



The role of fibrinogen: a new paradigm in the treatment of coagulopathic bleeding

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ABSTRACT

Fibrinogen is involved in both primary and secondary hemostasis, playing an important role in platelet aggregation and the establishment of a fibrin network. Recent evidence suggests that very high levels of fibrinogen act as antithrombin and can reduce endogenous thrombin potential and compromise clot stability, particularly following a low tissue factor stimulus. Several laboratory methods for measuring plasma fibrinogen concentrations are available, but results vary depending on the type of method and the use of artificial colloid plasma expanders. Adopting only the Clauss method can provide erroneously high levels when used in patients who have received colloid plasma expanders. This may contribute to a hazardous delay or complete lack of treatment. Multiple *in vitro* experiments, animal studies, and proof-of-principle randomized, clinical studies have recently suggested that hemostatic intervention with a fibrinogen concentrate may be efficient and safe in controlling perioperative bleeding. In particular, fibrinogen concentrate has a key role in improving clotting function and reducing blood loss in settings such as trauma and cardiothoracic surgery. However, prospective studies are needed to determine the clinical efficacy and safety of fibrinogen concentrate when used as a hemostatic intervention for patients with massive bleeding due to trauma or surgery.

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Abbreviations

CPB: cardiopulmonary bypass surgery

FFP: fresh frozen plasma

HES: hydroxyethyl starch

MCF: maximum clot firmness

PCC: prothrombin complex concentrate

rFVIIa: recombinant activated factor VII

Fibrinogen has long been known to play an essential role in hemostasis, and fibrinogen concentrates have been commercially available for decades for use in patients with hypofibrinogenemia, dysfibrinogenemia, and afibrinogenemia [1]. Recent insights into the mechanism and effects of fibrinogen and fibrin polymerization suggest that fibrinogen contributes substantially to achieving and maintaining hemostasis, particularly in patients with massive bleeding caused by trauma or surgery. This article summarizes the current understanding of the role of fibrinogen in hemostasis, the pathophysiology of acquired fibrinogen deficiency, issues in measuring fibrinogen levels and function, and preclinical and clinical evidence on the use of fibrinogen concentrate as a hemostatic intervention in patients with traumatic or post-operative bleeding.

Fibrinogen and fibrin polymerization: pathophysiology

Fibrinogen is a 300-kDa soluble plasma glycoprotein consisting of two identical subunits, each of which contains three polypeptide chains ($A\alpha$, $B\beta$, and γ) (Figure 1) [1]. It is synthesized in hepatocytes. Fibrinogen plays a role in both primary and secondary hemostasis. It facilitates platelet aggregation by binding to glycoprotein IIb/IIIa receptors on the surface of activated platelets, effectively forming a spider's web between activated platelets in the initial platelet plug. When fibrinogen is cleaved by thrombin, two fibrinopeptides (A and B) are released, followed by spontaneous polymerization of fibrin. Of note, fibrinogen is also an acute phase protein that is markedly upregulated by cytokine-mediated inflammatory reactions [1].

Beyond clot formation, fibrinogen has several other important functions that indicate that its role in hemostasis is complex. We have shown that increasing concentrations of fibrinogen beyond baseline reduces endogenous thrombin potential, irrespective of the degree of tissue factor stimulus [2]. In contrast, the impact of fibrinogen levels on clot stability appears to depend on tissue factor stimulus: following a high tissue factor stimulus, increasing levels of fibrinogen offer increasing clot stability; after an intermediate tissue factor stimulus, however, higher levels of fibrinogen exhibit antithrombin-like effects, reducing clot stability; this effect is even more pronounced after a low tissue factor stimulus, whereby the antithrombin-like effect is evident even at low levels of fibrinogen.

Trauma-induced coagulopathy

Approximately 25% of trauma patients present in emergency departments with acute coagulopathy, and these patients have a 4-fold higher risk of mortality than those without coagulopathy [3,4]. Trauma-induced tissue

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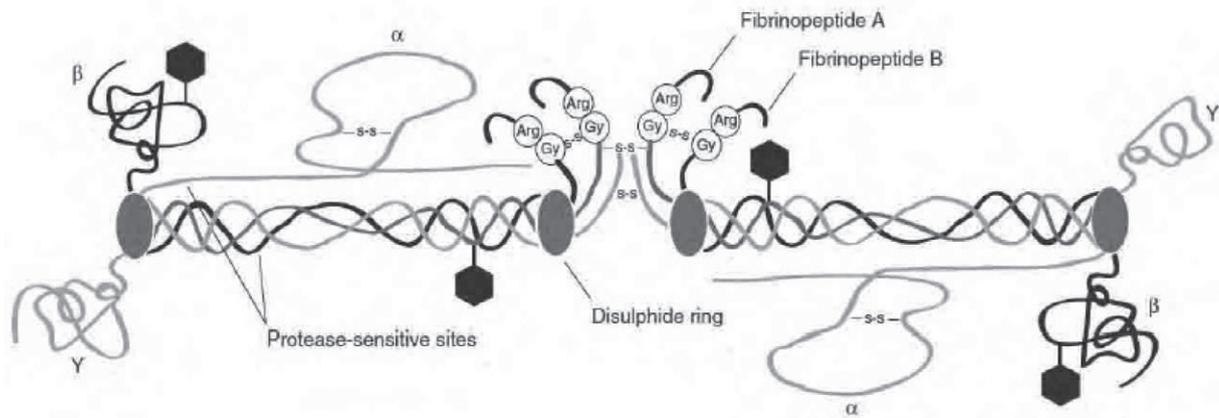


Fig. 1. Fibrinogen protein structure. Reproduced with permission from Fenger-Eriksen C, Ingerslev J, Sørensen B. Fibrinogen concentrate – a potential universal hemostatic agent. *Expert Opin Biol Ther* 2009;9:1325-33 [1]. Copyright © 2009, Informa Healthcare.

damage produces an acute stress reaction, vessel damage, and the release of tissue factor – all of which contribute to excessive activation of the coagulation system. In a porcine model of severe intracranial hypertension, we demonstrated that brain injury induced a hypercoagulable state, characterized by rapid clot initiation, increased clot propagation, increased thrombin generation, and reduced prothrombin time [5]. It appeared that release of tissue factor following brain injury was the primary trigger of this hypercoagulable state.

Areas of tissue trauma can become hypoperfused due to bleeding and vessel leakage, and this can lead to acidosis [4]. Hypoperfusion acidosis causes endothelial cells to release tissue-type plasminogen activator, which in turn leads to hyperfibrinolysis. Importantly, induction of acidosis has been shown to decrease fibrinogen levels by approximately 30% and platelet counts by approximately 50% [6]. These reductions appear to be irreversible, despite correction of pH. Paradoxically, while hypoperfusion initially causes excessive thrombin generation, the excess thrombin interacts with and upregulates thrombomodulin, and activates protein C; activation of this pathway can cause an inappropriate reduction of thrombin generation at a time when it is needed most to prevent bleeding [4].

Volume substitution is another important source of trauma-related coagulopathy. Volume resuscitation can lead to dilutional coagulopathy, and the use of synthetic colloid plasma expanders, such as hydroxyethyl starch (HES), gelatin, or dextran, can further impair coagulation beyond what is expected from dilution alone [7]. Fibrinogen appears to play a key role in this process: hemodilution with gelatin has been shown to produce an altered fibrin structure due to a reduction in fibrinogen concentration and interference with fibrin polymerization (Figure 2) [8]. In a study of whole blood coagulation profiles after hemodilution with isotonic saline, HES, or dextran, we showed that volume substitution reduced clot firmness and compromised clot propagation. *Ex vivo* addition of washed platelets or factor VIII had minimal impact on coagulation profiles, but the addition of fibrinogen corrected the impaired clot firmness caused by hemodilution [7]. Similar results were observed using a porcine model, in which the addition of fibrinogen after hemodilution with gelatin returned clot firmness and formation to baseline values [7]. It is important to note that the impact of volume substitution on coagulation is more complicated than a simple dilution of coagulation factors: hemodilution produces a specific pattern of derangement that affects some (but not all) coagulation factors [9]. Fibrinogen and factors II, X, and XIII, for example, are significantly reduced, while no significant change is seen in hematocrit, platelet count, factors VII or VIII, or thrombin generation. This evidence confirms that fibrinogen plays a key role in coagulopathy caused by volume substitution.

Coagulopathy after cardiac surgery

Changes in fibrinogen and fibrin polymerization leading to coagulopathy are time-specific. One example of this phenomenon is the coagulopathy seen perioperatively in patients undergoing cardiopulmonary bypass surgery

(CPB). We recently demonstrated that the magnitude of deterioration in fibrin polymerization immediately after CPB was greater than the deterioration in thrombin generation [10]. Tang and colleagues [11] also reported the results of a 48-hour longitudinal study, in which whole blood clot formation by thromboelastometry and thrombin generation was assessed following CPB. Maximum clot firmness (MCF) and thrombin generation decreased gradually for the first 6 hours after CPB, but were restored by hour 12. The restoration in these parameters is likely due to an inflammatory related increase in fibrinogen. Subsequent initiation of thromboprophylaxis had no effect on fibrin polymerization and clot firmness, but it inhibited thrombin generation, as expected. Notably, the most effective method for correcting MCF during bleeding immediately after CPB was fibrinogen substitution; platelet administration had a modest effect and the use of fresh frozen plasma (FFP), prothrombin complex concentrate (PCC), or recombinant activated factor VII (rFVIIa) had limited effects on clot firmness [12]. The most effective way to correct impaired thrombin generation in cases of late bleeding was with prothrombin complex concentrate. These and other findings [13] confirm that fibrinogen modulates postoperative blood loss after CPB.

These results raise some interesting questions regarding the optimal approach to correcting coagulopathy following CPB. While correction of thrombin generation is effective in cases where fibrin polymerization is normal, it may have limited hemostatic efficacy in cases where the coagulopathy is due primarily to early defective fibrin polymerization. For the past 2 decades, the focus of traumatic or perioperative bleeding management has been on restoring and facilitating thrombin generation with products such as rFVIIa, PCC, and FFP [1]. However, these findings suggest the need for a more critical look at the effects of early reductions in fibrinogen and defective fibrin polymerization, and the potential role of fibrinogen substitution as a hemostatic agent.

Measurement of fibrinogen

Two basic methods are available for measuring levels of fibrinogen in plasma: immunologic/quantitative methods and functional methods. Immunologic/quantitative methods measure antigen levels of fibrinogen – including dysfunctional fibrinogen. Several techniques are available for functional fibrinogen assessment. The most commonly used technique is the Clauss method, whereby thrombin is added in excess to citrated plasma, and the clotting time is proportional to the fibrinogen concentration [14]. The Clauss method has some important limitations, as detailed below. Alternatively, thromboelastometry can be used to measure fibrin polymerization, which has been shown to correlate with functional fibrinogen concentration [15,16]. With this method, platelets in citrated blood are inhibited with cytochalasin D, a potent inhibitor of the platelet cytoskeleton, and thromboplastin is used as an activator.

Traditionally, a fibrinogen level of 1.0 g/L has been the trigger level for intervention with fibrinogen. Recently, however, some evidence has been

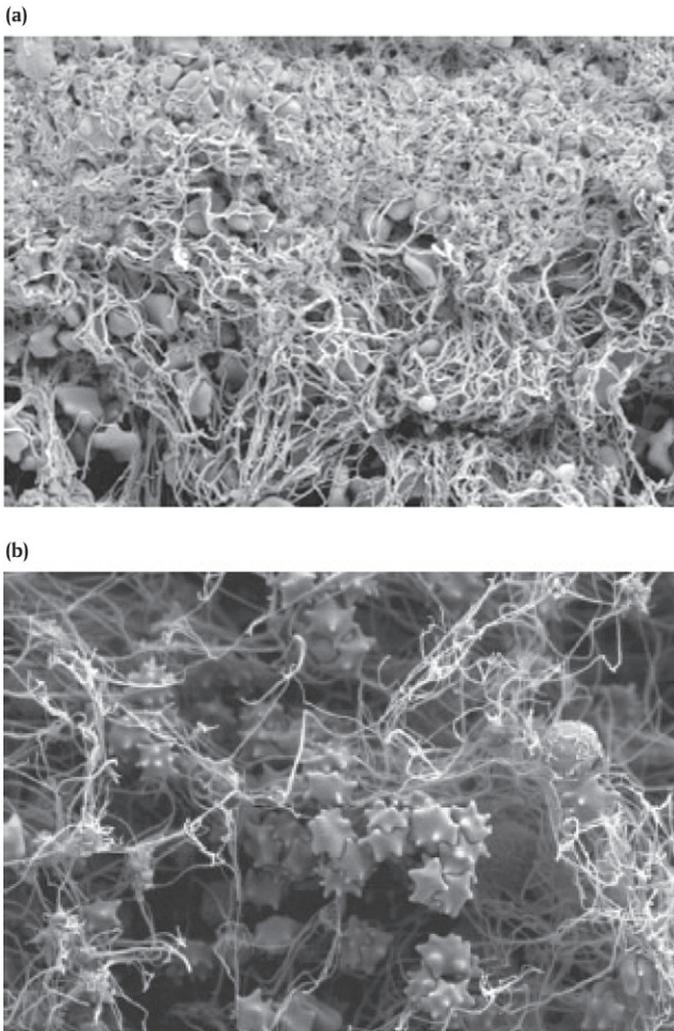


Fig. 2. Electron microscopy scans ($\times 2000$) of blood clots in a porcine model. (a) Blood clot in a non-diluted state, exhibiting a dense, branched, and meshed reticular fibrin network. (b) Blood clot following 65% hemodilution with gelatin, characterized by a clearly rarefied fibrin network. Reproduced with permission from Fries D, Krismer A, Klingler A, Streif W, Klima G, Wenzel V, et al. Effect of fibrinogen on reversal of dilutional coagulopathy: a porcine model. *Br J Anaesth* 2005;95:172-7 [8]. Copyright 2005, Oxford University Press.

reported that suggests that the optimal trigger level may be higher (e.g. 1.5 g/L or 2.0 g/L); thus, patients with higher levels may also benefit from fibrinogen substitution [17].

We recently compared the results of fibrinogen assessment using various methods to determine whether outcomes differed depending on the type of method used and the presence of colloid plasma expanders [18]. Methods included an immunologic/quantitative method and several functional methods, including thromboelastometry and multiple photometric and mechanical techniques that relied on the Clauss method. Samples were diluted 50% with isotonic saline, human albumin, or HES 130/0.4. We found a marked discrepancy in outcomes when HES was used in photometric techniques (Figure 3). Dilution with HES produced erroneously high estimates of fibrinogen level compared with results using the same analyzer and dilution with isotonic saline. The impact of HES on fibrin function was most evident in thromboelastometry assay: HES led to considerably lower levels of fibrin polymerization and estimates of fibrinogen level. The magnitude of the discrepancy could have important clinical consequences if fibrinogen levels are at or near the trigger level for intervention.

Based on these findings, we concluded the following [18]:

- results should be interpreted with caution when the Clauss method is used to assess plasma fibrinogen levels in patients with massive bleeding and fluid resuscitation with colloids;
- with the Clauss method, use of mechanical detection techniques appears to be more reliable than photometric methods;
- the thromboelastometry assay was the most optimal method for measuring fibrin polymerization defects induced by HES;
- for patients with trauma or perioperative bleeding, thromboelastometry should be performed to ensure sufficient levels of fibrinogen and fibrin polymerization.

Fibrinogen substitution as a hemostatic intervention

Interest in the use of fibrinogen concentrate as a hemostatic agent has risen based on recent preclinical data indicating that fibrinogen concentrate can correct coagulopathy caused by volume substitution with artificial colloids [7,8]. A retrospective review of outcomes following fibrinogen intervention for various types of clinical conditions suggested that fibrinogen significantly improved activated partial thromboplastin time and prothrombin time [19]. Notably, treatment with fibrinogen was also associated with a marked reduction in the need for transfusion of red blood cells, FFP, and pooled platelets.

These findings prompted the initiation of a randomized, placebo-controlled, proof-of-principle study to determine the effect of fibrinogen

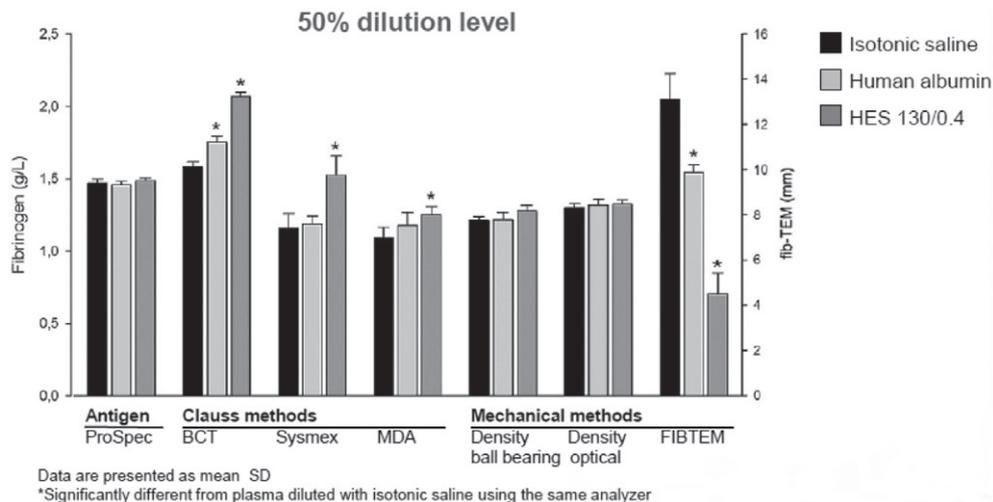


Fig. 3. Assessment of plasma fibrinogen level using multiple techniques. Adapted from [18].

BCT, blood-clotting time; FIBTEM, fibrin thromboelastometry; HES, hydroxyethyl starch; MDA, MDA II coagulation analyzer; SD, standard deviation.

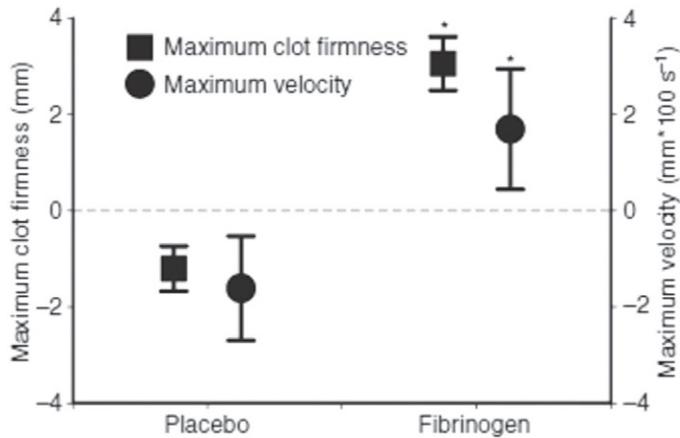


Fig. 4. Absolute change in maximum clot firmness and velocity in surgical patients treated with fibrinogen or placebo after 30% hemodilution with hydroxyethyl starch 130/0.4. Data presented as mean \pm SD. * $p < 0.05$ versus placebo. Reproduced with permission from Fenger-Eriksen C, Jensen TM, Kristensen BS, Jensen KM, Tønnesen E, Ingerslev J, Sørensen B. Fibrinogen substitution improves whole blood clot firmness after dilution with hydroxyethyl starch in bleeding patients undergoing radical cystectomy: a randomized, placebo-controlled clinical trial. *J Thromb Haemost* 2009;7:795-802 [20]. © 2009 International Society on Thrombosis and Haemostasis.

concentrate on hemostasis following surgery [20]. A total of 20 patients who underwent elective cystectomy were given HES 130/0.4 for blood loss. When the dilution level was 30%, patients were randomized to placebo or fibrinogen. The primary endpoint was MCF as determined by thromboelastometry, and change in transfusion requirement was a secondary endpoint.

Hemodilution with HES was associated with significantly impaired MCF compared with baseline values, although measures of clot initiation and propagation were not affected [20]. Administration of fibrinogen significantly improved MCF, while MCF continued to deteriorate in patients given placebo (Figure 4). A similar pattern was observed with the maximum velocity of clot formation. As expected, plasma fibrinogen levels increased after administration of fibrinogen concentrate and persisted for up to 6 hours; after 24 hours, fibrinogen levels were increased in both treatment groups, presumably due to inflammatory response-mediated release of fibrinogen. Notably, two of 10 patients treated with fibrinogen required transfusions in the first 48 hours after surgery, compared with eight of 10 patients treated with placebo ($p < 0.05$). These results indicate that administration of fibrinogen concentrate significantly improved clot strength and reduced postoperative transfusion requirement compared with placebo in patients with massive bleeding hemodiluted to 30% with HES 130/0.4.

Summary and future directions

Fibrinogen plays an important role in hemostasis, and reduction of plasma fibrinogen concentration to critically low levels occurs at an early stage in patients with traumatic or postoperative bleeding. Fibrinogen is unique in that it contributes to both primary and secondary hemostasis, which suggests that it may be particularly well suited for use as a hemostatic intervention. On the other hand, the hemostatic effects of fibrinogen depend in part on sufficient thrombin generation, and the inhibitory effects of high fibrinogen concentrations on thrombin generation and clot stability suggest that the full effects of fibrinogen on hemostasis are not yet fully understood. Data from laboratory studies, animal models, and a small randomized proof-of-principle trial indicate that administration of fibrinogen concentrate can correct dilutional coagulopathy. The available evidence also suggests that fibrinogen

concentrate can reduce transfusion requirement in the postoperative setting. While these preliminary results are compelling, larger prospective studies are needed to determine the clinical efficacy and safety of fibrinogen concentrate. In addition, efforts are needed to optimize and standardize the assessment of fibrinogen levels and function, as some standard laboratory tests using the Clauss method for determining plasma fibrinogen concentration have been shown to provide erroneously high levels when used in patients with bleeding treated with synthetic colloid plasma expanders.

Conflict of interest

BS has participated in advisory boards and/or received speaker honoraria from Novo Nordisk, Baxter, CSL Behring, Quintiles, Bayer, Pentapharm, and SOBI. The Haemostasis Research Unit receives unrestricted research support from Novo Nordisk, Grifols, CSL Behring, LFB, Baxter, SOBI, Bayer and Octapharma.

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