Acute coagulopathy of trauma: hypoperfusion induces systemic anticoagulation and hyperfibrinolysis


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Abstract

BACKGROUND: Coagulopathy is present at admission in 25% of trauma patients, is associated with shock and a 5-fold increase in mortality. The coagulopathy has recently been associated with systemic activation of the protein C pathway. This study was designed to characterize the thrombotic, coagulant and fibrinolytic derangements of trauma-induced shock. METHODS: This was a prospective cohort study of major trauma patients admitted to a single trauma center. Blood was drawn within 10 minutes of arrival for analysis of partial thromboplastin and prothrombin times, prothrombin fragments 1 + 2 (PF1 + 2), fibrinogen, factor VII, thrombomodulin, protein C, plasminogen activator inhibitor-1 (PAI-1), thrombin activatable fibrinolysis inhibitor (TAFI), tissue plasminogen activator (tPA), and D-dimers. Base deficit was used as a measure of tissue hypoperfusion. RESULTS: Two hundred eight patients were studied. Systemic hypoperfusion was associated with anticoagulation and hyperfibrinolysis. Coagulation was activated and thrombin generation was related to injury severity, but acidosis did not affect Factor VII or PF1 + 2 levels. Hypoperfusion-induced increase in soluble thrombomodulin levels was associated with reduced fibrinogen utilization, reduction in protein C and an increase in TAFI. Hypoperfusion also resulted in hyperfibrinolysis, with raised tPA and D-Dimers, associated with the observed reduction in PAI-1 and not alterations in TAFI. CONCLUSIONS: Acute coagulopathy of trauma is associated with systemic hypoperfusion and is characterized by anticoagulation and hyperfibrinolysis. There was no evidence of coagulation factor loss or dysfunction at this time point. Soluble thrombomodulin levels correlate with thrombomodulin activity. Thrombin binding to thrombomodulin contributes to hyperfibrinolysis via activated protein C consumption of PAI-1.
Title:

Acute coagulopathy of trauma: hypoperfusion induces systemic anticoagulation and hyperfibrinolysis

Short Title: Acute Traumatic Coagulopathy

Authors:

Karim Brohi, FRCS, FRCA, Mitchell J. Cohen, MD, Michael T. Ganter, MD, Marcus J. Schultz, MD, PhD, FCCS, Marcel Levi, MD, PhD, Robert C. Mackersie, MD, and Jean-François Pittet, MD

Author affiliations:

From the Department of Surgery, The Royal London Hospital (K.B.), London, United Kingdom; Departments of Surgery (M.J.C., R.C.M., J.F.P.) and Anesthesia (M.T.G., J.F.P.), San Francisco General Hospital, University of California San Francisco, San Francisco, California; and the Departments of Intensive Care, Laboratory of Experimental Intensive Care and Anesthesiology (M.J.S.) and Internal Medicine (M.L.), Academic Medical Center, Amsterdam, The Netherlands.

Corresponding Author:

Karim Brohi

Department of Surgery, The Royal London Hospital

Whitechapel Road, London, E1 1BB, United Kingdom

Phone: 020 7377 7695    Fax: 020 7377 7044

E-mail: karim@trauma.org
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Abstract

**Background:** Acute traumatic coagulopathy, present on admission in 25% of trauma patients, is associated with shock and a 5-fold increase in mortality. The coagulopathy has recently been associated with systemic activation of the protein C pathway. This study was designed to fully characterize the coagulopathy of shock and the resultant thrombotic, coagulant and fibrinolytic derangements.

**Methods:** This was a prospective cohort study of major trauma patients admitted to a single trauma center. Blood was drawn within 10 minutes of arrival for analysis of partial thromboplastin and prothrombin times, prothrombin fragments 1+2 (PF1+2), fibrinogen, factor VII, thrombomodulin, protein C, plasminogen activator inhibitor-1 (PAI-1), thrombin activatable fibrinolysis inhibitor (TAFI), tissue plasminogen activator (tPA) and D-dimers. Base deficit was used as a measure of tissue hypoperfusion.

**Results:** 208 patients were studied. Systemic hypoperfusion was associated with anticoagulation and hyperfibrinolysis. Thrombin generation was related to injury severity, but acidosis did not affect Factor VII or PF1+2 levels. Hypoperfusion-induced increase in soluble thrombomodulin levels was associated with reduced fibrinogen utilization, reduction in protein C and an increase in TAFI. Hypoperfusion also resulted in hyperfibrinolysis, with raised tPA and D-Dimers. This was associated with the observed reduction in PAI-1 and not alterations in TAFI.
**Conclusions:** Acute traumatic coagulopathy is due to systemic hypoperfusion and is characterized by systemic anticoagulation and hyperfibrinolysis. There was no evidence of coagulation factor loss or dysfunction at this time point. Soluble thrombomodulin levels appear to correlate with thrombomodulin activity. Thrombin-thrombomodulin leads to hyperfibrinolysis via activated protein C consumption of PAI-1.
Introduction

Acute traumatic coagulopathy is present immediately on admission in 25% of trauma patients and is associated with a 5-fold increase in mortality. Accepted causes of traumatic coagulopathy are consumption of clotting factors, acidosis and hypothermia leading to reduced activity, and dilution from intravenous fluids and packed cell administration. However acute coagulopathy is present early in the post-injury phase, prior to fluid administration and in normothermic patients. Further, while acidosis per se affects coagulation protease function, clot time and maximum clot firmness are only impaired at very low pH (<6.8).

We have recently demonstrated that only patients who are in shock are coagulopathic on admission. Increased severity of hypoperfusion was associated with an increase in plasma thrombomodulin and a reduction in protein C levels. This suggests that acute coagulopathy is due to systemic anticoagulation due to activation of the protein C pathway.

The overall goal of this study was to fully characterize the coagulopathy of shock, and in particular to examine the interplay of shock, anticoagulation and the fibrinolytic system. Secondly, we wished to determine whether coagulation factor consumption or dysfunction due to acidosis were responsible for coagulopathy prior to massive fluid and blood transfusion. Third, we hypothesized that thrombomodulin has a central role in traumatic coagulopathy, complexing thrombin and resulting in anticoagulation and hyperfibrinolysis. Finally, there has
been some debate from basic science studies as to whether the hyperfibrinolysis resulting from thrombin-thrombomodulin formation is due to activation of Thrombin Activatable Fibrinolysis Inhibitor (TAFI)\(^4\) or activated protein C consumption of PAI-1\(^5,6\). We sought to understand which pathway was more important in clinical practice.

**Methods**

This was a prospective cohort study of consecutive major trauma patients admitted to a single level 1 trauma center. The methodology has been described previously\(^3\). Briefly, a 10 ml sample of blood was drawn by a designated member of the trauma team immediately on admission to the emergency department. The sample was spun down, plasma extracted and frozen at -80\(^\circ\)C. For this study we assayed plasma levels of Factor VII (++++)\(^3\), tissue Plasminogen Activator (tPA) - Asserachrom tPA, Diagnostica Stago (normal range 3-13 ng/ml) and TAFI ELISA, Enzyme Research Laboratories (normal range: 2.8 - 9.2 mcg/ml) in addition to previously described measurements of Prothrombin Fragments 1+2 (PF1+2), Fibrinogen, soluble Thrombomodulin (sTM), protein C, Plasminogen Activator Inhibitor-1 (PAI-1) and D-Dimer levels. For the D-Dimer assay, levels above 0.22 \(\mu\)g/ml were reported as 0.22 \(\mu\)g/ml.

Data were collected prospectively on patient demographics, injury time, mechanism (blunt or penetrating) and severity, prehospital fluid administration, the time of arrival in the trauma room and admission vital signs. In the absence
of a biochemical marker, the injury severity score was used as a surrogate measure of the degree of tissue injury. A full blood count, coagulation profile and arterial blood gas were drawn at the same time as the research sample as part of the standard management of major trauma patients. The degree of shock and systemic tissue hypoperfusion was assessed with the base deficit. Admission base deficit is a clinically useful early marker of tissue hypoperfusion in trauma patients and an admission base deficit greater than 6 mEq/l has previously been identified as predictive of worse outcome in trauma patients.  

Statistical analyses were performed with Microsoft Excel 2003 with the WinSTAT plug-in. Normal-quantile plots were used to test for normal distribution. Parametric data are expressed as mean ± 95% confidence intervals. Non-parametric data are given as median (inter-quartile range). Two-group analysis was performed with a two-tailed unequal variance Student’s t-test. The $\chi^2$ test was used for dichotomous data analysis. A $p$-value of 0.05 was chosen to represent statistical significance.

Results

Blood was drawn on 208 consecutive patients immediately on arrival to the trauma room. Median prehospital time was 28 minutes (interquartile range - IQR: 23-29) and 150 mls of fluids (0-200) were administered in the field. Median injury severity score was 17 (9-26) and 25% were penetrating.
Patients without shock (base deficit - BD≤6) were not coagulopathic, regardless of injury severity (Fig 1, A&B). 2.6% of patients with a BD≤6 had a prolonged PT (>18s) compared to 19.6% of patients with BD>6 (p=0.001, \( \chi^2 \)) and only 1.9% of patients with a BD≤6 had a prolonged PTT (>60s) compared to 12.5% of patients with BD>6 (p=0.007, \( \chi^2 \)). For patients in shock, both PT and PTT were prolonged as injury severity increased (Fig 1, A&B). In contrast, there was activation of fibrinolysis without shock (Fig 1C). However shock increased the degree of fibrinolysis in all patients (Fig 1D). Acute coagulopathy is therefore a consequence of shock and is characterized by systemic anticoagulation and hyperfibrinolysis.

Prothrombin fragments 1+2 (PF1+2) were assayed to assess the degree of thrombin generation. PF1+2 increased with injury severity (Fig 2A). There was no reduction in PF1+2 levels as acidosis increased (fig 2B), suggesting that reduction in coagulation factor activity due to acidosis does not make a significant contribution to the coagulopathy of shock. Factor VII levels were also unchanged by acidosis (Fig 2C), and sufficient for clot generation. Combined, these data also suggest that consumption of factors is not clinically significant at this time point and does not contribute to acute coagulopathy.

We previously demonstrated that increasing shock with hypoperfusion was associated with a rise in plasma levels of soluble thrombomodulin (sTM) and a decrease in protein C levels\(^3\). Thrombomodulin complexes with thrombin and
switches it to an anticoagulant function. Thrombin is therefore not free to cleave fibrinogen to form fibrin. As thrombomodulin levels rise, fibrinogen levels also rise (figure 3A). Figure 3B shows how with low thrombomodulin levels, there is a dose-dependent reduction in fibrinogen levels. This effect is abolished when thrombomodulin is high. Thus in the presence of shock and high thrombomodulin levels, fibrin production is minimal, regardless of clotting factor activity.

Further consequences of formation of the thrombin-thrombomodulin complex include activation of protein C. We did not measure activated protein C in this study but can demonstrate falling protein C levels with increasing sTM (Fig 3C) and have previously shown that this is likely to represent protein C activation due to observed effects on anticoagulation³. Thrombin complexed to thrombomodulin also activates TAFI, and we can demonstrate an increase in TAFI levels with increasing sTM (Fig 3D). Together these results support a central role for thrombomodulin in acute traumatic coagulopathy.

We have demonstrated that systemic fibrinolysis is also a component of this coagulopathy (Fig 1C). Tissue Plasminogen Activator (tPA) is released from the endothelium, and was significantly elevated in patients with shock, irrespective of the amount of thrombin generated (Fig 4, A&B). tPA levels were significantly higher when PAI-1 was low (Fig 4C) and increasing tPA levels were correlated with increasing D-Dimers, as expected (Fig 4D).
When present in excess, activated protein C is a potent inhibitor of PAI-1⁴ and we have previously shown that patients in shock have low levels of PAI-1 and a direct correlation between protein C and PAI-1 levels, suggesting that protein C activation leads to PAI-1 consumption³. It has previously been suggested that the de-inhibition of fibrinolysis seen with protein C is not due to this mechanism but to a competitive reduction in TAFI activation by the thrombin-thrombomodulin complex⁵,⁶. We can demonstrate this competitive binding of T-TM to either protein C or TAFI by an inverse correlation between protein C and TAFI levels (Fig 5A). However while we can demonstrate an inverse relationship between PAI-1 and the D-Dimer level (Fig 5B) there is no such correlation between TAFI and D-Dimers (Fig 5C) suggesting that in the clinical setting the protein C - PAI-1 interaction is more important for the observed hyperfibrinolytic state.

**Discussion**

Acute traumatic coagulopathy occurs in patients who are shocked and is not due to coagulation factor consumption or dysfunction due to acidosis or dilution. These factors may be important later in the clinical course, after massive transfusion or the development of severe acidosis. However shock itself is associated with a coagulopathy that is due to the systemic activation of anticoagulant and fibrinolytic pathways.
We have demonstrated previously that the protein C pathway is implicated in this process\textsuperscript{3}, and show here the central role of thrombomodulin in the conversion of thrombin from its coagulant role to a regulator of clot formation. Thrombomodulin is an endothelial protein that is present in normal endothelial cells. Theoretically, activation leads to increased thrombomodulin expression on the surface of the endothelium, where it complexes thrombin which cleaves protein C at the endothelial protein C receptor. This ‘anticoagulant thrombin’ is no longer available to cleave fibrinogen to form fibrin, as we have demonstrated. This has significant implications for current practice. All efforts to correct traumatic coagulopathy are currently directed at augmenting the clotting factor pathway, through the administration clotting factors and platelets, e.g. fresh frozen plasma\textsuperscript{9} or recombinant Factor VIIa\textsuperscript{10}. In theory, while patients are shocked and thrombomodulin is present in excess, thrombin that is generated will be anticoagulant, and stable clot will not be formed. Although it may be possible to overwhelm thrombomodulin with massive thrombin generation, this would also be associated with widespread activation of protein C. This would lead to consumption of PAI-1 and increased fibrinolysis, breaking down the clot that had formed. Further, activated protein C has a relatively long half-life\textsuperscript{11}, and the anticoagulant environment might persist and result in re-bleeding. Further studies will be needed to ascertain whether this is mechanism is important following augmentation of the extrinsic pathway during shock.
There has been some debate in the literature about the relationship between soluble thrombomodulin (sTM) and endothelial-bound thrombomodulin (eTM). Studies variously suggest that sTM does not reflect endothelial TM activity but is simply a marker of endothelial injury\textsuperscript{12}, is itself active\textsuperscript{13,14} or that it is in fact inhibitory to the accepted role of thrombomodulin\textsuperscript{15,16}. Our data would suggest that plasma sTM levels do reflect overall thrombomodulin activity, as increased sTM levels appear to be associated with decreased fibrinogen utilization and activation of protein C & TAFI.

Finally, we have demonstrated that the increased fibrinolysis associated with injury is also due to shock and is mediated through de-inhibition of tPA through the consumption of PAI-1. As mentioned above, it has been suggested that TAFI is the main driver of fibrinolysis inhibition, and that reduction in TAFI activation by the competitive binding of protein C to thrombin-thrombomodulin is the mechanism for de-repression of fibrinolysis with activation of protein C. Although we can demonstrate an increase in TAFI levels with thrombomodulin, and a competition between TAFI and protein C, there was no observable correlation between TAFI and D-Dimer levels. Thus the consumption of PAI-1 by activated protein C appears to be the more important cause of hyperfibrinolysis.

This study has several limitations that have been alluded to previously\textsuperscript{3}. The PT and PTT are very crude methods of identifying coagulopathic patients, and do not describe the global fibrinolytic state at all. Comparing these biochemical
markers to more functional methods such as viscoelastic coagulation analyses (e.g., thrombelastography™) might reveal more clinically relevant changes in coagulation. Further, this is an investigation of the state of the coagulation system at a single time point. Alterations in response to continued hemorrhage or successful resuscitation are worthy of further study. Previous investigations at later time points have identified that patients become hypercoagulable\textsuperscript{17,18} and are at risk of thromboembolic complications\textsuperscript{19}. It is possible that this is a result of depletion of protein C following systemic activation, indeed a previous study has identified admission coagulopathy as an independent risk factor for later venous thromboembolism\textsuperscript{20}.

In summary we have identified that acute traumatic coagulopathy occurs only in the presence of shock and is characterized by systemic anticoagulation and hyperfibrinolysis mediated through the activation of thrombomodulin. This has significant implications for the management of traumatic hemorrhage, and suggests that hypoperfusion must be corrected before the coagulation system’s hemostatic balance can be restored.
References


Fig 1: Shock induces anticoagulation and hyperfibrinolysis

A

B

C

Injury Severity Score

D-Dimer

Prothrombin Time (s)
Fig 2: Early Thrombin Generation is related to injury severity & not affected by acidosis

A

B

C
Fig 3: Increased soluble thrombomodulin is associated with reduced fibrinogen utilization, reduction in protein C (activation) and an increase in TAFI.
Fig 4: Activation of fibrinolysis

A

B

C

D

0 10 20 30 40 50 60

≤2.1 2.2-4.1 4.2-7.6 ≥7.7

BD

0 10 20 30 40 50 60

<2 2-4 >4

PF1+2

0 10 20 30 40 50 60

<1.1 1.1-5.1 5.2-13.1 >13.1

PAI-1

0 2 4 6 8 10 12

<12.3 12.3-18.6 18.7-28.7 >28.7

D-Dimers

0 2 4 6 8 10 12

<12.3 12.3-18.6 18.7-28.7 >28.7

tPA

D-Dimers

Fig 4: Activation of fibrinolysis

A

B

C

D

0 10 20 30 40 50 60

≤2.1 2.2-4.1 4.2-7.6 ≥7.7

BD

0 10 20 30 40 50 60

<2 2-4 >4

PF1+2

0 10 20 30 40 50 60

<1.1 1.1-5.1 5.2-13.1 >13.1

PAI-1

0 2 4 6 8 10 12

<12.3 12.3-18.6 18.7-28.7 >28.7

D-Dimers

0 2 4 6 8 10 12

<12.3 12.3-18.6 18.7-28.7 >28.7

tPA
Fig 5: Hyperfibrinolysis is due to consumption of PAI-1, not a reduction in TAFI