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ORIGINAL ARTICLE

The incidence and magnitude of fibrinolytic activation in trauma patients

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Summary. *Background:* Trauma is a global disease, with over 2.5 million deaths annually from hemorrhage and coagulopathy. Overt hyperfibrinolysis is rare in trauma, and is associated with massive fatal injuries. Paradoxically, clinical trials suggest a much broader indication for antifibrinolytics. *Objective:* To determine the incidence and magnitude of fibrinolytic activation in trauma patients and its relationship to clot lysis as measured by thromboelastometry. *Methods:* A prospective cohort study of 303 consecutive trauma patients admitted between January 2007 and June 2009 was performed. Blood was drawn on arrival for thromboelastometry (TEM) and coagulation assays. Follow-up was until hospital discharge or death. TEM hyperfibrinolysis was defined as maximum clot lysis of > 15%. Fibrinolytic activation (FA) was deterined according to plasmin–antiplasmin (PAP) complex and D-dimer levels. Data were collected on demographics, mechanism, severity of injury, and baseline vital signs. The primary outcome measure was 28-day mortality. The secondary outcome measures were 28-day ventilator-free days and 24-h transfusion requirement. *Results:* Only 5% of patients had severe fibrinolysis on TEM, but 57% of patients had evidence of 'moderate' fibrinolysis, with PAP complex levels elevated to over twice normal (> 1500 μg L^{-1}) without lysis on TEM. TEM detected clot lysis only when PAP complex levels were increased to 30 times normal ($P < 0.001$) and antiplasmin levels were < 75% of normal. Patients with FA had increased 28-day mortality as compared with those with no FA (12% vs. 1%, $P < 0.001$), fewer ventilator-free days, and longer hospital stay. *Conclusions:* FA occurs in the majority of trauma patients, and the magnitude of FA correlates with poor clinical outcome. This was not detected by conventional TEM, which is an insensitive measure of endogenous fibrinolytic activity.

Keywords: coagulopathy, fibrinolysis, thromboelastometry, trauma.

Introduction

Trauma is a global public health problem, leading to over 6 million deaths each year [1]. Forty per cent of trauma deaths result from bleeding, and occur in the first few hours after injury [2]. Hemostatic dysfunction is common, and up to 25% of severely injured trauma patients have an established coagulopathy when they arrive in the emergency department (ED) [3]. This early coagulopathy is independently associated with mortality, increased transfusion requirements, organ injury, septic complications, and critical care stays [4]. Understanding the etiology of acute traumatic coagulopathy and all aspects of hemostatic dysfunction associated with trauma is a current imperative in the management of these patients.

Fibrinolysis is known to occur during traumatic coagulopathy, but the true incidence and magnitude of fibrinolytic activity (FA) in trauma has never been studied in detail [5]. Screening laboratory tests of coagulation (e.g. prothrombin time [PT] and partial thromboplastin time) do not assess fibrinolysis, and near-patient global assays such as thromboelastometry (TEM) are not in widespread routine use. The few small studies that have investigated the use of TEM in trauma suggest that the measure of FA used, known as TEM hyperfibrinolysis, is rare, with an admission incidence of 3–6% [6–8]. However, abnormal laboratory coagulation results are common in trauma patients, and we have previously shown their association with an increased fibrinolytic potential [5]. This suggests that the underlying incidence and magnitude of fibrinolysis may be more common than considered previously.

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Hyperfibrinolysis is classically defined as fibrinolytic activity being potentially greater than fibrin formation, such that clot integrity is threatened. Hyperfibrinolysis is identified on TEM when maximum clot lysis (ML) exceeds 15% of the maximum clot firmness [9]. However, there has never been an attempt to correlate this definition with classic laboratory tests of fibrinolysis. Trauma patients with TEM hyperfibrinolysis have mortality rates of 80–100%, even when treated [6]. However, clinical trials of antifibrinolytic therapy suggest that activation of fibrinolysis is more prevalent. Trauma patients randomized to tranexamic acid in the CRASH-2 trial had a 9% improved survival as compared with those who received placebo [10]. Despite these findings, some trauma units administer antifibrinolytics only if 'TEM hyperfibrinolysis' is present. On the basis of the results from CRASH-2, we hypothesized that trauma patients have increased FA that is undetectable by TEM, which might explain the benefit from tranexamic acid administration.

The objectives of the current study were: first, to determine the prevalence and severity of FA in trauma as measured by increases in plasmin generation, determined according to plasmin–antiplasmin (PAP) complex levels; second, to determine the ability of TEM to detect FA; and third, to assess whether FA was associated with the severity of injuries, admission physiology, and clinical outcomes.

Materials and methods

We conducted a prospective observational cohort study of trauma patients presenting to a single major trauma center. The study was reviewed and approved by the UK Regional Ethics Committee. Between January 2007 and June 2009, all adult trauma patients (> 15 years) who met the local criteria for trauma team activation were eligible for enrollment into the study. Exclusion criteria were: arrival in the ED > 2 h following injury; > 2000 mL of intravenous fluid before hospital admission; transfer from another hospital; and burns covering > 5% of total body surface area. Patients were retrospectively excluded if they declined to give consent to use research samples, were receiving anticoagulant medications (not including aspirin), or had moderate or severe liver disease or a known bleeding diathesis.

We categorized patients according to the degree of increased fibrinolytic activity, based on the level of PAP complexes and TEM hyperfibrinolysis. Expected normal values for PAP complex levels as given by the manufacturer were 120–700 μg L^{-1} (2.5–97.5% percentiles). PAP complex thresholds were arbitrarily set at approximately twice the upper limit of normal for moderate FA (> 1500 μg L^{-1}), in order to exclude minor elevations that might be expected in these patients. TEM hyperfibrinolysis was defined as ML > 15% [9]. Patterns of FA were classified as: 'normal' (PAP complex ≤ 1500 μg L^{-1} and ML < 15%); 'moderate' with FA only (PAP complex > 1500 μg L^{-1} and ML < 15%); 'severe' with FA and TEM lysis (PAP complex > 1500 μg L^{-1} and ML > 15%); and TEM-only lysis (PAP complex ≤ 1500 μg L^{-1} and ML > 15%).

A 20-mL research sample of blood was drawn from either the femoral vein or antecubital fossa, and the standard trauma laboratory tests were performed within 20 min of arrival in the ED. Blood for TEM analysis was drawn into a 2.7-mL citrated vacutainer (0.109 M buffered sodium citrate, 3.2%; Becton Dickinson, Plymouth, UK). Samples for PT determination were collected in 4.5-mL glass vacutainers (0.109 M buffered sodium citrate, 3.2%; Becton Dickinson), 9 : 1 (v/v). The PT was determined with the Sysmex CS2100i automated analyzer (Sysmex UK, Milton Keynes, UK). The sample for hemostatic assays was placed in a citrated tube, and spun down within 2 h of blood draw. The sample was first spun at 1750 × *g* for 10 min; the supernatant was then extracted, and respun at 1750 × *g* for a further 10 min. The extracted plasma was stored in aliquots at − 80 °C. Arterial blood analysis for base deficit (BD) was performed simultaneously with the research sample collection.

Rotational TEM (ROTEM) samples were processed within 1 h of blood draw at 37 °C on a ROTEM delta instrument (TEM International, Munich, Germany), and all treating clinicians were blinded to the results. The methodology and the parameters of ROTEM have previously been described in detail [11]. Two separate ROTEM assays were performed for each patient: the EXTEM, measuring tissue factor-initiated clotting; and the APTEM, with the addition of an antifibrinolytic. For the EXTEM, 20 μL of 0.1 M $CaCl_2$ (STARTEM) and 20 μL of tissue factor derived from rabbit brain were placed into the test cuvette, after which 300 μL of the blood sample was added. The APTEM test analysis was performed in the presence of 20 μL of aprotinin. Mild activation with tissue factor is performed to standardize the in vitro coagulation process and produce a more rapid result. All pipetting steps and the mixing of reagents with samples were guided by the electronic pipette program. Clot breakdown was determined by rate of ML during a 60-min assay time for each sample analyzed. Plasma activity levels of α_2-antiplasmin (Siemens Berichrom α_2-antiplasmin; Sysmex, UK) were assayed with a Sysmex CS2100i automated analyzer. Latex immunoassays were used to quantify the levels of D-dimer (Siemens Innovance D-dimer; Sysmex, UK), also with a Sysmex CS2100i automated analyzer. ELISAs were used to quantify tissue-type plasminogen activator (t-PA) (Asserachrom tPA; Diagnostica Stago, France), plasminogen activator inhibitor-1 (PAI-1) (Asserachrom PAI-1; Diagnostica Stago), prothrombin fragments 1 + 2 (PF_{1+2}) (Enzygnost F1 + 2 monoclonal; Siemens Healthcare Diagnostics, Germany), PAP complex (DRG PAP micro, Germany), thrombin-activatable fibrinolysis inhibitor (TAFI) (Asserachrom TAFI; Diagnostica Stago), and activated TAFI (TAFIa) (Asserachrom TAFIa; Diagnostica Stago).

Data were collected prospectively on patient demographics, time of injury, mechanism (blunt or penetrating), prehospital fluid administration, time of arrival in the ED, and baseline vital signs. Injury was classified according to the injury severity score (ISS) [12] as moderate (< 16) severe (16–24) or critical (> 24) trauma. Arterial BD was used as a marker of tissue

hypoperfusion. Patients were followed until hospital discharge or death. Outcome measures recorded were 28-day mortality, 28-day ventilator-free days, and all transfusions required in the first 24 h.

Normal-quantile plots were used to test for normal distribution in patients with mild injuries and no shock. Parametric data are expressed as mean (confidence intervals). For the parametric data, two-group analysis was performed with a two-tailed unequal variance Student's *t*-test. Injury severity and its components (abbreviated injury score [AIS]), ventilator-free days and hospital length of stay were not normally distributed, and are expressed as median (interquartile range). For the non-parametric data, one-way ANOVA (Kruskal–Wallis) was used for analysis. Univariate and multiple regression analyses were used to assess correlation. For analysis of contingency data, a χ^2-test for trend was used. A *P*-value of < 0.05 was chosen to represent statistical significance throughout.

Results

Three hundred and twenty-three trauma patients were eligible for enrollment into the study over an 18-month period. Four declined consent, three were discharged early with no contact details, six were unable to give consent and had no next of kin, five were unable to consent and their next of kin declined on their behalf, and a further two were excluded because of protocol violation. Of the 303 remaining patients, 10 did not have ROTEM studies performed and five were unable to have ROTEM studies performed, leaving 288 patients for inclusion in the study. Patient demographics and injury characteristics are shown in Table 1. The rate of blunt trauma and mortality increased with the magnitude of lysis; all of those with severe lysis had blunt trauma, and they had a mortality rate of 40%. The admission temperature in all patients was slightly below the normal range, but did not vary significantly between the groups.

Rates of FA and TEM lysis

Fifteen patients (5%) had TEM hyperfibrinolysis, and, of these, four (1%) had ML $> 50\%$ (Fig. 1A). One hundred and eighty patients (59%) had evidence of FA, in that they had PAP complex levels > 1500 μg L^{-1}. One hundred and sixty-five patients (57%) had increased PAP complex levels (> 1500 μg L^{-1}) without TEM hyperfibrinolysis (Fig. 1B). Significant increases in ML occurred when PAP complex levels were $> 20\,000$ μg L^{-1} (Fig. 1C). When aprotinin was added in vitro to the plasma sample, there was an improvement in ML only in this group. Active fibrinolysis was shown in the moderate group by markedly increased D-dimer levels

Table 1 Patient demographics, injuries, admission parameters, and outcomes

	All patients	Normal	Moderate	Severe	TEM lysis only
Number (%)	303	100 (35)	165 (57)	15 (5.2)	8 (2.7)
Age (years)	37 (36–39)	32 (30–35)	40 (37–43)*	40 (33–47)	44 (36–53)
% Male	81.9	85.0	80.0	73.3	87.5
Injuries					
% Blunt trauma	79.5	63.0	88.5*	93.3*	50
ISS	10 (4–25)	6 (1–10)	17 (9–28)*	25 (17–38)*	5 (2–9)
% ISS > 15	41.9	12.2	55.8*	86.7*	0
AIS extremity	1 (0–3)	0 (0–2)	2 (0–3)*	3 (1–3)*	1 (0–2)
AIS thorax	0 (0–3)	0 (0–1)	2 (0–3)*	3 (0–5)*	0 (0–0)
AIS head	0 (0–2)	0 (0–1)	0 (0–3)*	0 (0–3)	0 (0–0)
Admission parameters					
Temperature (°C)	35.8 (35.6–36.0)	36.0 (35.8–36.2)	35.7 (35.3–36.0)	35.1 (34.0–36.3)	36.0 (35.3–36.6)
SBP (mmHg)	130 (127–133)	130 (125–136)	130 (125–136)	119 (100–138)	141 (128–155)
Base deficit (mM)	2.4 (1.8–2.9)	0.5 (− 0.1 to 1.1)	2.9 (2.1–3.7)*	8.6 (5.2–12.0)*†	0 (–2.3–2.6)
PT (s)	11.6 (11.5–11.8)	11.2 (11.1–11.4)	11.7 (11.5–12.0)*	13.6 (11.8–15.4)*	11.3 (10.8–11.7)
% PT ratio > 1.2	9.4	3.0	10.9*	40.0*	0.0
TEM CT	70.0 (64.8–75.1)	72.0 (65.5–78.6)	67.4 (59.6–75.1)	84.7 (61.5–108.0)	69.9 (44.0–95.7)
TEM CA5	42 (41–43)	43 (41–45)	42 (41–43)	35 (30–41)*†	41 (29–51)
24-h transfusions					
PRBCs (units)	1.7 (0.6–2.7)	0.2 (0.0–0.7)	2.0 (1.3–2.7)*	6.5 (3.0–10.0)*†	0 (0–0)
FFP (units)	0.8 (0.1–1.5)	0.1 (0.0–0.9)	1.0 (0.6–1.4)*	2.9 (1.2–4.5)*	0 (0–0)
Platelets (units)	0.2 (0.0–0.4)	0.0 (0.0–0.0)	0.2 (0.1–0.3)*	0.7 (0.3–1.0)*†	0 (0–0)
Cryoprecipitate (units)	0.2 (0.0–0.4)	0.0 (0.0–0.0)	0.2 (0.1–0.4)*	0.6 (0.2–1.0)*	0 (0–0)
Outcomes					
28-day mortality (%)	8.9	1.0	12.1*	40.0*	0.0
28-day ventilator-free days	28 (27–28)	28 (28–28)	28 (27–28)*	28 (27–28)	28 (27–28)
Hospital stay (survivors)	15 (12–18)	2 (1–6)	11 (4–25)*	26 (15–32)*	4 (1–9)

AIS, abbreviated injury score; FFP, fresh frozen plasma; ISS, injury severity score; PRBC, packed red blood cell; PT, prothrombin time; SBP, systolic blood pressure; TEM CT, clotting time on rotational thromboelastometry (ROTEM); TEM CA5, clot amplitude at 5 min on ROTEM. Values are number (%), mean (confidence intervals), or percentage of patient group. ISS, AIS, ventilator-free days and hospital stay are given as median (interquartile range). *$P < 0.05$ vs. no lysis; †$P < 0.05$ for moderate vs. severe lysis.

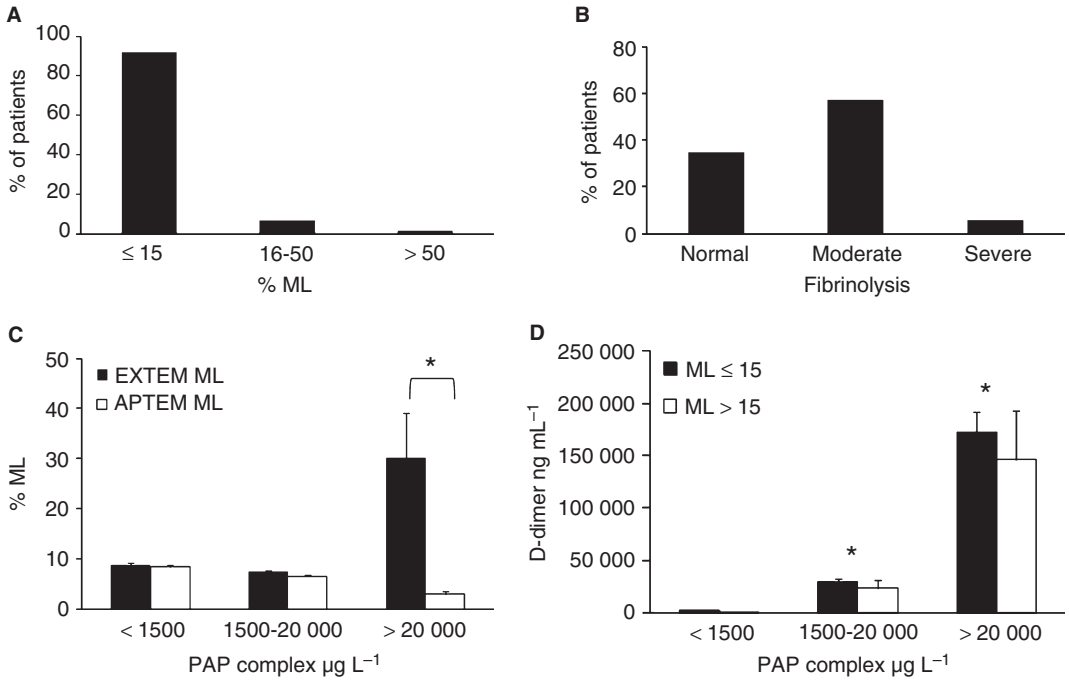

Fig. 1. Thromboelastometry (TEM) underestimates the incidence and severity of fibrinolysis. (A) Incidence of hyperfibrinolysis as defined by functional lysis on TEM (maximum clot lysis [ML]). (B) Incidence of moderate and severe hyperfibrinolysis (normal, ROTEM ML < 15% and plasmin–antiplasmin (PAP) complex < 1500 μg L^{-1}; moderate, ML < 15% and PAP complex > 1500 μg L^{-1}; severe, ML > 15% and PAP complex > 1500 μg L^{-1}). (C) Functional clot lysis (dark bars) was only measurable with very high PAP complex levels (%ML: PAP complex < 1500 μg L^{-1} – 8.8% vs. PAP complex > 20 000 μg L^{-1} – 30.3%). Functional clot lysis improvement with aprotinin (white bars) was only identified at high plasmin levels (%ML: PAP complex < 1500 μg L^{-1} – 0.5% vs. PAP complex > 20 000 μg L^{-1} – 27.3%; *P < 0.001 vs. PAP complex < 1500 μg L^{-1}). (D) D-dimer levels were equivalent whether or not lysis was detected on TEM for given plasmin generation levels (D-dimer: for PAP complex range 1500–20 000 μg L^{-1} and ML ≤ 15% 29 167 vs. ML > 15% 23 390 P = 0.85; for PAP complex > 20 000 μg L^{-1} and ML ≤ 15% 171 971 vs. ML > 15% 146 093 P = 0.62; *P < 0.05 as compared with PAP < 1500 μg L^{-1}). Values are % or mean + 95% confidence interval.

(Table 2). Plasmin generation was associated with significantly increased fibrin breakdown as measured by D-dimer production, regardless of whether lysis was visible on TEM (Fig. 1D). There was a TEM-only lysis group with eight patients (2.7%) that showed no FA. They had low mean PAP complex levels (1011 μg L^{-1}) and no evidence of fibrin breakdown as characterized by D-dimer levels (mean: 865 ng mL^{-1}). In fact, this was lower than the level in the normal group.

Table 2 TEM and coagulation factor assays

	All patients	Normal	Moderate	Severe	TEM lysis only
ML	9.4 (8.0–10.8)	8.1 (7.4–8.7)	6.6 (6.0–7.1)*	45.5 (28.0–63.0)*†	18.4 (16.5–20.3)*
PAP complex (μg L^{-1})	4690 (3887–5494)	928 (862–993)	5844 (4835–6854)*	17 503 (10 502–24 503)*†	1011 (794–1227)
t-PA (ng mL^{-1})	11.8 (10.2–13.5)	8.0 (6.8–9.1)	12.4 (10.8–14.0)*	39.1 (14.5–63.7)*	8.3 (3.8–12.7)
D-dimer (ng mL^{-1})	30 544 (22 822–38 266)	2576 (1727–3426)	38 687 (30 502–46 872)*	88 831 (31 348–146 315)*	865 (488–1243)*
Antiplasmin (IU dL^{-1})	122.8 (119.6–126.1)	131.4 (127.6–135.2)	120.4 (116.0–125.0)*	86.7 (66.0–107.3)*†	130.4 (119.9–141.0)
PAI-1 (pmol L^{-1})	32.7 (28.0–37.4)	38.3 (27.7–48.9)	29.6 (24.6–34.6)	28.6 (21.4–35.8)	43.7 (0.1–87.4)
PF_{1+2} (pmol L^{-1})	1865 (1562–2168)	596 (489–703)	2314 (1911–2716)*	5062 (2546–7577)*	316 (234–399)*
Fibrinogen (g L^{-1})	2.1 (2.0–2.1)	2.2 (2.1–2.3)	2.1 (1.9–2.1)*	1.5 (1.1–1.9)*†	2.1 (1.7–2.4)
TAFI (μg mL^{-1})	12.6 (12.2–13.0)	13.5 (12.9–14.1)	12.0 (11.5–12.5)*	10.6 (9.0–12.4)*	13.3 (11.1–15.3)
TAFIa (ng mL^{-1})	132 (117–147)	84 (66–103)	153 (133–173)*	303 (179–427)*†	66.9 (58.2–75.5)

ML, ROTEM maximum clot lysis; PAI-1, plasminogen activator inhibitor-1; PAP complex, plasmin–antiplasmin complex; PF_{1+2}, prothrombin fragments 1 + 2; TAFI, thrombin-activatable fibrinolysis inhibitor; TAFIa, activated thrombin-activatable fibrinolysis inhibitor; t-PA, tissue plasminogen activator. Values are means (confidence intervals). α_2-Antiplasmin: normal range, 76–126 IU dL^{-1}; intra-assay and inter-assay variability, 0.5% and 3.2%. D-dimer: normal range, < 550 ng mL^{-1}; intra-assay and inter-assay variability, 4.1% and 4.3%. PAI-1: normal range, 4–43 ng mL^{-1}; intra-assay and inter-assay variability, 6.0% and 5.6%. PAP complex: normal range, 120–700 μg L^{-1}; intra-assay and inter-assay variability, 4.2% and 7.3%. PF_{1+2}: normal range, 69–229 pmol L^{-1}; intra-assay and inter-assay variability, 4.5% and 7.65%. TAFI: normal range, 7.6–10.6 μg mL^{-1}; intra-assay and inter-assay variability, 4.3% and 6.9%. TAFIa: normal range, 8.53–22.07 ng mL^{-1}; intra-assay and inter-assay variability, 3.4% and 3.15%. t-PA: normal range, 2–12 ng mL^{-1}; intra-assay and inter-assay variability, 4.5% and 6.4%. *P < 0.05 vs. no lysis; † P < 0.05 for moderate vs. severe lysis.

Relationship to injury characteristics

Increasing ISS was associated with increasing FA, as measured by both PAP complex levels and ML (Fig. 2A). However, TEM ML increased only minimally, from 6% with ISS ≤ 15 to 12% for ISS > 24 ($P = 0.10$). In contrast, PAP complex levels increased just over five-fold, from 2036 μg L^{-1} (ISS ≤ 15) to 10 697 μg L^{-1} (ISS > 24, $P < 0.001$). Ninety per cent of patients with ISS > 24 had PAP complex levels > 1500 μg L^{-1}, but only 11.6% had TEM hyperfibrinolysis (Fig. 2A), showing that PAP complex was more sensitive to injury severity than TEM. In a multiple regression analysis of all organ injuries, PAP complex levels correlated with the severity of head, chest and extremity trauma ($P < 0.001$ for all three regions; $r^2 = 0.28$), whereas %ML was very weakly correlated with the severity of extremity injury only ($P = 0.008$, $r^2 = 0.05$). PAP complex levels correlated with the degree of shock and injury severity (BD, $P < 0.001$; ISS, $P < 0.001$; $r^2 = 0.36$; Fig. 2B). Eighty-eight per cent of patients with BD > 6 mM had moderate fibrinolysis, as compared with 58% of patients with BD < 6 mM ($P < 0.0001$; Fig. 2C). D-dimer levels were high even with relatively low levels of shock (Fig. 2D). TEM ML levels were not correlated with the degree of shock as measured by the BD

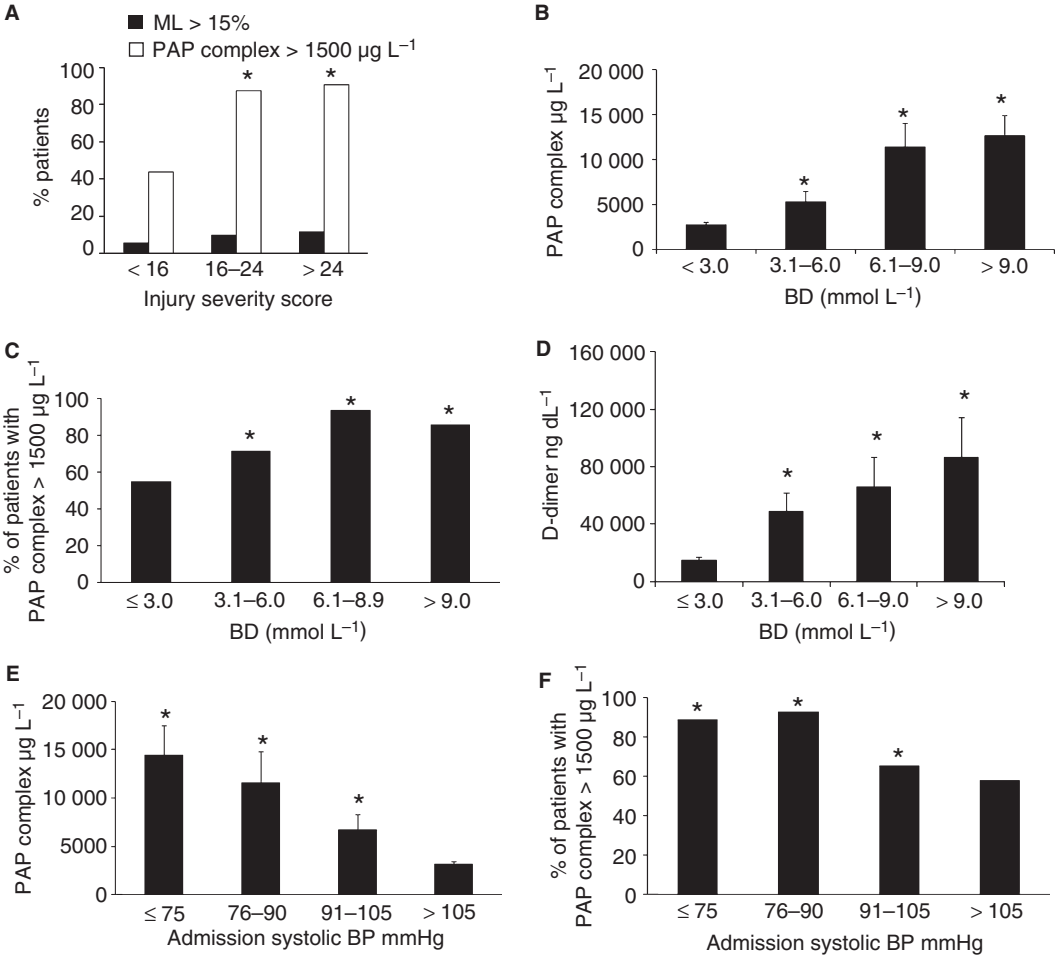

Fig. 2. Fibrinolytic activation is associated with injury severity and the degree of shock. (A) The proportion of patients presenting with high maximum clot lysis (ML) and plasmin–antiplasmin (PAP) complex levels by injury severity score (ISS). The percentage of patients with overt fibrinolysis (ML > 15%) rose slightly with injury severity (ISS < 16, 5.8%; ISS 16–24, 10.0%; ISS > 24, 11.6%; $P > 0.05$), whereas the percentage of patients with PAP complex > 1500 μg L^{-1} rose substantially with injury severity (ISS < 16, 43.8%; ISS 16–24, 87.5%; ISS > 24, 90.9%; $P < 0.001$) (B) PAP complex levels increased with the degree of systemic hypoperfusion (*$P < 0.001$ vs. base deficit [BD] <3.0 mM). (C) The proportion of patients presenting with high PAP complex levels (> 1500 μg L^{-1}) increased with systemic hypoperfusion (BD < 3.0, 55%; BD 3.1–6.0, 71%; BD 6.1–9.0, 93%; BD > 9.0, 86%; $P < 0.001$ vs. BD < 3.0 mM). (D) D-dimer levels increased significantly with increasing hypoperfusion. Even with moderate hypoperfusion (BD 3.1–6.0, D-dimer 49 387 ng mL^{-1}), levels were very high, and they increased substantially as hypoperfusion increased (BD 6.1–9.0, D-dimer 66 233 ng mL^{-1}, BD > 9.0, D-dimer 86 892 ng mL^{-1}; $P < 0.001$ vs. BD < 3.0 mM). (E) PAP complex levels were inversely related to systolic blood pressure (SBP). Even at borderline SBP, PAP complex levels were significantly higher, and they continued to rise as SBP fell (SBP > 105 mmHg, 3170 μg L^{-1}; SBP 91–105 mmHg, 6776 μg L^{-1}; SBP 76–90 mmHg, 11 029 μg L^{-1}; SBP ≤ 75, 14 876 μg L^{-1}; $P < 0.001$ vs. SBP > 105 mmHg). (F) The proportion of patients with high PAP complex levels (> 1500 μg L^{-1}) increased with falling blood pressure (SBP > 105 mmHg, 58%; SBP 91–105 mmHg, 66%; SBP 76–90 mmHg, 92%; SBP ≤ 75 mmHg, 89%; $P < 0.0001$ vs. SBP > 105 mmHg). Values are percentages or means + 95% confidence intervals.

or systolic blood pressure (SBP). Low SBP was also associated with increasing FA (Fig. 2E), and 91% of patients with SBP <90 mmHg had moderate or worse FA, as compared with 59% with SBP > 90 mmHg ($P \leq 0.0001$; Fig. 2F). Mild FA occurred at moderate levels of both injury severity and shock, whereas severe hyperfibrinolysis only occurred in association with the most severe extremity injuries. TEM-only lysis followed the same injury pattern as no lysis, with low levels of shock and low injury severity.

Mechanisms of activation

Both t-PA and PAP complex levels were significantly incrementally elevated, as expected in all those with FA (Table 2). Levels of PF_{1+2} (Table 2) also increased with the same pattern as FA, suggesting that thrombin might be a key stimulator of t-PA release from the endothelium. Levels of t-PA correlated with hypoperfusion and with injury severity (ISS, $P = 0.002$; BD, $P < 0.001$; $r^2 = 0.19$). Severe FA was associated with massive t-PA production and PAP complex levels, such that there was significant consumption of antiplasmin (Fig. 3A). Severe hyperfibrinolysis was only seen in association with α_2-antiplasmin activity below 75 IU dL^{-1} (Fig. 3B). However, significant D-dimer generation continued to occur, whatever the level of α_2-antiplasmin activity (up to 125 IU dL^{-1}; Fig. 3C). TAFI levels did not seem to strongly affect the regulation of fibrinolysis, as D-dimer levels were negatively correlated with TAFI levels and positively correlated with TAFIa levels (Fig. 3D). TEM-only lysis again followed the pattern of normal lysis: t-PA and PAP complex levels were not raised, and the α_2-antiplasmin level was not reduced.

Clinical outcomes

The small number of patients with severe FA had a high mortality rate (6/15, 40%), but those with moderate FA were a larger group, and, despite having a lower mortality rate, had a higher number of deaths overall (20/165, 12%). Patients with moderate FA had a 12-fold increase in 28-day mortality as compared with patients with no lysis (Fig. 4A; Table 1). PAP complex levels were independently associated with 28-day mortality in a multiple regression analysis including age, mechanism of injury, injury severity, admission SBP, and BD ($r^2 = 0.24$; PAP complex, $P < 0.001$). For the patients who died in the severe FA group, the average time to death was < 1 day and all deaths occurred within 48 h.

There was an association between severity of fibrinolysis and the average number of blood transfusions per patient (Fig. 4B). PAP complex levels were independently associated with 24-h red cell requirements in a multiple regression analysis including mechanism of injury, injury severity, admission SBP, BD, and prothrombin ratio ($r^2 = 0.24$; PAP complex, $P < 0.001$). Patients with moderate FA were also more likely to receive plasma (20% vs. 2%, $P < 0.001$; Fig. 4C), platelet (12.7% vs.

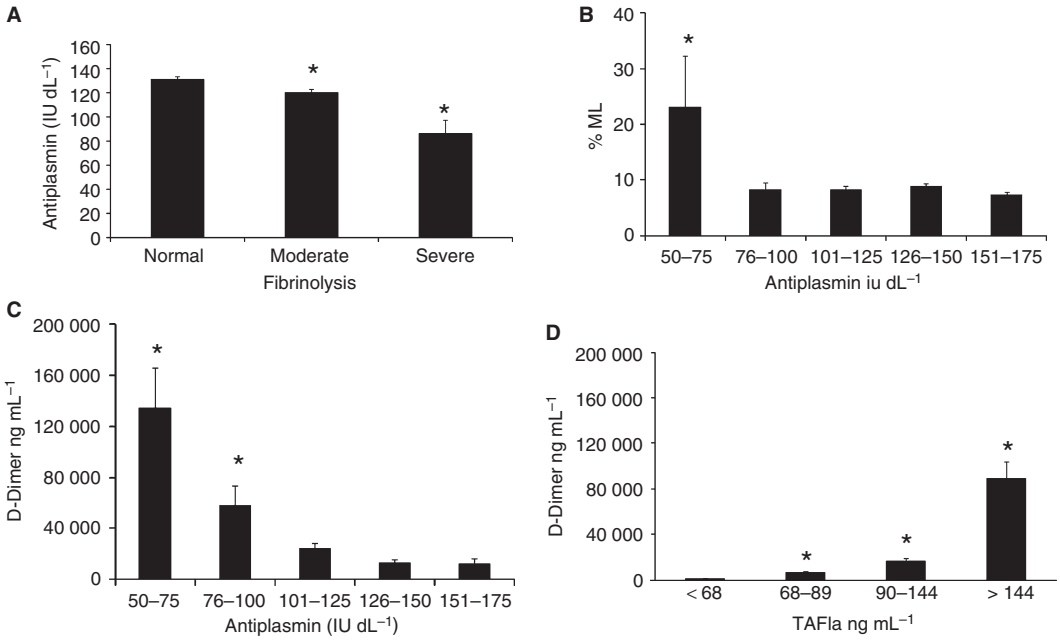

Fig. 3. Mechanisms of activation of moderate and severe fibrinolysis. (A) Antiplasmin (AP) activity fell in the moderate fibrinolysis group, but was only 66% of normal in the severe group (normal, 131 IU dL^{-1}; moderate, 120 IU dL^{-1}; severe, 87 IU dL^{-1}; $^*P < 0.001$ vs. normal). (B) Rotational thromboelastometry hyperfibrinolysis was only seen when antiplasmin activity fell below 75 IU dL^{-1} (percentage maximum clot lysis [%ML]: AP 50–75 IU dL^{-1}, 23.1%; AP 151–175 IU dL^{-1}, 7.4%; $^*P < 0.01$ vs. AP 151–175 IU dL^{-1}). (C) In contrast, D-dimer levels were raised with relatively normal activity of antiplasmin (D-dimer: AP 50–75 IU dL^{-1}, 135 161 ng mL^{-1}; AP 76–100 IU dL^{-1}, 58 606 ng mL^{-1}; AP 101–125 IU dL^{-1}, 24 713 ng mL^{-1}; $^*P < 0.05$ vs. AP 150–175 IU dL^{-1}, 12 547 ng mL^{-1}). (D) Activated thrombin-activatable fibrinolysis inhibitor (TAFIa) levels were positively correlated with D-dimer production (TAFIa in quartiles, $^*P < 0.001$ vs. first quartile).

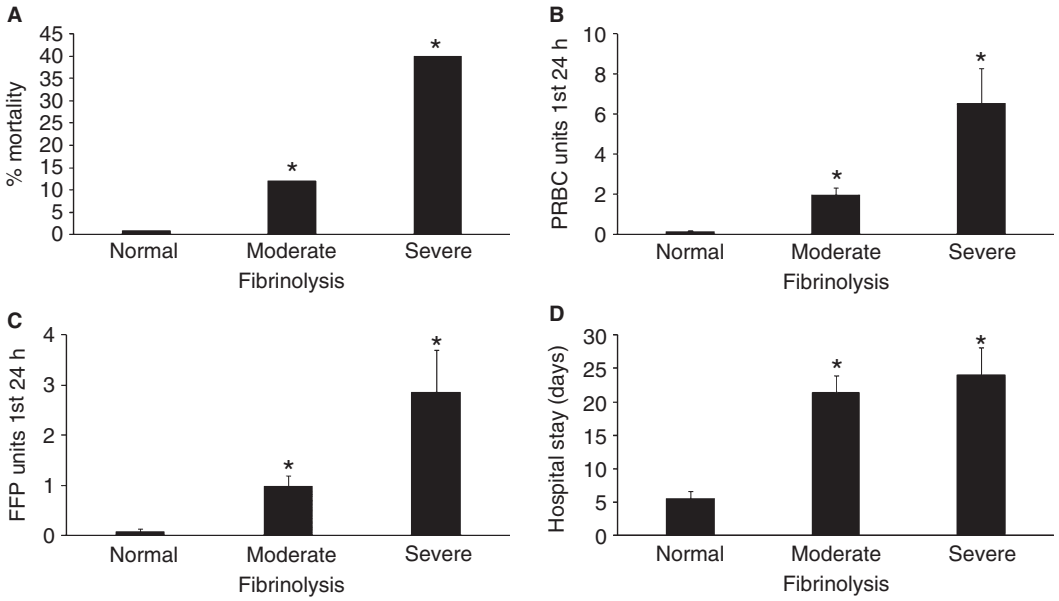

Fig. 4. Outcomes associated with moderate and severe fibrinolysis. (A) Significantly higher mortality was seen in the moderate group (12%, $P < 0.001$) and the severe group (40%, $P < 0.05$) than in the normal group (1%). (B) Moderate and severe fibrinolysis was associated with higher packed red blood cell (PRBC) transfusion (moderate vs. normal and severe vs. normal, $P < 0.001$ for both groups). (C) There were higher fresh frozen plasma (FFP) requirements in the moderate group (1.0 units) and severe group (2.87 units) (moderate vs. normal and severe vs. normal, $P < 0.001$ for both groups). (D) Hospital length of stay for survivors was increased vs. the normal group (99 survivors, 5.7 days) in the moderate group (145 survivors, 21.5 days) and the severe group (nine survivors, 24.1 days) (*$P < 0.001$). Values are percentages or means ± 95% confidence intervals.

0%, $P < 0.001$) and cryoprecipitate (11.5% vs. 0%, $P < 0.001$) transfusions. Survivors with moderate FA had a higher incidence of septic complications (48% vs. 3%, $P < 0.001$), had fewer 28-day ventilator-free days (27 vs. 28 days, $P < 0.028$) and spent longer in hospital (11 vs. 2 days, $P < 0.001$; Fig. 4D) than patients in the normal group. The TEM-only lysis group was again a relatively uninjured cohort, with 0% mortality, no use of blood or products, and a median length of stay of just over 48 h.

Discussion

We have shown that FA, as measured by increased levels of PAP complex, t-PA, and D-dimer, occurs in almost two-thirds of trauma patients, and that the degree of FA is associated with increasing injury and with significantly worse transfusion requirements, morbidity, and mortality. This high rate of FA has not been identified previously, but it is consistent with previous work [5] and the efficacy of tranexamic acid in reducing mortality in bleeding trauma patients [10].

Moreover, > 90% of FA in trauma patients is not detected with the current definition of TEM hyperfibrinolysis. Six in every 10 patients presented with moderate FA, but only one in 20 showed TEM hyperfibrinolysis. We have shown that increased FA occurs without visible clot lysis by TEM, despite similar levels of D-dimer generation as in those patients with TEM hyperfibrinolysis, and that patients with even moderate FA have significantly increased transfusion requirements and other adverse outcomes. The observed FA was not a consequence of hypothermia (at least at the levels seen in our study [13]). FA is therefore a real pathophysiologic entity with important consequences for trauma patients. This is of concern in the pragmatic management of bleeding trauma patients. The group with moderate FA is larger and accounts for more deaths than patients with TEM hyperfibrinolysis, and would have potentially benefited from the use of antifibrinolytics.

It is unclear under what circumstances TEM detects fibrinolytic activity. It is possible that TEM is simply insensitive until FA reaches a certain threshold. Free plasmin is rapidly hydrolyzed, and has a very short half-life. In the TEM cup, free t-PA is required to generate new plasmin for clot breakdown. This was only seen when t-PA levels were nearly five times normal and α_2-antiplasmin levels were significantly reduced to below 75%. TEM hyperfibrinolysis may only occur when there is insufficient antiplasmin to block the action of all free plasmin generated in the sample well. Certainly, the finding that the addition of aprotinin, a stoichiometric inhibitor of plasmin, to the TEM cups improved the TEM ML trace of those with TEM hyperfibrinolysis, but did not alter the traces of others with FA, suggests that generation of free plasmin is necessary to produce the TEM hyperfibrinolysis. Further research is required to examine this and to identify methods with which to increase the sensitivity of TEM.

There are a number of limitations to our study. Although we have shown that trauma patients have increased FA and that the degree of activation is associated with increased transfusion requirements and worse outcomes, we have not shown that these levels are associated with an increase in clot breakdown or fragility. We used D-dimer levels to measure the overall results of fibrinolysis, but D-dimer levels are a marker of prothrom-

botic fibrin generation as well as of fibrinolytic activity. We found significantly higher levels of PF_{1+2} in the severe group, and it is not possible to separate the effects of these two processes on the basis of D-dimer levels alone. The PAP complex levels probably represent a more robust marker of the activity of the fibrinolytic system itself. We did not investigate the value of the various stimuli that drive the generation of FA, and these mechanisms will require further basic and clinical research. Finally, although TAFI did not appear to be a major factor in the systemic regulation of fibrinolysis, it may have an important role in intrinsic clot stability, and its role needs further elucidation.

In summary, we have identified FA in the majority of trauma patients, and shown that the degree of FA is associated with significantly worse outcomes in terms of transfusion requirements, mortality, and morbidity. These findings provide a logical explanation for the apparent paradox that antifibrinolytic therapy appears to have broad application in trauma and elective surgery, but massive near-fatal trauma is required to produce detectable changes in TEM. The discovery of FA in two-thirds of trauma patients presents new opportunities for the development of diagnostic modalities and targeted therapeutic intervention to improve outcomes after trauma.

Addendum

K. Brohi and R. Davenport: overall prospective cohort study design and ethical approval; I. Raza, R. Davenport, C. Rourke, J. Manson, C. Spoors, H. De'ath, and S. Khan: patient enrollment, sampling, follow-up, and data collection; R. Davenport, C. Rourke, and S. Platton: initial sample analysis; K. Brohi, S. Allard, D. P. Hart, S. Stanworth, P. MacCallum, and K. J. Pasi: cohort preliminary data review, fibrinolysis substudy development, and sample assay plan; I. Raza, C. Rourke, R. Davenport, and S. Platton: additional assays; I. Raza, K. Brohi, B. Hunt, R. Davenport, S. Allard, D. P. Hart, S. Stanworth, P. MacCallum, and K. J. Pasi: data analysis and interpretation; I. Raza, K. Brohi, and R. Davenport: writing of the manuscript; K. Brohi, I. Raza, B. Hunt, S. Stanworth, and P. MacCallum: manuscript editing and revisions.

I. Raza and K. Brohi certify that we had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure of conflict of interests

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