

ORIGINAL ARTICLE

Fibrinogen levels during trauma hemorrhage, response to replacement therapy, and association with patient outcomes

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Summary. *Background:* Low fibrinogen levels are known to occur in trauma. However, the extent of fibrinogen depletion during trauma hemorrhage, the response to replacement therapy and association with patient outcomes remain unclear. *Objectives:* The study aims were to: characterize admission fibrinogen level and correlate it with factors associated with injury; describe the time course of fibrinogen depletion and response to replacement therapy; determine the correlation of fibrinogen level with rotational thromboelastography (ROTEM) parameters; evaluate the effect of fibrinogen supplementation *ex vivo*; and establish the association between fibrinogen level and clinical outcomes. *Methods:* This was a prospective cohort study of 517 patients. Blood samples were drawn on admission and after administration of every 4 units of packed red blood cells. Fibrinogen levels were determined with the Clauss method, and global hemostatic competence was assessed with thromboelastometry. The effect of fibrinogen supplementation was assessed in a subgroup of coagulopathic patients. *Results:* Low admission fibrinogen level was independently associated with injury severity score ($P < 0.01$), shock ($P < 0.001$), and prehospital fluid volume ($P < 0.001$). Fibrinogen supplementation during transfusion maintained but did not augment fibrinogen levels. Administration of cryoprecipitate was associated with improved survival. ROTEM parameters correlated with fibrinogen level, and *ex vivo* fibrinogen administration reversed coagulopathic ROTEM parameters. Fibrinogen level was an independent predictor of mortality at 24 h and 28 days ($P < 0.001$). *Conclusions:* Fibrinogen level is decreased in injured patients on admission and is associated with poor outcomes. ROTEM is a rapid means of assessing hypofibrinogenemia. Earlier admin-

istration of specific fibrinogen replacement may improve outcomes, and prospective controlled trials are urgently needed.

Keywords: coagulopathy, fibrinogen, hemorrhage, ROTEM, transfusion, trauma.

Introduction

Uncontrolled bleeding is a leading cause of death in trauma patients, and, despite advances in trauma care, the mortality rate of patients requiring large volume blood transfusion remains in excess of 30% [1]. Until recently, it was thought that traumatic coagulopathy, which invariably accompanies major hemorrhage, was a late consequence of hemodilution, acidemia, hypothermia, and loss of coagulation proteins through bleeding and consumption [2]. The recent identification of an endogenous coagulopathy (acute traumatic coagulopathy [ATC]) [3], occurring very early in the clinical course, has led to the development of new resuscitation paradigms that specifically target ATC [4]. The contribution of fibrinogen to ATC and the potential benefits of early fibrinogen replacement have not been fully examined.

Fibrinogen levels are reduced in experimental models of acute traumatic coagulopathy [5]. Small retrospective clinical studies also suggest a fall [6,7], although not to levels that would have historically triggered fibrinogen replacement (below 0.8–1.0 g L⁻¹ [8]). Although fibrinogen replacement has been used as first-line treatment for trauma-induced coagulopathy [7,9], and recent massive transfusion guidelines suggest maintaining higher levels of fibrinogen (above 1.5 g L⁻¹), the supporting evidence is weak [10–12]. The time course of fibrinogen depletion in trauma hemorrhage has not been described, and the ability of fibrinogen replacement therapy to correct trauma-induced coagulopathy remains unclear.

The overall objective of this study was to characterize the changes and role of fibrinogen in victims of major trauma. Specific aims were to: describe the changes in fibrinogen level on admission; describe the changes in fibrinogen concentration during damage control resuscitation; determine whether

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rotational thromboelastography (ROTEM) parameters correlated with fibrinogen level and could potentially guide fibrinogen replacement; evaluate the effect of different fibrinogen replacement regimens on trauma patient blood *ex vivo*; and establish the relationship between admission fibrinogen levels and clinical outcomes in terms of survival and blood transfusion requirements. We conducted a prospective cohort study of trauma patients presenting directly to two major trauma centers.

Materials and methods

Patient selection

Between January 2008 and December 2010, all adult trauma patients (≥ 16 years) who met the local criteria for trauma team activation were eligible for enrollment. Exclusion criteria were: arrival in the emergency department more than 2 h following injury; administration of more than 2000 mL of intravenous fluid prior to hospital arrival; transfer from another hospital; and burns on $> 5\%$ the total body surface area. Patients were retrospectively excluded if they: declined to give consent for the research study; were receiving anticoagulant medications (not including aspirin); or had moderate or severe liver disease or a known bleeding diathesis. Emergency consent was obtained from the trauma team leader (a physician independent of the research study), who acted as the patient's legally authorized representative. Written consent from the patient or next of kin was obtained as soon after enrollment as appropriate. The study was reviewed and approved by the Regional Ethics Committee.

Major hemorrhage protocol

Trauma patients with major blood loss were routinely managed according to a Major Hemorrhage Protocol (MHP). The MHP is activated when a patient has presented with a systolic blood pressure (SBP) of < 90 mmHg, has demonstrated a poor response to initial fluid resuscitation, and/or has suspected active hemorrhage. Protocols at both trauma centers advocated initial administration of plasma with red cells following a strategy based on blood component ratios. Fresh frozen plasma (FFP)/packed red blood cell (PRBC) ratios for trauma patients receiving blood according to protocol would therefore be 0 : 4, then 4 : 8 and finally 8 : 12 at the transfusion intervals prespecified by this study (i.e. after 4, 8 and 12 units of PRBCs, respectively). Protocols at both centers also defined early use of platelets (PLTs) and cryoprecipitate, if major bleeding persisted, again according to a strategy based on ratios (6 units of PRBCs, 4 units of FFP, 1 unit of PLTs, and two pools [10 units] of cryoprecipitate). One unit of PLTs relates to either a pool of 4 units of buffy coat PLTs or a single donor apheresis unit. PLT/PRBC and cryoprecipitate/PRBC ratios would be the same at each interval: 0 : 4 at the 4-unit interval; 0 : 8 at the 8-unit interval; and 1 : 12 at the 12-unit interval. Estimated expected fibrinogen doses given at each transfusion interval,

assuming adherence to the MHP, would be 0, 3.6 and 9.6 g (see calculation method below) with fibrinogen (g)/PRBC ratios of 0 : 1, 0.45 : 1 and 0.8 : 1 at the same transfusion intervals. Fibrinogen concentrate (FgC) was not used at either center, and autologous cell salvage was not used in any of these patients.

Sampling technique

Twenty milliliters of blood was drawn from either the femoral vein or antecubital fossa and the standard trauma laboratory tests (peripheral blood count, clotting screen, and arterial blood gas) were performed within 20 min of arrival in the emergency department (ED). Blood for ROTEM analysis and fibrinogen level determination was drawn into a 2.7-mL citrated vacutainer (0.109 M buffered sodium citrate, 3.2%; Becton Dickinson, Plymouth, UK) and processed in the trauma research and hospital laboratories, respectively. For those patients with active bleeding who required transfusion, further blood samples were taken after the fourth, eighth and 12th units of PRBCs had been administered.

Sample analysis

ROTEM samples were processed within 2 h of blood draw, with a ROTEM delta instrument (TEM International, Munich, Germany), at 37 °C. The methodology and the parameters of ROTEM have been described previously [13]. Two separate ROTEM assays were performed for each patient, the EXTEM, measuring tissue factor-initiated clotting, and the FIBTEM, with the addition of cytochalasin D, a platelet inhibitor. All pipetting steps and the mixing of reagents with samples were performed with an automated electronic pipette program. Fibrinogen levels (STA Fibrinogen [Stago, Asnières sur Seine, France] or Siemens Thrombin [Sysmex UK, Milton Keynes, UK] reagents) and prothrombin times (STA Neoplastine R [Stago] or Siemens Innovin [Sysmex UK] reagents) were determined in the hospital laboratories with the STA-R evolution analyzer (Stago) and the Sysmex CS2100i (Sysmex UK) analyzer according to standard protocols.

Definition of coagulopathy

We defined ATC on the ROTEM instrument [14] as a 5-min EXTEM clot amplitude (CA) of ≤ 35 mm. We have shown that this definition can accurately identify ATC and predict the need for massive transfusion (defined as ≥ 10 units of PRBCs in 24 h) [15]. Unless otherwise stated, this definition of ATC has been used. We also assessed the relationship between admission fibrinogen values and a prothrombin time ratio (PTr) of > 1.2 [16].

Ex vivo evaluation of fibrinogen replacement therapies

Ex vivo analysis of the response to fibrinogen replacement therapy was performed on admission blood samples from

trauma patients with ATC. ROTEM results from a cohort of patients with minor injury (defined by an injury severity score [ISS] of ≤ 4) were used as a baseline comparator. Citrated whole blood samples were analyzed after the *ex vivo* addition of two doses of FgC (60 mg mL⁻¹) (Bio Products Laboratory, Elstree, UK) and two doses of cryoprecipitate (fibrinogen concentration of 7.4 g L⁻¹) (NHSBT, Watford, UK).

The two FgC doses were selected to be equivalent to administering 6 g and 12 g of FgC to a 70-kg man. Assuming a 5-L blood volume, a 6-g dose and a 12-g dose reach final blood concentrations of 1.2 mg mL⁻¹ and 2.4 mg mL⁻¹, respectively. The two doses (6 and 12 g) were selected on the basis of pilot data obtained with a dilutional model of coagulopathy [17].

The standard dose of cryoprecipitate was chosen to reflect clinical practice in the UK. Ten units is the most frequently used dose for adult patients with major blood loss, and has an average volume of approximately 350 mL (<http://www.transfusionguidelines.org.uk> [accessed 17 January 2012]). Assuming a 70-kg man with a 5-L blood volume, the standard dose and the high dose equate to the delivery of 2.6 g and 7.8 g, respectively. Preprepared aliquots of each product were thawed in a water bath at 37 °C when needed, and whole blood was added and mixed thoroughly. Analysis of each sample was performed within 2 h of blood draw, with EXTEM and FIBTEM channels, and run for 1 h at 37 °C.

Data collection

Data were collected prospectively on patient demographics, time of injury, mechanism of injury (blunt or penetrating), prehospital fluid administration, time of arrival at the ED, and admission vital signs. Blood transfusion requirements were collected prospectively for each patient, at the time of transfusion during the first bleeding episode. Fluid requirements were collected for the first 24 h. Total quantities of fibrinogen administered from FFP, PLT and cryoprecipitate units transfused were calculated retrospectively, using data of mean fibrinogen concentrations of blood products from the National Health Service Blood & Transplant (UK BTS, 2005). We calculated the total fibrinogen dose given to patients as (FFP units \times 0.9 g) + (PLTs \times 0.4 g) + (cryoprecipitate \times 2 g), using similar methods to those previously published [11]. These data were then used to calculate fibrinogen/PRBC ratios.

Outcome measures

Patients were followed until hospital discharge or death. Outcome measures recorded were 24-h mortality, 28-day mortality, 12-h PRBC requirements, and fibrinogen/PRBC ratio measures.

Statistical analysis

Statistical analysis was performed with GRAPHPAD PRISM v5 (GraphPad Software, San Diego, CA, USA), Microsoft Excel

2003 (Microsoft, Redmond, WA, USA), and SAS v9.1 (SAS Institute, Cary, NC, USA). Normal-quartile plots were used to test for normal distribution. Parametric data are expressed as means (95% confidence intervals [CIs]). Non-parametric data are given as medians (interquartile ranges [IQRs]). Correlation was assessed with the Pearson method. Receiver operating characteristic curve analysis was performed to evaluate the ROTEM instrument's performance with respect to discriminating patients with admission fibrinogen levels of < 1.5 g dL⁻¹. Multiple linear regression was used to assess the injury and prehospital treatment parameters influencing fibrinogen levels on admission. The predefined variables included in the models were: ISS, SBP, base deficit, PTr, activated partial thromboplastin time (APTT), gender, age, mechanism of injury, total volume of clear fluids administered prior to admission blood sample, and administered PRBC volume. Logistic regression was used to establish admission fibrinogen level as an independent predictor of transfusion and mortality. Forward selection was used to derive each model. A *P*-value of < 0.05 was chosen to represent statistical significance throughout.

Results

Five hundred and fifty-five trauma patients were enrolled into the study over the 36-month period. Thirty-eight subjects were excluded: 21 after personal or legal representative consent was declined following enrollment; eight for retrospective exclusion criteria (five were excluded because of > 2 L of fluid administration before hospital arrival; two patients were subsequently found to be < 16 years old; and one patient was a suicide by hanging); and nine patients because admission fibrinogen levels were not available. This left a total of 517 patients for evaluation. The median time from injury (estimated as the time from when the emergency services were alerted) to blood sampling was 90 min (IQR, 69–110 min). The demographics and injury characteristics of all patients are shown in Table 1.

Fibrinogen levels on admission

The proportions of patients with admission fibrinogen levels below 1.5 g L⁻¹, 1.0 g L⁻¹ and 0.8 g L⁻¹ were 14%, 5% and 3%, respectively. Fibrinogen levels were reduced by 33% in coagulopathic patients (Fig. 1A) and by 58% in patients with a prolonged PTr (> 1.2) (Fig. 1B). Hypotension was associated with decreased fibrinogen levels (Fig. 1C): 41% of hypotensive patients (SBP < 90 mmHg) had fibrinogen levels below 1.5 g L⁻¹, as compared with only 10% of normotensive patients ($P < 0.001$). Increasing shock severity (as measured by the base deficit) was also associated with a reduction in fibrinogen levels (Fig. 1D). Fibrinogen values were significantly decreased in patients with the highest degrees of injury (ISS ≥ 25 ; Fig. 1E). Higher volumes of prehospital fluid therapy were also associated with a reduction in admission fibrinogen levels (Fig. 1F).

Table 1 Clinical characteristics of patients ($n = 517$)

Patients	All patients	Minor injury	Non-coagulopathic	Coagulopathic
Age	36 (23–51)	34 (22–42)	37 (23–51)	33 (24–44) ^{NS}
Male	405 (78)	79 (77)	328 (79)	70 (77) ^{NS}
Injuries				
ISS	14 (8–27)	1 (1–4)	13 (6–25)	25 (13–34) ^{***}
ISS > 15	255 (50)	0	187 (45)	64 (70) ^{***}
Blunt injury	429 (83)	70 (67)	339 (81)	80 (88) ^{NS}
Admission parameters				
Systolic blood pressure < 90 mmHg	56 (11)	5 (5)	35 (8)	19 (22) ^{***}
Base deficit > 6 mEq L ⁻¹	94 (19)	6 (6)	59 (15)	33 (37) ^{***}
PT (s)	11.2 (10.7–12.3)	10.9 (10.5–11.3)	11.1 (10.6–11.9)	12.1 (11.3–13.4) ^{***}
PTr > 1.2	38 (8)	2 (2)	17 (4)	20 (22) ^{***}
Intravenous fluids prior to baseline sample (mL)	0 (0–500)	0 (0–0)	0 (0–350)	500 (0–875) ^{***}
Fluid requirements (first 12 h)				
Crystalloid (mL)	1095 (0–2750)	0 (0–775)	1000 (0–2303)	1800 (500–4000) ^{**}
Colloid (mL)	0 (0–500)	0	0 (0–500)	0 (0–1000) [*]
PRBC units	0 (0–2)	0	0	0 (0–6) ^{***}
PRBC ≥ 10 units	26 (5)	0	12 (3)	11 (12) ^{***}
FFP units	0	0	0	0 (0–4) ^{***}
Platelet units	0	0	0	0 (0–0.5) ^{***}
Cryoprecipitate units	0	0	0	0 (0–0.5) ^{***}

FFP, fresh frozen plasma; ISS, injury severity score; NS, not significant; PRBC, packed red blood cell; PT, prothrombin time; PTr, prothrombin time ratio. Values are given as number (%) or median (interquartile range). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (vs. non-coagulopathic group).

According to multiple regression analysis, low admission fibrinogen levels were independently associated with injury severity ($P = 0.002$), shock as measured by base deficit ($P = 0.002$), and the volume of prehospital fluid resuscitation ($P < 0.001$), but not SBP ($P = 0.06$); $r^2 = 0.27$.

Changes in fibrinogen concentration during hemorrhage and transfusion

We analyzed fibrinogen levels in bleeding patients requiring PRBC transfusions during resuscitation. The results were tabulated at three transfusion points (after 4, 8 and 12 units of PRBCs), and subsets from the cohort were then analyzed by blood components administered (Table 2). The median time from hospital admission to receiving fibrinogen from any source (cryoprecipitate, FFP, or PLTs) was 40 min (IQR, 20–65 min; $n = 75$). Patients who received fibrinogen supplementation maintained their admission fibrinogen level (1.60 g L^{-1}) throughout transfusion of the first 8 units of PRBCs (1.54 g L^{-1}), with a small, non-significant reduction by the 12th unit (1.31 g L^{-1}). In comparison, fibrinogen levels rapidly decreased in patients receiving no transfusion source of fibrinogen supplementation to 0.5 g dL^{-1} by transfusion of 8 units of PRBCs (Table 2).

Those patients who received cryoprecipitate as a concentrated source of fibrinogen were first transfused with cryoprecipitate at a median of 103 min (IQR, 78–134 min) from admission ($n = 39$). Patients transfused with cryoprecipitate maintained their admission fibrinogen level (1.60 g L^{-1}) throughout transfusion of the first 12 units of PRBCs (1.60 g L^{-1}). In comparison, those patients not receiving cryoprecipitate had a steady fall in fibrinogen levels during hemorrhage and transfusion, with a 38% decrease from admission levels (1.60 g L^{-1}) by the 12th

PRBC unit (1.00 g L^{-1}), although this did not reach statistical significance (Table 2).

Relationship between EXTEM/FIBTEM and fibrinogen levels

We evaluated the ability of ROTEM analysis to assess the fibrinogen levels in ATC. EXTEM and FIBTEM measures of CA5 and maximal clot formation (MCF) were significantly correlated with Clauss fibrinogen levels (Fig. 2A–D). The CA5 showed stronger correlations than MCF for both FIBTEM ($r^2 = 0.44$, $P < 0.001$) and EXTEM ($r^2 = 0.35$, $P < 0.001$). The sensitivity and specificity of EXTEM CA5 < 36 mm for discriminating patients with admission fibrinogen levels below 1.5 g L^{-1} were 53% and 87%, respectively (Fig. 2E). FIBTEM CA5 < 9.5 mm had a sensitivity of 78% and a specificity of 70% (Fig. 2F).

Effect of ex vivo fibrinogen replacement on coagulation function

We analyzed the potential for early FgC and cryoprecipitate supplementation to correct ATC by examining ROTEM changes after *ex vivo* addition to whole blood in admission samples from 19 consecutive trauma patients with ATC. As compared with the minor injury cohort ($n = 96$), patients with ATC had a 36% reduction of their admission EXTEM CA5 (ATC, 28 mm vs. 44 mm, $P < 0.001$; Fig. 4A). The addition of a 6-g equivalent dose of FgC partially improved this value, but only the 12-g equivalent dose significantly increased the EXTEM CA5 above admission levels and restored it to the level of the minor injury cohort. Cryoprecipitate achieved correction of ATC and restoration to the level of the minor injury group

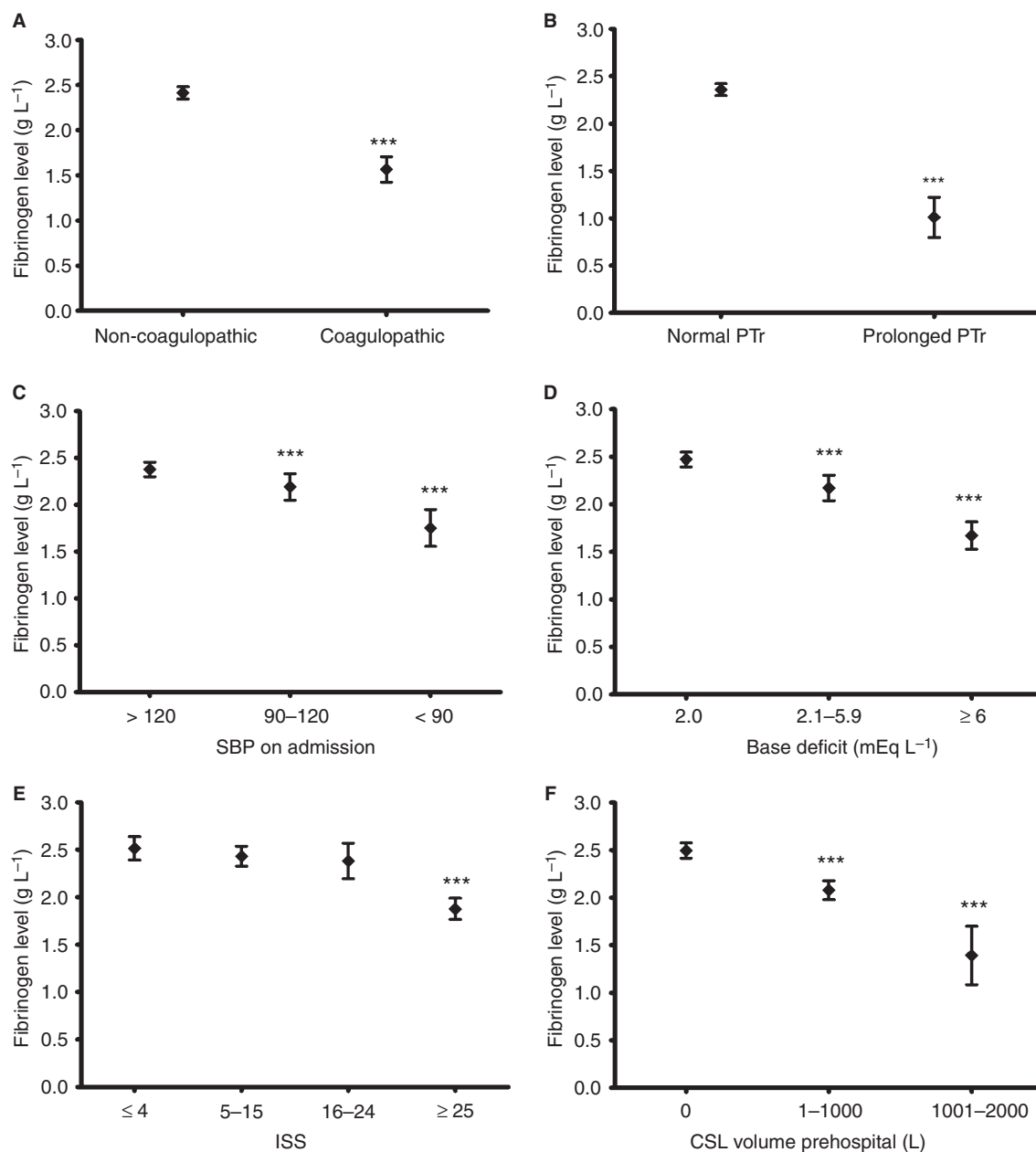


Fig. 1. Characterization of fibrinogen levels on admission in relation to factors associated with injury. (A) Decreased fibrinogen levels with acute traumatic coagulopathy incidence (1.6 g L^{-1} vs. 2.4 g L^{-1} , $P < 0.001$). (B) Decreased fibrinogen levels with prolonged prothrombin time ratio (PTr) (1.0 g L^{-1} vs. 2.4 g L^{-1} , $P < 0.001$). (C) Decreasing fibrinogen level with decreasing systolic blood pressure (SBP) in tertiles. (D) Decreasing fibrinogen level with increased degree of shock as measured by base deficit in tertiles. (E) Decreased fibrinogen levels with increasing injury severity score (ISS) in quartiles. (F) Decreased fibrinogen levels with increased prehospital fluid resuscitation. Values are means \pm 95% confidence intervals. CSL, crystalloid.

after the addition of the high-dose replacement equivalent (Fig. 3A). A similar pattern was seen with the EXTEM MCF results (Fig. 3B).

For the FIBTEM measures, the 6-g equivalent FgC concentration restored CA5 and MCF values to those of the minor injury cohort, and the 12-g equivalent concentration resulted in supramaximal CA5 and MCF values (Fig. 3C,D). High-dose cryoprecipitate restored FIBTEM CA5 and MCF values, whereas standard cryoprecipitate values remained low (Fig. 3C,D).

Effect of fibrinogen levels on outcome

The overall mortality rate of the enrolled patients was 5% at 24 h and 12% at 28 days. In all patients, mean admission fibrinogen levels in survivors were 51% and 39% higher than in non-survivors at 24 h and 28 days, respectively ($P < 0.001$) (Fig. 4A,B). However, there was no difference in mortality between patients who received cryoprecipitate in the first 12 units and those who did not at either 24 h (22% vs. 18%; Fig. 4C) or 28 days (30% vs. 34%; Fig. 4D).

Table 2 Transfusion data for patients receiving ≥ 4 units of packed red blood cells (PRBCs)

	Four-unit window	Eight-unit window	Twelve-unit window
Total patient numbers	103	42	20
Transfusions administered			
Expected PRBC/FFP/PLT/cryoprecipitate† ratios	4 : 0 : 0 : 0	8 : 4 : 0 : 0	12 : 8 : 1 : 1
PRBC units administered	4 (4–4)	8 (8–9)	12 (12–15)
FFP units administered	1 (0–4)	4 (3–6)	5 (4–8)
PLT units administered	0 (0–0)	0 (0–1)	1 (0–1)
Cryoprecipitate pools administered	0 (0–0)	0 (0–2)	2‡ (1–2)
Actual PRBC/FFP/PLT/cryoprecipitate ratios	4 : 1 : 0 : 0	2 : 1 : 0 : 0	12 : 5 : 1 : 1
Fibrinogen (g)/PRBC (units) ratio	0 : 1 (0–0.9 : 1)	0.5 : 1 (0.3–1.0 : 1)	0.7 : 1 (0.3–0.8 : 1)
Total fibrinogen given (g)	0 (0–3.6)	3.6 (2.1–8.8)	8.4 (4.8–11.5)
Fibrinogen levels (g L ⁻¹)			
Whole cohort	1.5 (1.2–1.8)	1.5 (1.1–2.0)	1.3 (1.0–1.7)
Received PRBCs alone	1.2 (1.0–1.8)	0.5 (0.4–0.6)	1.0§
PRBCs plus fibrinogen in any form	1.6 (1.4–1.8) ^{NS}	1.5 (1.2–2.0)*	1.3 (1.0–1.7) ^{NS}
PRBCs plus fibrinogen from FFP or PLTs alone	1.6 (1.4–1.9)	1.5 (1.1–1.5)	1.0 (0.8–1.0)
PRBCs plus fibrinogen from cryoprecipitate and FFP/PLTs¶	1.4 (1.2–1.7) ^{NS1}	1.7 (1.3–2.0) ^{NS1}	1.6 (1.1–1.9) ^{NS1}

FFP, fresh frozen plasma; PLT, platelet; NS, not significant (vs. PRBC alone group); NS1, not significant (vs. FFP/PLT-only group). Values are given as median (interquartile range). * $P < 0.05$ (vs. PRBC-only group). †A cryoprecipitate ratio of 1 relates to two pools of cryoprecipitate (one adult dose). ‡Two pools of cryoprecipitate equates to one adult dose of cryoprecipitate. §One patient received PRBCs alone by 12 units.

Multiple logistic regression models were developed to examine the influence of fibrinogen level at admission and fibrinogen/PRBC ratio administered within the first 12 h on the three outcomes of major blood loss of ≥ 4 units of PRBCs in the first 12 h, and survival at 24 h and 28 days. Data from 422 patients were available for modeling (95 patients were excluded because of missing data for at least one of the variables of interest).

Admission fibrinogen level was not found to be a significant predictor of major blood loss ($P = 0.8$), but was an independent predictor of mortality at both 24 h and 28 days ($P < 0.001$). Other significant independent variables were: APTT (odds ratio [OR] 1.03, 95% CI 1.00–1.06), ISS (OR 1.03, 95% CI 1.00–1.06), female gender (OR 2.94, 95% CI 1.04–5.81), and age (OR 1.05, 95% CI 1.02–1.07). The odds of death reduced by a factor of 0.22 during the first 28 days for every 1 g L⁻¹ increase in admission fibrinogen level (Table 3).

We also examined the effect of the final fibrinogen/PRBC ratio on transfusion requirements and survival for those patients who survived the first 12 h of hospital admission. The median fibrinogen/PRBC ratio for patients who received PRBCs ($n = 135$) was 0.7 : 1 (IQR, 0 : 1–1.2 : 1). For the patients who received blood products, the fibrinogen/PRBC ratio was found to be highly significant when added to the fitted model. The odds of a patient requiring ≥ 4 units of blood in the first 12 h increased by a factor of 2.67 for every unit increase in the first 12-h fibrinogen ratio ($P < 0.001$). The odds of death during the first 28 days, conditional on 12-h survival, decreased by a factor of 0.91 for every 1-g increase in fibrinogen therapy administered within the first 12 h ($P = 0.08$) (Table 4).

Discussion

We have shown that fibrinogen depletion occurs in acute traumatic coagulopathy and progresses during trauma hem-

orrhage. Fibrinogen levels do not normalize during damage control resuscitation, despite the provision of high ratios of plasma and PLTs. Supplementation of fibrinogen, either as cryoprecipitate or concentrate *ex vivo*, was able to correct this coagulopathy. Fibrinogen depletion was associated with poor outcomes, and outcomes tended to improve with increasing total amounts of fibrinogen administered. Patients who received cryoprecipitate maintained their fibrinogen levels, and had lower mortality rates than those who did not. Overall, this study suggests that bleeding patients with ATC have identifiable and measurable fibrinogen depletion, and may benefit from early high-dose fibrinogen supplementation.

Normal hemostasis is critically dependent on fibrinogen as a substrate for clot formation [14]. Although fibrinogen levels are known to decrease during massive hemorrhage [5,18], there is very limited evidence to support a specific effective fibrinogen concentration during active bleeding [10]. Traditionally, fibrinogen was only supplemented when levels fell below 1.0 g L⁻¹ [8]. More recent evidence suggests that patients with fibrinogen levels below 2 g L⁻¹ have worse outcomes [19], constituting nearly 40% of our study population. Bleeding trauma patients with ATC present with established fibrinogen depletion, with levels below those currently recommended for therapeutic supplementation [10].

Management of trauma hemorrhage requires rapid identification of ATC and guidance of transfusion therapy, which may be provided by whole blood viscoelastic tests of coagulation (i.e. ROTEM and TEG) [20–22]. Clauss fibrinogen levels were strongly correlated with both CA5 FIBTEM and CA5 EXTEM in our study, suggesting that ROTEM can estimate functional fibrinogen levels in a clinically useful time frame [15,21]. Our data suggest that ROTEM may have a role in identifying patients requiring supplementation and guiding the dose and timing of fibrinogen administration [7,23].

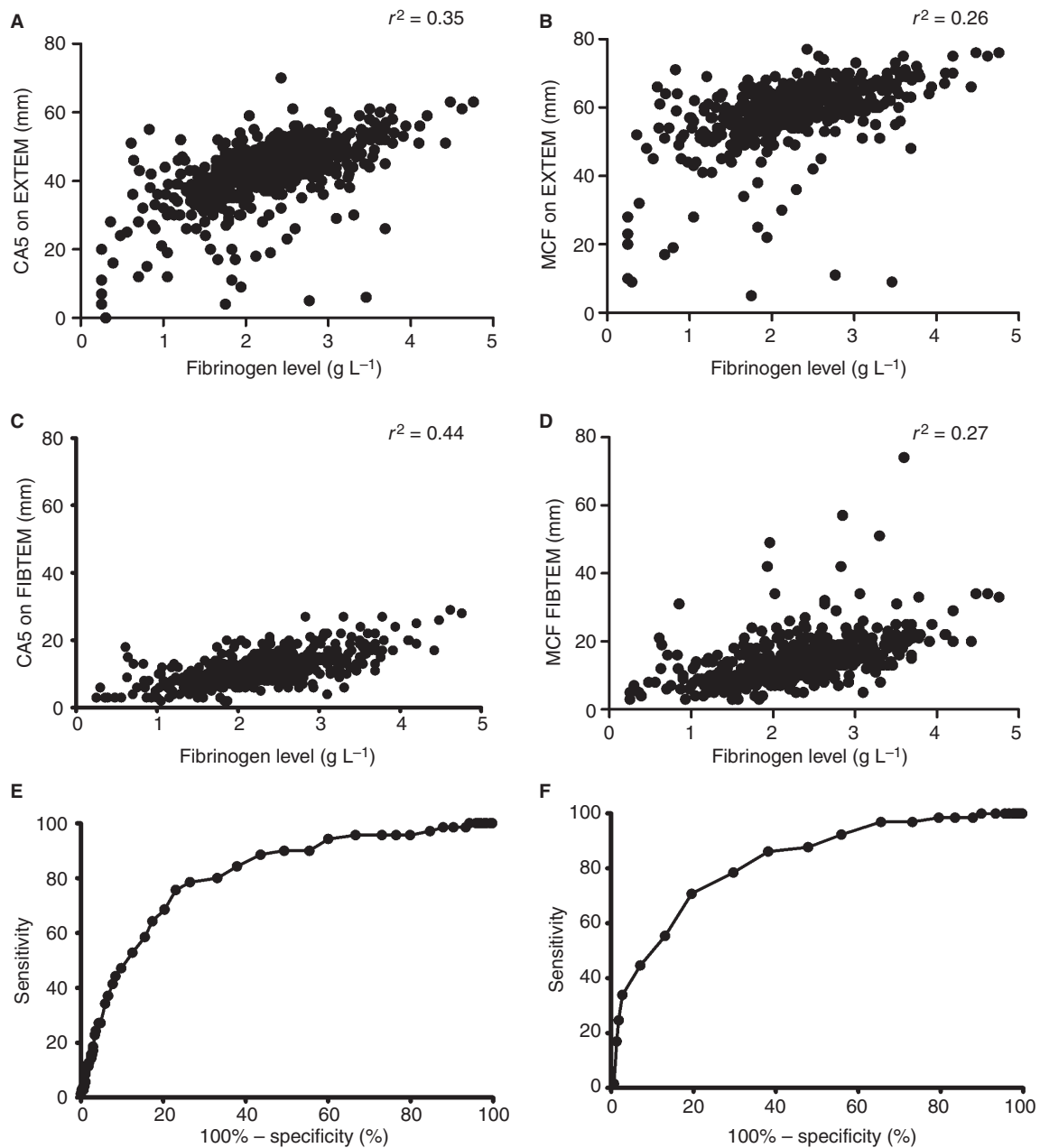


Fig. 2. Association of admission fibrinogen levels with rotational thromboelastography analysis. Clot amplitude (CA)5 measurements had a higher correlation coefficient ([A] $r^2 = 0.35$, $P < 0.001$; [B] $r^2 = 0.44$, $P < 0.001$) than maximal clot formation measurements ([C] $r^2 = 0.26$, $P < 0.001$; [D] $r^2 = 0.27$, $P < 0.001$) with admission fibrinogen levels. EXTEM (E) and FIBTEM (F) tests gave a receiver operating characteristic curve area of 0.8 (95% confidence interval 0.7–0.9, $P < 0.001$).

The effects of fibrinogen replacement were evaluated in this study by a series of *ex vivo* spiking experiments, which indicated that the ROTEM changes that characterize ATC [15] were reversed in a stepwise manner, depending on the dose of fibrinogen administered. Both cryoprecipitate and FgC were effective at reversing traumatic coagulopathy, but differences were seen in the degree of reversal achieved, with suggestions that lower cryoprecipitate concentrations (representative of the doses often used in clinical practice) were less effective. Cryoprecipitate and FgC are the two concentrated sources of

fibrinogen for replacement, but there are differences in composition; cryoprecipitate additionally contains factor VIII, von Willebrand factor, FXIII, and fibrinogen, but requires thawing, and this may cause delays in transport from the blood bank. FgC may be more suited to delivering the higher and earlier fibrinogen doses that may be required to reverse fibrinogen depletion in trauma hemorrhage.

The association of low admission fibrinogen levels with increased mortality, and that of fibrinogen treatment with improved patient survival, are consistent with previous reports

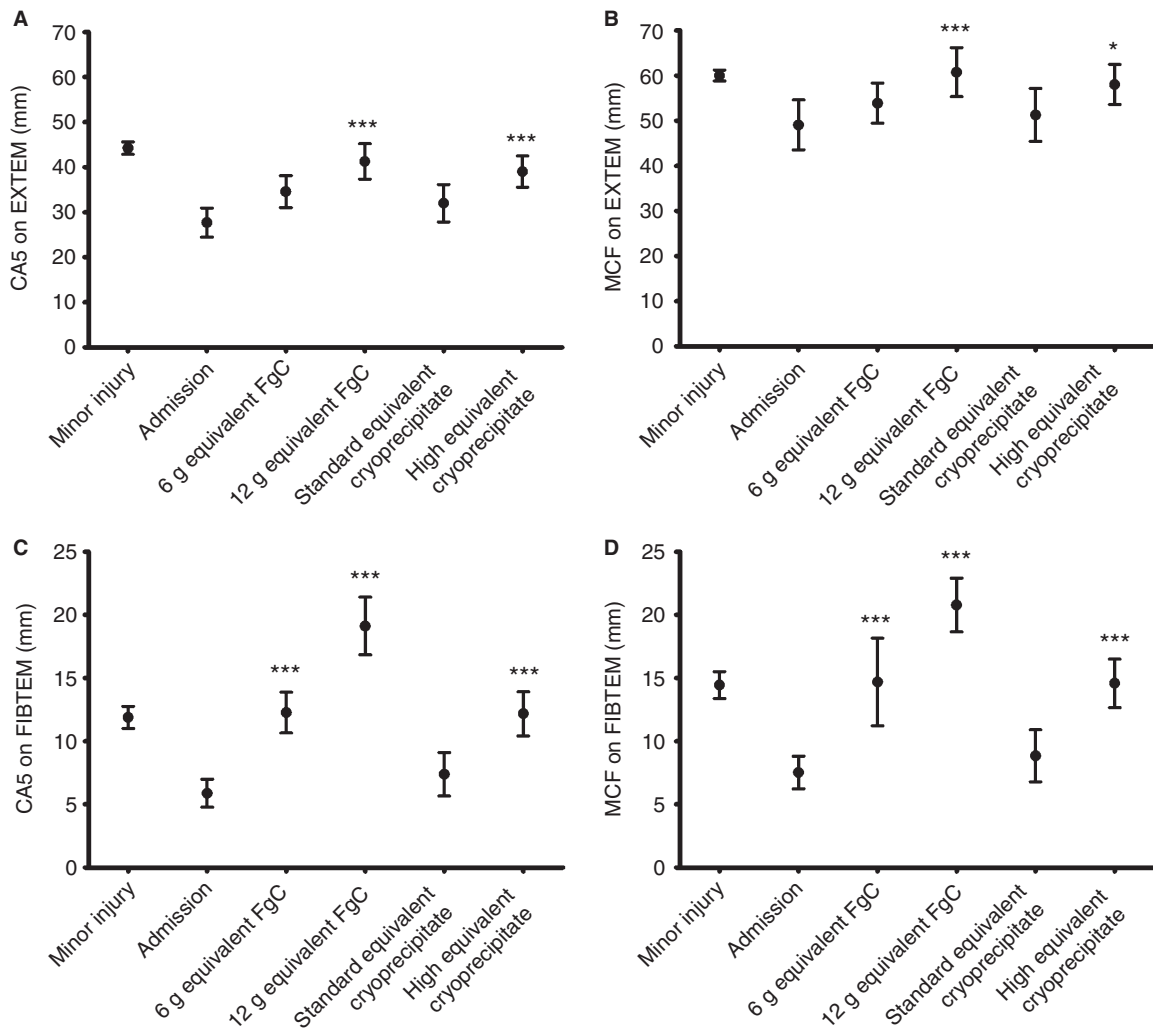


Fig. 3. Effects of fibrinogen concentrate (FgC) and cryoprecipitate *ex vivo* on rotational thromboelastography changes associated with acute traumatic coagulopathy. (A) Clot amplitude (CA)5 on EXTEM increases with administration of 6 g (28 mm vs. 35 mm, $P < 0.05$) and 12 g (28 mm vs. 41 mm, $P < 0.001$) of FgC and high-dose cryoprecipitate (28 mm vs. 39 mm, $P < 0.001$). (B) Administration of 12 g of FgC (49 mm vs. 61 mm) and high-dose cryoprecipitate (49 mm vs. 58 mm) significantly ($P < 0.001$) increased maximal clot formation (MCF) on EXTEM. (C) Administration of 6 g of FgC (6 mm vs. 12 mm, $P < 0.001$) and high-dose cryoprecipitate (6 mm vs. 12 mm, $P < 0.001$) restored the CA5 on FIBTEM to a level comparable that in uninjured patients (12 mm). There was a three-fold increase in CA5 following administration of 12 g of FgC (6 mm vs. 19 mm, $P < 0.001$). (D) Reversal of impairment in MCF on FIBTEM with 6 g of FgC (8 mm vs. 15 mm, $P < 0.001$) and an equivalent high dose of cryoprecipitate (8 mm vs. 15 mm, $P < 0.001$). Administration of 12 g of FgC produced a 2.5-fold increase in MCF on FIBTEM (8 mm vs. 21 mm, $P < 0.001$). Values are means \pm 95% confidence intervals.

[11,12]. Although these observational findings suggest that fibrinogen may play a causal role in reducing mortality, it is possible that fibrinogen levels are a marker for some other aspect of transfusion therapy rather than exerting a direct effect on mortality. Although other lines of evidence, including work on the effects of fibrinogen replacement in animal models [24] and in non-randomized studies of trauma patients [7,23,25], are also suggestive of a role for fibrinogen therapy in traumatic hemorrhage, randomized controlled trials will be required to definitively address this question.

There are limitations to this study. We defined major blood loss as the requirement for ≥ 4 units PRBCs in the first 12 h, but transfusion requirements are a surrogate measure of bleeding and blood loss. We were also unable to determine the relative

benefits of the different transfusion sources of fibrinogen from our data, owing to the structure of the major hemorrhage protocol and insufficient numbers of bleeding patients to allow detailed subgroup analysis. The experimental data in our study examining the addition of fibrinogen concentrate or cryoprecipitate to coagulopathic samples were obtained with an *ex vivo* model, which can only suggest what the clinical effects of fibrinogen replacement might be. The fibrinogen conversion factor was calculated in line with published methodology [11], but does not take into account the dilutional effect of the volume of each blood component added. This partial dilutional effect is also likely to have a similar effect on final fibrinogen levels *in vivo*. Finally, the relationships defined in the regression analyses are potentially subject to confounding by factors that

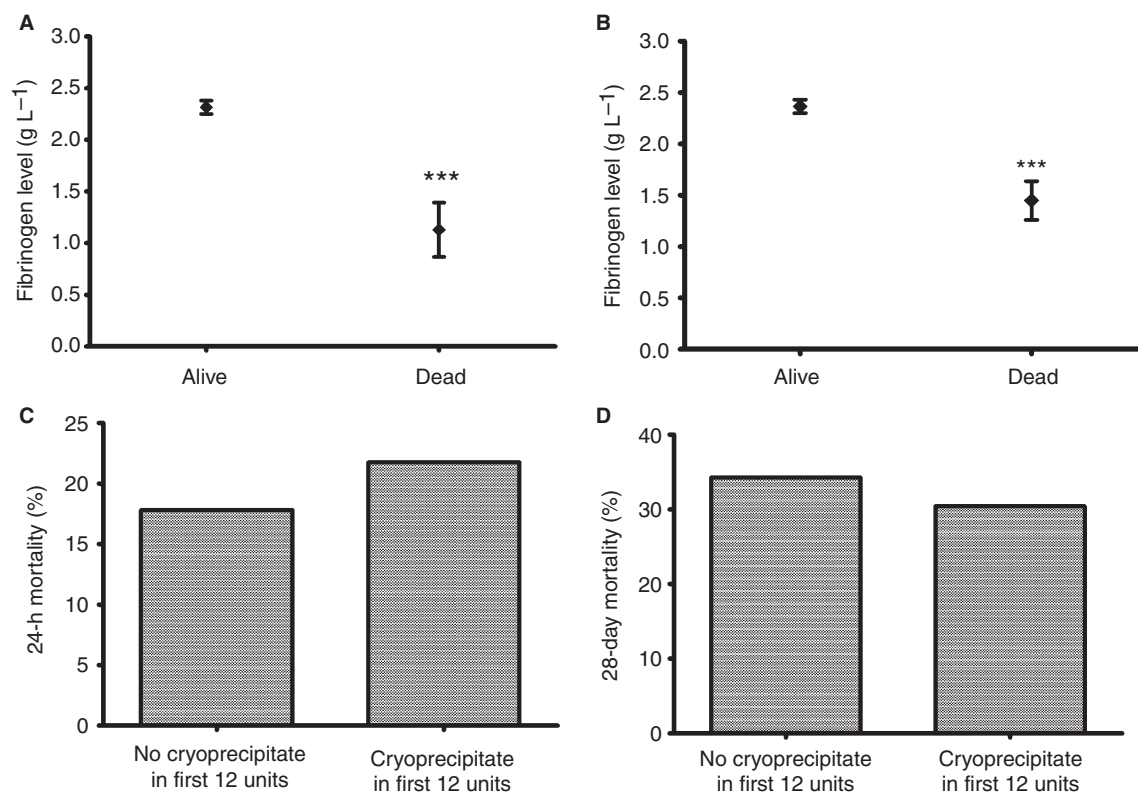


Fig. 4. Association of admission fibrinogen levels and supplementation with mortality. (A) Decreased admission fibrinogen levels in non-survivors at 24 h (1.1 g L^{-1} vs. 2.3 g L^{-1} , $P < 0.001$). (B) Decreased admission fibrinogen levels in non-survivors at 28 days (1.4 g L^{-1} vs. 2.4 g L^{-1} , $P < 0.001$). (C) No change in 24-h mortality with lower fibrinogen supplementation in the first 12 packed red blood cell (PRBC) units (18% vs. 22%). (D) No change in 28-day mortality with lower fibrinogen supplementation in the first 12 PRBC units (34% vs. 30%). Values are means \pm 95% confidence intervals or percentages.

Table 3 Independent variables associated with mortality

Parameter	Odds ratio	95% CI	<i>P</i> -value
Fibrinogen level	0.22	0.10–0.47	< 0.001
Injury severity	1.03	1.00–1.06	0.07
APTT	1.05	1.01–1.09	0.02
Gender (female)	2.46	1.04–5.81	0.04
Age	1.05	1.02–1.07	< 0.001

APTT, activated partial thromboplastin time; CI, confidence interval.

Table 4 Effect of fibrinogen administration on 28-day mortality (conditional on 12-h survival)

Parameter	Odds ratio	95% CI	<i>P</i> -value
Fibrinogen given in first 12 h	0.91	0.81–1.01	0.08
Injury severity	1.06	1.03–1.10	< 0.001
APTT	1.10	1.04–1.16	0.001
Age	1.03	1.01–1.05	0.005
Gender (female)	3.03	1.24–7.39	0.01

APTT, activated partial thromboplastin time; CI, confidence interval.

we did not measure or adequately adjust for in our regression models, and must be interpreted cautiously.

In summary, bleeding trauma patients arrive in hospital with an established coagulopathy that includes significant fibrinogen depletion. Contemporary damage control resuscitation does

not maintain normal fibrinogen levels, despite high-dose FFP therapy. Fibrinogen supplementation is able to correct coagulopathy *ex vivo*, and patients who receive additional fibrinogen supplementation appear to have better outcomes. Our study therefore suggests that there may be a role for early, high-dose fibrinogen supplementation in trauma hemorrhage, and provides important data for the design and conduct of future randomized controlled trials.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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