RBC ratio (*i.e.*, less than 1:2) relative to a high

ratio (more than 1:1).<sup>111,112</sup> On the contrary, two other retrospective studies found no benefit of a high FFP:RBC ratio.<sup>113,114</sup> Differences in patient demographics, inclusion criteria, and transfusion protocols may have contributed to these conflicting findings. Nevertheless, the introduction of massive transfusion protocols resulting in more aggressive resuscitation may further improve survival in severe trauma.<sup>115</sup> Therefore, recently updated guidelines of the American Association of Blood Banks and the European task force recommend early intervention with FFP but without a preset FFP:RBC ratio.<sup>67,116</sup>

From a mechanistic point of view, FFP increases the procoagulant, anticoagulant, and antifibrinolytic potential<sup>10</sup> when given in adequate amounts<sup>117</sup> at an early stage of dilution.<sup>109</sup> However, there are safety concerns about the routine use of FFP that limit its therapeutic benefits.<sup>118,119</sup> First, there is a potential, although low, risk of viral transmission with FFP. Such risks may be further reduced in the future as more virus inactivated plasma products become available.<sup>120</sup> The incidence of transfusion-related acute lung injury has recently decreased after the adoption of male-only donor policies for FFP.<sup>121</sup> However, large volumes of FFP are required to raise factor levels, and the administration of FFP may increase the incidence of volume overload, nosocomial infections, multiple organ failures, and possible mortality.<sup>119,122,123</sup> Therefore, FFP should not be considered as a fluid replacement therapy,<sup>1,64,67,124</sup> but if it is clinically proven effective, the use of FFP in massive hemorrhage may be a notable exception because of acute hypovolemia.<sup>109,110</sup>

### Cryoprecipitate, Fibrinogen Concentrate, and FXIII Concentrate

Cryoprecipitate is the plasma component that is prepared after partially thawing FFP. Because cryoprecipitate is rich in fibrinogen, FXIII, von Willebrand factor, and FVIII, it has been used for the treatment of bleeding in acquired fibrinogen or FXIII deficiency. In European countries, the use of cryoprecipitate has largely ceased, and specific plasma-derived factor concentrates are administered instead for fibrinogen or FXIII deficiency. Because FFP transfusion is insufficient to raise plasma fibrinogen in the United States and United Kingdom, cryoprecipitate is an alternative for the replacement of low plasma fibrinogen. One unit (15 ml) of cryoprecipitate per 10 kg of body weight is estimated to increase plasma fibrinogen by 0.5 g/l in the absence of continuing bleeding. The plasma fibrinogen level can be increased proportionally to the transfused amount of cryoprecipitate or fibrinogen concentrate,<sup>125</sup> whereas 30 ml/kg FFP is required to raise the plasma fibrinogen level by 1 g/l.<sup>117</sup>

Although there is a paucity of data on the safety and efficacy of cryoprecipitate in the massive transfusion setting, roles for fibrinogen in hemostasis have been previously suggested (table 2). A high ratio of fibrinogen to transfused erythrocyte units has been associated with a reduction in mortality in combat trauma patients.<sup>126</sup> High plasma fibrinogen levels (more than 3 g/l) may even compensate for low platelet counts.<sup>11,91</sup> There are

increasing clinical data that support the use of fibrinogen concentrate to reduce blood loss and transfusion of erythrocytes and platelets after major surgery without increasing thrombotic complications.<sup>68,71,72,127</sup>

Decreased levels of FXIII have been associated with an increased bleeding tendency after major cancer surgery and neurosurgery, and FXIII supplementation has been proven to decrease blood loss after major cancer surgery.<sup>27,56,74</sup> *In vitro* studies suggest that FXIII can improve clot stability,<sup>88,128</sup> but FXIII may be less efficacious in cases of low fibrinogen levels. However, cryoprecipitate with high concentrations of fibrinogen, FXIII, and FVIII may be a valuable alternative for a single coagulation factor transfusion. To conclude, restoring fibrinogen and FXIII levels seems to be advantageous in bleeding management after major surgery or trauma, but the choice between FFP, cryoprecipitate, and fibrinogen in massive hemorrhage remains controversial, and further investigations are required.

### Prothrombin Complex Concentrate

Prothrombin complex concentrate (PCC) contains FII, FVII, FIX, and FX, as well as proteins C and S, and trace amounts of heparin and antithrombin, depending on the product. PCC has been used conventionally for the treatment of hereditary deficiency of FII, FVII, FIX, and FX, but individual (plasma-derived or recombinant) factor concentrates may be available for this indication. In most European countries and Canada, PCC is approved for a rapid reversal of vitamin K antagonists (coumarin derivatives).<sup>129</sup> In contrast to FFP (1 unit, 250 ml) which contains 0.5–1.0 IU/ml of all plasma factors, the factors contained in PCC (about 500 IU, 20 ml) are highly concentrated, at up to 25 times the levels found in FFP.<sup>129</sup> Without the need for cross-matching/thawing, it is possible to replace vitamin K-dependent factors rapidly without the risk of volume overload, exposure to immunoglobulins, and additional hemodilution (particularly for erythrocytes and platelets).<sup>130,131</sup>

However, there is a paucity of data on the use of PCC in coagulopathy due to hemodilution, trauma, or hepatic dysfunction. In a porcine hemodilution model, PCC (35 units/kg) improved PT and showed a trend of decreasing blood loss after splenic injury.<sup>132</sup> In several small retrospective studies, PCC was shown to be hemostatic in postcardiac surgical patients who developed coagulopathy refractory to platelets, FFP, and cryoprecipitate.<sup>133–135</sup> In an *in vivo* study in 16 critically ill patients with acquired deficiency of coagulation factors caused by various conditions, PCC was shown to reverse PT and restore factor levels.<sup>136</sup> In trauma patients, the use of PCC after the initial treatment with fibrinogen concentrate was shown to reduce the need for FFP without affecting survival rate.<sup>90</sup> In summary, several lines of evidence suggest that PCC is beneficial in treating bleeding after hemodilution by increasing thrombin generation, which optimizes fibrin generation and possibly antifibrinolytic properties. Although the use of PCC is presumably safe for acute reversal of coumarins, there is a paucity of data on its safety in

the setting of massive hemorrhage and hemodilution. The prothrombotic risk of PCC may be increased in the presence of antithrombin deficiency caused by hemodilution.<sup>21,137</sup> Additional clinical studies are necessary to establish optimal indications and dosages for PCC in perioperative settings.

### Recombinant Activated Factor VII

Two prospective randomized trials of recombinant activated FVII in massive transfusion (more than 8 units of erythrocytes) from blunt or penetrating injury demonstrated no differences in erythrocyte transfusion within 48 h (primary endpoint) between patients who received recombinant activated FVII (400 µg/kg in three divided doses) and those who had the placebo.<sup>138</sup> However, in the subgroup analysis of blunt trauma patients who survived beyond 48 h, less erythrocyte transfusion (reduction of 2.6 units;  $P = 0.02$ ) and reduced incidence of massive transfusion (14% vs. 33%;  $P = 0.03$ ) were observed with recombinant activated FVII treatment relative to placebo. A trend favoring recombinant activated FVII for reducing massive transfusion was also observed in penetrating trauma cases (7% vs. 19%;  $P = 0.08$ ). In addition, positive effects of recombinant activated FVII in obstetric hemorrhage patients without relevant numbers of thromboembolic complications were recently reported.<sup>139</sup> Recombinant activated FVII after hemodilution may only be efficacious when fibrinogen levels are supplemented first.<sup>67,140</sup> Because of accelerated thrombin generation together with low antithrombin levels after hemodilution, the administration of recombinant activated FVII may potentially increase the risk of thromboembolic complications.<sup>141</sup> However, a small randomized study in 30 blunt trauma patients with traumatic brain injury did not show an increased rate of thromboembolic complications after administration of recombinant activated FVII (400 µg/kg in three divided doses).<sup>142</sup>

### Platelet Concentrates

In hemorrhage after trauma or major surgery, the administration of platelet concentrates has to be considered if platelet count falls below  $50 \times 10^3/\mu\text{L}$ .<sup>1,64,67</sup> However, because of margination of platelets under *in vivo* flow conditions<sup>102</sup> and possible release from sequestered platelets in the spleen, lungs, and bone marrow,<sup>15</sup> the threshold for administration of platelets, especially in cases of dilutional coagulopathy, remains unclear. Additional prospective studies are warranted to evaluate the efficacy of administering RBC:FFP:platelets at a 1:1:1 ratio in severely injured patients with massive bleeding.<sup>143,144</sup>

Platelet dysfunction induced by drug therapy (acetylsalicylic acid, glycoprotein IIb/IIIa inhibitors, and others) can cause excessive bleeding with normal platelet counts. When platelet dysfunction is identified or strongly suggested, transfusion of platelet concentrates is strongly advised, even when platelet counts are normal.<sup>8</sup> Potential limitations of platelet transfusion include serious adverse events, such as transfu-

sion-associated viral or bacterial infections, transfusion-associated lung injury, stroke, or even death.<sup>8,145</sup>

Desmopressin acetate, an analog of endogenous vasopressin, has been shown *in vitro* to antagonize platelet dysfunction induced by glycoprotein IIb/IIIa inhibitors and aspirin.<sup>146</sup> Desmopressin acetate has also been reported to be effective in reducing blood loss after cardiac surgery<sup>147</sup>; however, subsequent studies failed to show marked benefits in improving perioperative hemostasis.<sup>148</sup> A systematic review showed that desmopressin acetate was able to reduce perioperative blood loss but did not minimize perioperative allogeneic erythrocyte transfusion.<sup>149</sup> Data on the use of desmopressin acetate in hemorrhage and dilution are lacking, but it may be speculated that there is a tachyphylaxis caused by high stress and endogenous exhaustion of procoagulant factors. A potential beneficial effect of factor VIII/von Willebrand factor concentrate on platelet function has yet to be proven.

### Antifibrinolytics

Fibrinolysis is frequent in severe trauma<sup>6,9,34,35,62</sup> and hemodilution,<sup>10</sup> but it is rarely diagnosed. Lysine analogues,  $\epsilon$ -aminocaproic acid and tranexamic acid, are currently available antifibrinolytics. It is not known whether antifibrinolytic therapy could actually lower the threshold levels of fibrin(ogen) in cases of severe hemodilution, but antifibrinolytics are presumably effective in preserving a weak fibrin clot that is otherwise susceptible to plasmin. Tranexamic acid has been shown to improve clot stability in hemophilic patients.<sup>87</sup> The overall reductions in blood loss and the need for allogeneic red cell transfusion by lysine analogues have been reported in cardiac, orthopedic, and hepatic surgery.<sup>150</sup> A prospective randomized placebo-controlled trial was recently conducted to investigate the effectiveness of tranexamic acid (1 g loading followed by 1 g over 8 h) in 20,211 trauma patients.<sup>151</sup> This study demonstrated significant reductions in all-cause mortality (14.5% *vs.* 16.0%; relative risk 0.91;  $P = 0.0035$ ), and in deaths due to bleeding (4.9% *vs.* 5.7%; relative risk 0.85;  $P = 0.0077$ ), without increasing vascular occlusive events, in the tranexamic acid group compared to the placebo group.<sup>151</sup>

### Conclusion

Hemodilution caused by trauma and major surgery induces complex hemostatic changes involving procoagulant factors as well as anticoagulant, fibrinolytic, and antifibrinolytic factors. The endothelial responses to shear stress, active proteases, and various inflammatory cells and cytokines add further complexity to the pathophysiology of massive hemodilution. In addition to the conventional transfusion products, which are often difficult to administer in a timely manner, purified factor concentrates of plasma origin and from recombinant synthesis are highly concentrated (*i.e.*, small volume) for a rapid restoration of targeted factor(s). The use of point-of-care testing is desirable to optimize the

dose and timing of such intervention. Additional clinical trials of different factor concentrate therapies are required to validate their efficacy and safety in patients after trauma or major surgery.<sup>152</sup> Further understanding of the time course of pathophysiological changes in massive hemodilution is necessary to optimally balance hemostatic and anticoagulant therapies.

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