

# Fibrinogen concentrate substitution therapy in patients with massive haemorrhage and low plasma fibrinogen concentrations

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**Background.** Patients experiencing massive haemorrhage are at high risk of developing coagulopathy through loss, consumption, and dilution of coagulation factors and platelets. It has been reported that plasma fibrinogen concentrations may reach a critical low level relatively early during bleeding, calling for replacement fibrinogen therapy. Cryoprecipitate has been widely used in the past, but more recently, a pasteurized fibrinogen concentrate has become available. We audited the effects of fibrinogen concentrate therapy on laboratory and clinical outcome in patients with massive haemorrhage.

**Methods.** We identified 43 patients over the previous 2 yr to whom a fibrinogen concentrate had been administered as treatment for hypofibrinogenaemia during serious haemorrhage. Platelet count, P-fibrinogen, activated partial thromboplastin time (APTT), prothrombin time (PT), D-dimer, and volume of blood lost were obtained from medical and laboratory records. Numbers of units of red blood cells (RBC), fresh frozen plasma (FFP), and pooled platelet concentrates were recorded before and after fibrinogen substitution.

**Results.** A significant increase in plasma fibrinogen concentration was observed after fibrinogen concentrate therapy. Platelet counts and fibrin D-dimer values remained unchanged, whereas the APTT and PT improved significantly. Requirements for RBC, FFP, and platelets were significantly reduced. Blood loss decreased significantly.

**Conclusions.** Off-label substitution therapy with a fibrinogen concentrate generally improved global laboratory coagulation results and as supplementary intervention, appeared to diminish the requirements for RBC, FFP, and platelet substitution in this patient cohort.

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Excessive bleeding and coagulopathy may occur in relationship with various conditions such as advanced liver disease, obstetric complications, disseminated intravascular coagulation, major trauma, or surgery and may in part be caused by insufficient plasma concentrations of functional fibrinogen. In more general terms, acquired fibrinogen deficiency may result from inadequate synthesis, increased loss, or dysfunction of the fibrinogen molecule compromising its polymerization into fibrin.

Diminished haemostatic capacity in a massively bleeding patient is caused by multiple factors such as loss,

consumption, or dilution of coagulation factors, platelets, and other blood cells involved in the overall regulation of haemostasis. The bleeding tendency may be further aggravated by anaemia, hypothermia, and acidosis.<sup>1</sup> Moreover, the final haemostatic clot may be less resistant to fibrinolysis, which further compromises the ability to establish and maintain haemostasis. Management of the excessively bleeding patient is multidisciplinary and involves correction of hypothermia and acidaemia, resuscitation with crystalloids or colloids, and infusion of red blood cells (RBC), fresh frozen plasma (FFP), and platelets.<sup>2</sup> Treatment

regimens may also include infusion of antifibrinolytic drugs and coagulation factor concentrates such as recombinant activated factor VII, prothrombin complex concentrates, cryoprecipitate, or fibrinogen. During progressive blood loss, fibrinogen appears to represent the coagulation factor first reaching a critical low threshold level at around 1 g litre<sup>-1</sup>;<sup>3</sup> the decrease in fibrinogen may emerge before the development of significant thrombocytopenia. In addition, haemodilution with colloid plasma expanders predisposes to the development of a functional fibrinogen deficiency as recently described by our own group<sup>4</sup> and others.<sup>5,6</sup> Fibrinogen is synthesized in the liver. Besides playing a crucial role in the final steps of the coagulation process, it is an acute-phase reactant protein. The concentration of fibrinogen in healthy persons displays a range of 2–4 g litre<sup>-1</sup> (6–13 µmol litre<sup>-1</sup>) with up to a 10-fold increase during tissue damage, infection, and cytokine-induced inflammatory responses. It seems reasonable to assume that seriously bleeding patients suffering from critically low levels of fibrinogen or a functional fibrinogen deficiency may benefit from substitution therapy. In the past, FFP or cryoprecipitate has been the mainstay in correction of fibrinogen deficits. However, since cryoprecipitate is produced from donor plasma pools and is not subjected to attenuation of possible viral contaminants, the use of cryoprecipitate has diminished considerably in the EU.<sup>7</sup> Instead, virally inactivated commercially available fibrinogen concentrates derived from human plasma<sup>8</sup> are being increasingly used, although none of the available fibrinogen concentrates has reached approval by the Mutual Recognition Procedure. The role of fibrinogen, a critical haemostatic coagulation factor in ensuring sufficient haemostasis during serious bleeding, has been a subject of increasing interest.<sup>9,10</sup> Although several experimental laboratory and animal studies strongly suggest a potent haemostatic effect of fibrinogen substitution, only few observational clinical data are available. This retrospective survey evaluated the laboratory and clinical outcome after substitution therapy with a fibrinogen concentrate (Haemocomplettan<sup>®</sup>, CSL Behring, Marburg, Germany) in continuously bleeding patients in our institution, where critically low plasma fibrinogen concentrations have been detected.

## Methods

This single-centre retrospective survey was approved by the Danish Data Protection Agency (Reference number 2007-41-1136). Patients in whom a fibrinogen concentrate had been administered to treat hypofibrinogenaemia (defined by a laboratory threshold value below 2 g litre<sup>-1</sup>) occurring during a course of serious bleeding were identified through the hospital dispensary and via report forms filed together with the off-label dispensing of a virally inactivated, pasteurized fibrinogen concentrate derived from plasma, Haemocomplettan<sup>®</sup> (CSL Behring Marburg, Germany). Patient's clinical data together with the amount

and the time of administration of Haemocomplettan<sup>®</sup> were recorded from examination of medical records. The product is licensed in some EU countries such as Germany, Austria, Portugal, and the Netherlands, but not in Denmark. Permission to use this fibrinogen concentrate for congenital and acquired fibrinogen deficiency was granted by the Danish Medicines Agency. Hence, the present survey reflects results following special license use of Haemocomplettan<sup>®</sup> administered via the Centre for Haemophilia and Thrombosis at Aarhus University Hospital, Skejby, Denmark. In order to correct plasma fibrinogen concentrations (to >2 g litre<sup>-1</sup>) and improve haemostasis, each patient was dosed according to our previously published pharmacokinetic observations which showed that an infusion of 2 g fibrinogen concentrate increases in plasma fibrinogen by 1 g litre<sup>-1</sup>.<sup>8</sup> The actual dose was selected individually based on the measured low level of plasma fibrinogen, severity of bleeding, body weight, and according to recommendations listed in the summary of product characteristics for Haemocomplettan<sup>®</sup> (adults: 1–2 g initially and cumulated amounts of 4–8 g if required in case of severe bleeding).<sup>11</sup>

Data on infused blood components were obtained from the electronic transfusion database of our University Hospital Blood Bank that records delivery of blood products (RBC, FFP, and platelet pools) in real-time mode. This tool enabled us to retrieve data on any Blood Bank product delivered before and after infusion of the fibrinogen concentrate. To ensure that each bleeding episode was properly assessed and to avoid underestimation on the use of blood products, the observation period for blood therapy was 48 h in total. Data on observed and measured blood losses were retrieved from the patient's medical records in which blood loss was reported in detail and continuously registered as drainage tube volume and weight of napkins used for wound drainage. RBC supplementation in the present study followed our national guidelines: Hb concentration <7.2 or <9.7 g dl<sup>-1</sup> in patients with ischaemic heart disease.

Laboratory coagulation parameters recorded were: platelet count, plasma fibrinogen concentration, activated partial thromboplastin time (APTT), prothrombin time (PT), and D-dimer. In all patients, a laboratory coagulation screen was available before and after administration of fibrinogen. The interval between blood samples varied between patients, ranging from 0 to 23 h (median=8 h). D-dimers were determined by an automatic immunoturbidimetric test based on changed turbidity after suspension of latex particles coated with antibodies against fibrin D-dimer (STAR-Liatest D-dimer). Remaining coagulation assays were performed on the STAR Evolution analyser (Diagnostica Stago, Asnières, France) using commercially available reagents: APTT (phospholipids reagent, Platelin LS, Organon, Munich, Germany), PT (calcium-thromboplastin reagent, STA-Neoplastin, Diagnostica Stago), and fibrinogen (Claus's method) (thrombin reagent, STA-Fibrinogen, Diagnostica Stago).

Primary outcome variables were transfusion requirements and changes in laboratory coagulation indices. In addition, the estimated volume of blood lost during a 24 h period before and after administration of fibrinogen concentrate was recorded from examination of the medical records. Records were also examined for adverse effects related to the administration of fibrinogen concentrate, with particular focus on anaphylactoid reactions and thromboembolic episodes.

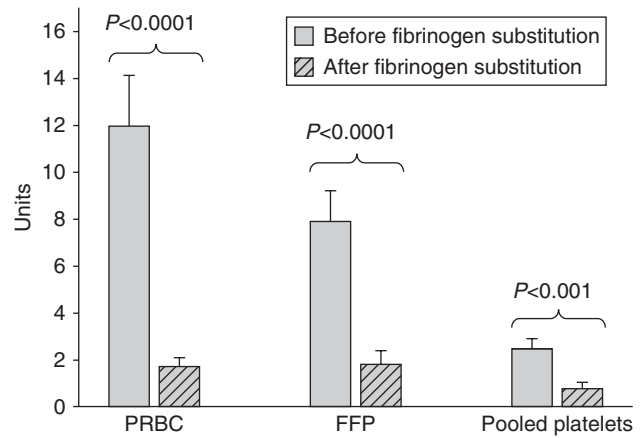
Statistical analysis was performed using statistical software GraphPad InStat (version 3.00, GraphPad Software, San Diego, CA, USA). Since the patient material expressed a pronounced heterogeneity, data before and after administration of fibrinogen concentrate were considered non-parametric and were evaluated using Wilcoxon's rank-sum test. A *P*-value of  $<0.05$  was considered statistically significant. Data are presented using median and quartiles or median and range or absolute data, respectively.

## Results

Fifteen males and 28 females with an average age of 49.5 yr (range 0.1–76 yr) were included. All patients underwent surgery. The majority of the haemorrhages were subsequent to surgery whereas in most of the obstetric cases, surgery was instituted as supplementary treatment to control the bleeding. Underlying clinical conditions were: 12 obstetric complications, four newborns with bleeding problems during operation for congestive heart disease, 14 patients had cardiothoracic surgery, six intra-abdominal surgery, six trauma patients, and one patient with post-operative pharyngeal bleeding (Table 1). Eight patients (18%) in the study group died in immediate continuation with the bleeding episodes. These eight patients were excluded from data analysis regarding transfusion requirements and blood loss.

Total transfusion requirements of RBC, FFP, and pooled platelet concentrates were significantly reduced in the 12 h after administration of fibrinogen concentrate (Fig. 1). Median total blood loss decreased significantly from 4000 to 50 ml in adults whereas a non-significant decrease from 265 to 30 ml was observed in the paediatric group.

The average amount of Haemocomplettan<sup>®</sup> administered was 2.0 g (range 1–5 g) to adults and 0.35 g (range



**Fig 1** Blood product transfusion requirements—quantitative results. Use of RBC, FFP, and pooled platelet concentrates before and after fibrinogen substitution in surviving patients.  $n=35$ . Data presented as mean (SE) obtained during a time period of 24 h before and 24 h after fibrinogen substitution.

0.2–0.5 g) to children. Laboratory, transfusion, and clinical data before and after fibrinogen administration are listed in Table 2. After fibrinogen substitution, a significant increase in plasma fibrinogen concentration (median increase  $1.0 \text{ g litre}^{-1}$ ) was observed; platelet count and fibrin D-dimer remained unchanged, whereas the APTT and PT decreased significantly.

After thorough investigation of the medical records, three episodes were identified within the first 48 h after fibrinogen substitution where a causal relationship to the use of fibrinogen could not be entirely excluded. (i) One patient was reported by nursing staff to show jitter and snoring respiration, though on arrival of the attending doctor, the patient was alert with normal respiration. Fibrinogen substitution had taken place  $\sim 24$  h previously; (ii) 24 h after fibrinogen substitution, one patient complained of attacks of shivering. (iii) One patient was discharged from the intensive care unit after  $\sim 18$  h with no further bleeding problems but died 8 h later in the ward. The cause of death was not specified. Fibrinogen had been administered  $\sim 24$  h before the time of death.

## Discussion

The main finding from this retrospective study is that substitution therapy with fibrinogen concentrate (Haemocomplettan<sup>®</sup>) was associated with a significant reduction in transfusion requirements for RBC, FFP, and pooled platelet concentrates and a significant reduction in blood loss. There was also a significant improvement in fibrinogen-dependent coagulation parameters, though the improvement in APTT (by 6 s) may not be clinically relevant. No serious adverse events were recorded that appeared to be causally associated with fibrinogen substitution therapy. As the numbers in this study were low and verified plasma concentrations of fibrinogen were low, the likelihood of recording thromboembolic events is also low.

**Table 1** Underlying clinical conditions. Age presented as median (range) or absolute.  $n=43$ . \*Congestive heart failure

|                          | Number | Age             |
|--------------------------|--------|-----------------|
| Obstetric complications  | 12     | 34 (27–44)      |
| Paediatric*              | 4      | 0.1 (0.04–0.17) |
| Cardiothoracic bleeding  | 14     | 58 (19–76)      |
| Intra-abdominal bleeding | 6      | 56 (34–74)      |
| Trauma                   | 6      | 54 (28–79)      |
| Other                    | 1      | 51              |

**Table 2** Laboratory data and blood loss before and after fibrinogen substitution. Laboratory data for all patients ( $n=43$ ) and blood loss for surviving patients ( $n=35$ ) noted in patient records during a time period of 12 h before and 12 h after fibrinogen substitution. Data presented as median (quartiles). APTT, activated partial thromboplastin time; PT, prothrombin time. \*  $P<0.05$ . Fibrinogen normal range: 2–4 g litre<sup>-1</sup>

|   | Before fibrinogen substitution | After fibrinogen substitution | P-value |
|---|--------------------------------|-------------------------------|---------|
| Laboratory data                                       |                                |                               |         |
| Platelet count (10 <sup>9</sup> litre <sup>-1</sup> ) | 80 (59–122)                    | 107 (85–131)                  | 0.10    |
| APTT (s)  | 46 (40–69)                     | 40 (35–45)                    | <0.05*  |
| PT (relative)   | 0.58 (0.5–0.7)                 | 0.75 (0.6–0.9)                | <0.05*  |
| Fibrin D-dimer (mg litre <sup>-1</sup> )              | 2.2 (0.5–8.3)                  | 2.6 (0.4–7.9)                 | 0.51    |
| P-fibrinogen (g litre <sup>-1</sup> )                 | 1.4 (1.0–1.8)                  | 2.4 (2.1–2.6)                 | <0.05*  |
| Blood loss (ml)                                       |                                |                               |         |
| Adult   | 4000 (1500–7750)               | 50 (0–425)                    | <0.05*  |
| Paediatric  | 265 (233–298)                  | 30 (16–47)                    | 0.19    |

The improvement of APTT and PT after fibrinogen substitution therapy corresponds well with findings in a recently published retrospective study which reported an average increase in plasma fibrinogen of 1.09 g litre<sup>-1</sup> after administration of a mean dose of fibrinogen of 4 g.<sup>12</sup> In our study, the average dose of fibrinogen administered in adults was 2 g which caused a median increase in plasma fibrinogen level of 1.01 g litre<sup>-1</sup>. This observation is in accord with our previous report proposing an expected increase in plasma fibrinogen level of 1 g litre<sup>-1</sup> after administration of 2 g to a person with a body weight of 60 kg (30 mg kg<sup>-1</sup>).<sup>8</sup> This difference may reflect different study populations and their individual clinical conditions at the time of fibrinogen administration, including the level of haemodilution.

The retrospective and uncontrolled design of our study has led to some limitations. Because registration of the patient's bodyweight in their medical records was incomplete, we were not able to present the corresponding increase in plasma fibrinogen concentrations per kilogram bodyweight. The critical threshold level of plasma fibrinogen is usually set at 1 g litre<sup>-1</sup> when measured by the methods of Claus. However, in patients who have received excessive amounts of colloid plasma expanders, the Claus method may artificially suggest higher fibrinogen concentrations than are actually present and call for fibrinogen substitution therapy even at levels above 1 g litre<sup>-1</sup>.<sup>13–15</sup> This laboratory paradigm may cause delay or exclusion of fibrinogen substitution in seriously bleeding patients. Furthermore, this phenomenon may also act as a possible confounder of the data in the present survey. Another limitation is that we cannot establish nor dismiss a causal relationship between fibrinogen administration and the observed improvements in laboratory values, clinical blood loss, and transfusion requirements as most patients received concomitant haemostatic interventions such as surgery, transfusion with FFP, or platelet administration. The accuracy of medical records, in particular recorded blood loss, might have been confounded by pronounced interobserver variability and in some cases, such data were not retrievable. To minimize the risk of overestimating the effect from fibrinogen substitution on transfusion requirements

and blood loss, the eight patients who did not survive were excluded from data analysis.

Fibrinogen constitutes an important component of the haemostatic process, including its roles in formation of platelet aggregates and generation of a sufficiently stable fibrin network. Adequate amounts of functional fibrinogen are also required to achieve an optimal effect of other haemostatic interventions such as infusion of antifibrinolytic drugs, recombinant activated factor VII,<sup>16</sup> prothrombin complex concentrate, and platelet transfusion. During progressive blood loss, fibrinogen appears to represent the coagulation factor first reaching a critical low threshold in continuous bleeding patients<sup>3</sup> and notably hypofibrinogenemia may emerge before the development of significant thrombocytopenia. In addition, haemodilution with colloid plasma expanders predisposes to development of a functional fibrinogen deficiency.<sup>4–6</sup> Hence, it seems reasonable to expect an overall beneficial haemostatic effect of fibrinogen administration as supplementary treatment in massively bleeding patients with low levels of plasma fibrinogen. Serious bleeding in patients with obstetric complications such as abruptio placentae and placenta praevia is often associated with excessive fibrinolysis and fibrinogenolysis, resulting in very low levels of plasma fibrinogen and high levels of fibrin D-dimer<sup>17</sup> that make fibrinogen substitution even more reasonable in this subgroup of patients. The origin of the observed hypofibrinogenemia in the study population here cannot be determined, but it seems reasonable that loss, consumption, colloid-induced dysfunction, and in at least some cases (e.g. obstetric bleeding) hyperfibrinolysis may be involved. Since all patients had documented hypofibrinogenemia, the impact on clinical and laboratory parameters points in the direction of a significant and pronounced haemostatic effect prompted by the fibrinogen substitution therapy.

Two adverse events and one serious adverse event were identified. It is unlikely that anaphylaxis could explain any of the symptoms observed accounting for the prolonged time interval between administration and symptoms. One patient died suddenly ~24 h after fibrinogen administration. After a thorough examination of the patient's



medical record, no causal correlation could be established. Therefore, we assess that the fatal outcome was not a suspected or unexpected serious adverse reaction related to the administration of the fibrinogen concentrate.

In conclusion, the present study suggests that substitution therapy with fibrinogen concentrate, as a supplementary intervention in bleeding patients with low plasma levels of fibrinogen, may contribute to reduce transfusion requirements, decrease blood loss, and lead to an overall improvement of laboratory coagulation tests. Prospective clinical trials aiming at evaluating the haemostatic potential of fibrinogen substitution therapy are highly desirable.

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