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Trauma-Induced Coagulopathy: an Investigation of Fibrinolysis and the Effect of Tranexamic Acid

Lewis Simpson Gall

Centre for Trauma Sciences, The Blizard Institute of Cell and Molecular Science,

Barts and The London School of Medicine and Dentistry, Queen Mary University of London

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Primary Supervisor

Professor Karim Brohi, Consultant Trauma and Vascular Surgeon

Secondary Supervisor

Dr Ross A Davenport, Consultant Trauma and Vascular Surgeon

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STATEMENT OF ORIGINALITY

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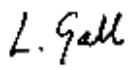

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RESEARCH ASSOCIATED PUBLICATIONS, PRESENTATIONS AND PRIZES

Published Articles

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Published Abstracts

Gall L, Davenport R, Brohi K. Effect of early tranexamic acid on the coagulation system in patients with suspected traumatic haemorrhage: a prospective cohort study. *Lancet* 2016; 387:S46

Gall L, Davenport R, Brohi K. Tranexamic acid is associated with enhanced clot strength, reduced fibrinolysis and reduced acute traumatic coagulopathy in bleeding trauma patients. *Shock* 2015; 44 Suppl 2

Oral Presentations

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THESIS ABSTRACT

Haemorrhage is a leading cause of trauma morbidity and mortality, with many deaths potentially preventable. Hyperfibrinolysis is a central characteristic of trauma-induced coagulopathy (TIC) which develops rapidly and is associated with poor outcomes. Tranexamic acid (TXA) improves survival in trauma haemorrhage but its uptake worldwide remains variable, in part because its effects on the coagulation system during trauma haemorrhage have not been described. Further uncertainty regarding patient selection for TXA therapy has emerged following the description of an early viscoelastic haemostatic assay (VHA) diagnosed hypofibrinolytic phenotype in whom TXA may potentiate thrombotic complications. The patient characteristics and mechanisms leading to this apparent hypofibrinolytic phenotype are poorly understood.

Over 900 trauma patients prospectively recruited to a multicentre observational cohort study had blood drawn within 2-hours of injury for VHA and fibrinolysis plasma protein analysis. Patients were categorised according to VHA maximum lysis (ML) and D-dimer (DD) levels. Patients with ML_{LOW} exhibited heterogeneity in clinical and injury characteristics and outcomes. Those who died were severely injured, with a high incidence of traumatic brain injury and a 7-fold higher D-dimer. Patients with ML_{LOW}+DD_{HIGH} had a hyperfibrinolytic biomarker profile, with the fibrinolytic mediator S100A10 identified as a potential driver of fibrinolysis, which can *ex-vivo* artificially reduce ML. Empiric TXA could benefit this occult hyperfibrinolytic phenotype.

Over two subsequent observational studies, the effects of TXA on the coagulation system during trauma haemorrhage and the effect of TXA infusion and timing of treatment on thrombotic events were investigated. Early empiric TXA avoided VHA-hyperfibrinolysis and provided a degree of protection from TIC. Whilst univariate analysis suggested increased thromboses with later TXA treatment in patients receiving TXA bolus+infusion, neither the TXA infusion nor time to bolus were associated with thrombotic events after multivariate analysis. A single TXA bolus may provide a lower effective therapeutic dose with reduced complications.

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LIST OF APPLIED ABBREVIATIONS

α2AP	α-2-antiplasmin
A5	Clot amplitude 5 minutes after clotting time
AAGBI	Association of Anaesthetists of Great Britain and Ireland
ABC	Avidin-biotin-peroxidase complex
ABG	Arterial blood gas
ACIT II	Activation of Coagulation and Inflammation in Trauma II study
ADP	Adenosine diphosphate
AIIt	Annexin A2 heterotetramer complex
AIS	Abbreviated Injury Score
ANXA2	Annexin A2
APL	Acute Promyelocytic Leukaemia
APTEM	ROTEM assay with fibrinolysis inhibited
APTT	Activated partial thromboplastin time
ASAP	As soon as possible
ATC	Acute Traumatic Coagulopathy
BD	Base deficit
bpm	Beats per minute
CA(x)	Clot amplitude at (X) minutes after clotting time
CFT	Clot Formation Time

CI	Confidence interval
CRASH-2	Clinical randomisation of an antifibrinolytic in significant haemorrhage trial
CRYO	Cryoprecipitate
CT	Clotting time or computerised tomography
CV	Coefficient of variation
DD	D-dimer
DIC	Disseminated intravascular coagulation
DVT	Deep vein thrombosis
EACA	Epsilon aminocaproic acid
ECLT	Euglobulin clot lysis time
ED	Emergency department
EDTA	Ethylenediaminetetraacetic acid
EFI	Enzymatic fibrinolysis index
ELISA	Enzyme-linked immunosorbent assay
EPL	Estimated percent lysis
EXTEM	ROTEM assay measuring activated clotting via the extrinsic pathway
FBC	Full blood count
FFP	Fresh frozen plasma
FFTEG	Functional fibrinogen thromboelastography
FIBTEM	ROTEM assay with platelet function inhibited
FP7	European Union research and innovation funding programme

G&S	Group & screen
GCS	Glasgow Coma Score
HALT-IT	Haemorrhage alleviation with tranexamic acid – intestinal system trial
HEMS	Helicopter emergency medical service
HR	Heart rate
hrs	Hours
INR	International normalised ratio
INTEM	ROTEM assay measuring activated clotting via the intrinsic pathway
INTRN	International Trauma Research Network
IQR	Inter-quartile range
ISS	Injury Severity Score
IV	Intravenous
LAA	London Air Ambulance
LED	Light-emitting diode
LI30	Lysis index 30 minutes after clotting time
LOS	Length of stay
LOT	Lysis onset time
LY30	% decrease in clot amplitude 30 minutes after maximal amplitude
LY60	% decrease in clot amplitude 60 minutes after maximal amplitude
M	Molar (Moles per litre)
MATTERs	Military application of tranexamic acid in trauma emergency resuscitation study

MCF	Maximum clot firmness
MHP	Major haemorrhage protocol
MI	Myocardial infraction
min	Minutes
ML	Maximum lysis
MODS	Multiple organ dysfunction syndrome
MOF	Multi-organ failure
n/a	Data not available/applicable
NEG	Negative
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
OD	Absorbance
OR	Odds ratio
PAI-1	Plasminogen activator inhibitor-1
PAP	Plasmin-α2-antiplasmin complex
PBS	Phosphate-buffered saline
PE	Pulmonary embolism
PLTS	Platelets
POS	Positive
PRBC	Packed red blood cells
Pro-uPA	Pro-enzyme of uPA

PT	Prothrombin time
PT Frag 1+2	Prothrombin fragments 1+2
PTr	Prothrombin time ratio
QMUL	Queen Mary University of London
RBC	Red blood cells
RCT	Randomised control trial
Resus	Resuscitation room
RLH	The Royal London Hospital
RNA	Ribonucleic acid
ROTEM	Rotational thromboelastometry
RT	Room temperature
SBP	Systolic blood pressure
sec	Seconds
sFMC	Soluble fibrin monomer complex
SOFA	Sequential Organ Failure Assessment score
SOP	Standard operating procedure
SpR	Specialist Registrar
TACTIC	Targeted Action for Curing Trauma Induced Coagulopathy
TAFI	Thrombin activatable fibrinolysis inhibitor
TBI	Traumatic Brain Injury
TEG	Thromboelastography

TIC	Trauma-Induced Coagulopathy
TMB	Tetramethylbenzidine
tPA	Tissue-type plasminogen activator
TXA	Tranexamic acid
U&E	Urea & electrolytes
UK CRN	United Kingdom Clinical Research Network
uPA	Urokinase plasminogen activator
uPAR	uPA receptor
VHA	Viscoelastic haemostatic assay
vs	Versus
VTE	Venous thromboembolism
WOMAN	World maternal antifibrinolytic trial

Conventional and SI units and abbreviations for physical quantities, constants and chemical symbols are used throughout.

Chapter 1

INTRODUCTION, THESIS AIMS & HYPOTHESES

1.1 BACKGROUND

Traumatic injuries account for the death of almost 5 million people worldwide each year[1] with this number projected to rise by 40% by the year 2030.[2] Haemorrhage is responsible for 30-40% of deaths following trauma and is the most common cause of early in-hospital mortality within the first few hours following major trauma.[3] Of those patients with potentially survivable injuries, who subsequently die, the majority do so because of haemorrhage.[4] Early coagulopathy exacerbates bleeding and is itself a poor prognostic indicator. Patients with Trauma-induced Coagulopathy (TIC) are eight times more likely to die within the first 24 hours[5] and more likely to require a massive transfusion[6], with increased risk of multi-organ failure (MOF) and longer critical care and hospital stay.[5] A greater understanding of the mechanisms underpinning TIC is key to the development of effective treatment strategies aimed at reducing the number of preventable deaths from haemorrhage.

Fibrinolysis activation occurs almost universally following severe injury and is of central importance to the coagulation dysfunction that develops following trauma.[7] Physiological fibrinolysis is a proportionate response to increased fibrin generation following tissue trauma[8], whereas excessive or systemic fibrinolytic activation is inappropriate and potentially lethal. Acute Traumatic Coagulopathy (ATC)[9] is an early and endogenous haemostatic abnormality of which global hypocoagulation, systemic hyperfibrinolysis[10,11], early fibrinogen depletion and platelet dysfunction[12] are key components. ATC is triggered by massive tissue injury in conjunction with hypoperfusion (shock) in the early phase after major trauma.[10] Hyperfibrinolysis is defined by disproportionately increased fibrinolytic activity with respect to fibrin formation[13], and is

associated with poor clot integrity, excessive bleeding and worse coagulopathy, in addition to increased morbidity and mortality.[3]

Controversy currently exists over patient selection for antifibrinolytic therapy[14] and is a reflection of our limited understanding of both the mechanism and dynamics of fibrinolytic activation after traumatic injury. Hyperfibrinolysis in trauma has yet to be properly defined with many different arbitrary definitions used in the literature (Table 1). Sensitive diagnostics for a rapid and current assessment of the fibrinolytic system are lacking[8] despite the increasing use of viscoelastic haemostatic assays (VHA) (e.g. rotational thromboelastometry (ROTEM; Tem International GmbH, Munich, Germany) and thromboelastography (TEG; Haemonetics, Braintree, USA)) to define fibrinolytic activation states. Correlation of lysis parameters with laboratory gold standards of fibrinolysis - e.g. plasmin-α2-antiplasmin complex (PAP) - levels has proven difficult to interpret, and therefore the clinical sequela of acute hyperfibrinolysis and/or early hypofibrinolysis has yet to be accurately described. In particular, the impact of a highly activated (or inhibited) fibrinolytic system on bleeding risk, organ failure and thrombotic complications is unclear. Understanding the drivers of trauma-induced fibrinolysis, the temporal relationship to injury and resuscitation, as well as optimal treatment strategies are priorities for the trauma research community.

Table 1 Summary of studies of hyperfibrinolysis in trauma

	Year	Definition of hyperfibrinolysis used	Number of patients studied	% patients with hyperfibrinolysis	Mortality - with hyperfibrinolysis (%)	Mortality - without hyperfibrinolysis (%)
ROTEM studies						
Levrat[15]	2008	ECLT < 90 minutes (and MCF ≤ 18 mm)	87	6%	100%	11%
Schochl[16]	2009	EXTEM ML = 100%	33	100%	88%	n/a
Theusinger[17]	2011	EXTEM ML > 15%	(552) [a]	13 patients [b]	77%	33%
Tauber[18]	2011	EXTEM ML > 15%	334	7%	57%	11%
Kutcher[19]	2012	EFI > 10%	115	20%	52%	13%
TEG studies						
Carroll[20]	2009	LY60 > 15%	161	2%	67%	8%
Kashuk[21]	2010	EPL > 15%	61	18%	64%	24%
Cotton[22]	2012	LY30 > 7.5%	1996	2%	76%	10%
Ives[23]	2012	EPL > 15%	118	11%	92%	10%
Chapman[24]	2013	LY30 ≥ 3%	73	15%	64%	18%
Pommerening[25]	2014	LY30 > 3%	1625	11%	18%	10%
Moore[26]	2014	LY30 ≥ 3%	180	18%	44%	n/a
Moore[27]	2016	LY30 ≥ 3%	2540	18%	34%	n/a
Biomarker studies						
Raza[7]	2013	PAP >1500 µg/L AND EXTEM ML < 15% (moderate) PAP >1500 µg/L AND EXTEM ML > 15% (severe)	303	Moderate 57% Severe 5%	Moderate 12.1% Severe 40%	1% [c]
Cardenas[28]	2014	PAP 1500 – 20000 µg/L (moderate) PAP >20000 µg/L (severe)	163	Moderate 45% Severe 10%	Moderate 25% Severe 31%	4.1% [c]

[a] Includes trauma and non-trauma patients presenting to the ED; [b] Denominator for trauma patients not available; [c] No fibrinolysis defined as PAP < 1500 µg/L. ECLT, euglobulin clot lysis time; EPL, estimated percent lysis; EFI, enzymatic fibrinolysis index (EXTEM ML – APTEM ML); LY30, clot lysis 30 minutes after maximal amplitude; ML, maximum lysis 60 minutes after the onset of clot formation; n/a, data not available from original publication

1.2 REGULATION OF FIBRINOLYSIS

The coagulation and fibrinolytic systems are both activated following trauma and exist in a dynamic equilibrium.[7,29] Plasmin is essential for vascular homeostasis, required for the timely breakdown of fibrin clots following coagulation in order to prevent propagation of the clot distant to the site of injury and maintain vascular patency. Plasminogen may be activated either by tissue-type or urinary-type plasminogen activators (tPA and uPA respectively) or by the contact pathway. tPA is a serine protease produced and secreted primarily by pre-capillary arteriole and post-capillary venule endothelial cells[30–32] and is the primary plasminogen activator in the vasculature. In response to vascular injury or the presence of thrombin, additional stores of tPA from endothelial Weibel-Palade bodies[33] and possibly by poorly characterized other small storage granules[34] are released.[35] Both tPA and plasminogen bind to the surface of fibrin which acts as a cofactor for tPA with the net effect being enhanced plasmin generation in the presence of fibrin.[36] In this manner, the fibrinolytic system is self-regulating with fibrin being both degraded by plasmin as well as initiating and accelerating fibrinolysis.

Fibrinolytic activation is tightly controlled by plasminogen activator inhibitor 1 (PAI-1) which principally inhibits tPA, α-2 antiplasmin (α2AP) which inhibits plasmin and thrombin activatable fibrinolysis inhibitor (TAFI) which indirectly limits fibrin breakdown.[36] PAI-1 is found within two distinct pools within the blood - plasma and platelets.[37] Circulating levels of PAI-1 are relatively low in comparison to the rich source within platelet α-granules.[37] Platelet-rich thrombi are resistant to tPA-mediated fibrinolysis[38], however, platelet contribution to plasma levels of PAI-1 and the degree to which platelets modulate trauma-induced hyperfibrinolysis is uncertain. Overall platelet count is typically preserved after injury, although platelet dysfunction contributes to ATC and is associated with increased

mortality.[12,39] Whilst initial studies suggested that only 10% of platelet PAI-1 was in an active configuration[40–42] subsequent research has discovered that platelets actually retain high levels of active PAI-1[43,44] capable of complexing and inactivating the plasminogen activators.

Moore *et al.*[45] demonstrated that platelet lysate mixed with whole blood *ex vivo*, resulted in a faster clotting time and reduced tPA-mediated fibrinolysis measured by TEG in accordance with earlier studies describing the anti-fibrinolytic function of platelet PAI-1.[38,46] Platelets are additionally a source of α2AP[38], TAFI[47] and factor XIII[48], all of which promote clot stabilization. Platelet-mediated fibrinolytic inhibition could in theory explain the improved outcomes associated with early transfusion of high ratios of platelets to red blood cells in patients with traumatic haemorrhage.[49–51] In our study of 161 bleeding trauma patients with serial blood sampling during active haemorrhage for multiple electrode aggregometry (Multiplate) and thromboelastometry (ROTEM)[52], we found platelet transfusions did not appear to influence platelet aggregation but were associated with a significant reduction in EXTEM maximum lysis (ML). Furthermore, platelet transfusions were associated with increased levels of circulating PAI-1 and reduced tPA levels which may represent a primary anti-fibrinolytic mode of action in conferring outcome benefit. Platelets, however, are not purely antifibrinolytic but contain plasminogen, plasminogen activators and a reservoir of plasmin on their surface and therefore have profibrinolytic potential.[53] The net effect of pro and anti-fibrinolytic actions of platelets and their effect in modulating TIC requires further exploration.

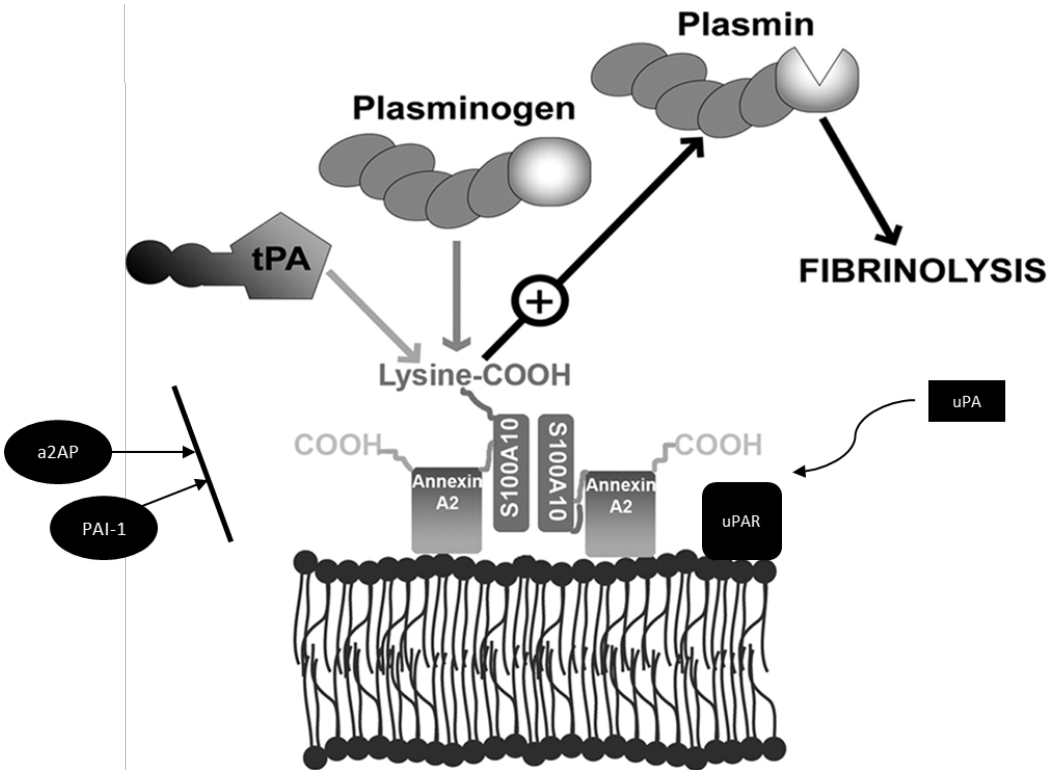


Figure 1 Schematic representation of the AIIt complex on the endothelial cell surface

Adapted from [54]

The vascular endothelium plays an active role in haemostasis, coagulation and fibrinolysis and endothelial surface plasminogen receptors play an important regulatory role. The protein complex annexin A2 heterotetramer (AIIt) is an endothelial cell surface plasminogen receptor composed of two annexin A2 (ANXA2) molecules bound by a dimer of the protein S100A10 positioned in the centre (Figure 1).[55] The role of ANXA2 appears to be to anchor S100A10 to the cell surface membrane via its phospholipid-binding sites. S100A10 then initiates the fibrinolytic process by binding both tPA and plasminogen via its C-terminal lysine residue. S100A10 additionally co-localises plasminogen with the uPA/uPA receptor (uPAR) complex.[56,57] The net effect of bound plasminogen being co-localised with its activators tPA and uPA on the endothelial cell surface is one of significantly increased plasmin formation. ANXA2 and S100A10 proteins subsequently bind plasmin. The binding of tPA and plasmin to the S100A10 subunit protects these proteins from otherwise rapid inactivation by

the circulating inhibitors PAI-1 and a2AP respectively. The plasmin formed by S100A10 additionally partakes in an exponential activation cycle by activating uPA from its pro-enzyme (pro-uPA) to its active form which is then in turn capable of further plasmin generation.[58] The S100A10 protein therefore plays an important role in plasminogen binding and plasmin generation at the endothelial cell surface.

1.3 THE FIBRINOLYTIC SYSTEM IN TRAUMATIC COAGULOPATHY

ATC is an endogenous process, driven by the combination of endothelial hypoperfusion and tissue injury, with hyperfibrinolysis, hypocoagulation, increased thrombin generation and early fibrinogen depletion identified as key components (Figure 2).[10,59] Present in up to 25% of trauma patients, ATC occurs in the first hour after injury, before significant fluid resuscitation or haemodilution has occurred.[9,60] During resuscitation of the bleeding trauma patient, ATC is compounded by ongoing blood loss and treatment strategies which utilise hypocoagulable fluids e.g. crystalloids, with ensuing haemodilution, hypothermia and acidaemia development contributing to a global failure of the coagulation system. Trauma-induced coagulopathy (TIC)[61] describes collectively the innate component of ATC and all subsequent coagulopathies which develop, typically iatrogenic and related to suboptimal fluid resuscitation or delayed haemorrhage control.[62] A temporal switch in coagulation status from the initial hypocoagulability of TIC to a hypercoagulable response occurs over hours to days after major trauma.[63,64] A biphasic response has similarly been described in the fibrinolytic system[65,66] with an initial acceleration of clot lysis lasting for several hours after injury, followed by prolongation of clot lysis lasting between four to eleven days.[67]

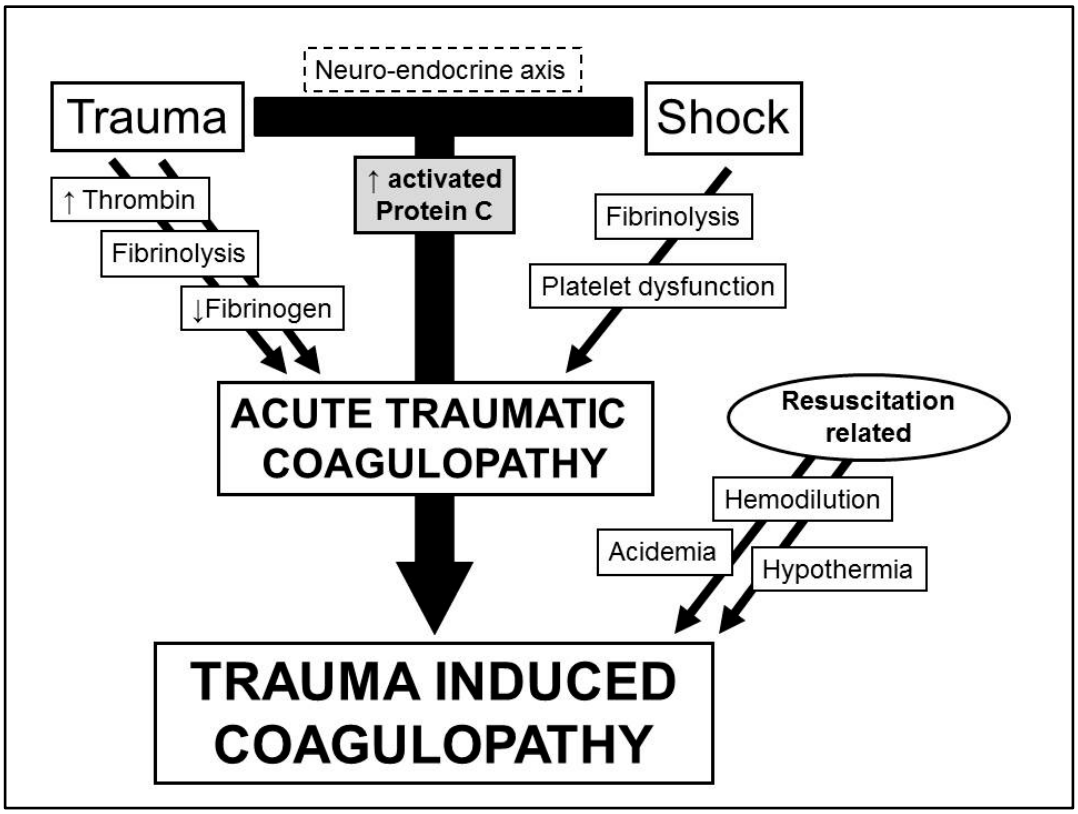


Figure 2 Pathogenesis of trauma-induced coagulopathy [62]

Hypofibrinogenaemia is commonly encountered in bleeding trauma patients, with levels below 2.29 g/L strongly associated with increased mortality.[68] Fibrinogen replacement can *ex vivo* correct coagulopathy[59] with pilot trial data[69] finding a potential outcome benefit for patients treated with early fibrinogen supplementation. Several larger randomised trials are now underway to assess the efficacy of fibrinogen concentrate and cryoprecipitate in the early phase of haemostatic resuscitation[70–72]. The exact mechanism of early fibrinogen loss is not clear but hypothermia and acidosis are known to disrupt fibrinogen metabolism[73] and more recently direct fibrinogenolysis[74,75] has been postulated as a potential mechanism.

Activation of the Protein C (PC) pathway has been identified in both clinical human studies and experimental animal models as a potential mediator of hyperfibrinolysis in ATC.[76–79] Recently published mechanistic data demonstrates an important role for activated protein C (aPC) in driving both systemic fibrinolysis and possibly fibrinogen depletion.[11] In addition to its inhibitory effect on thrombin generation, aPC in excess binds to and neutralizes PAI-1, resulting in de-repression of shock-mediated tPA release from the endothelium leading to uninhibited activation of fibrinolysis.[6,76,80] PAI-1 rather than TAFI appears to exert greater control over the fibrinolytic state after trauma.[7,10] In an experimental model of trauma haemorrhage using transgenic mice with reduced capacity to activate PC, fibrinolysis and fibrinogen depletion were significantly attenuated with relative protection from ATC, confirming a mechanistic role for aPC in hyperfibrinolysis.[11]

Gando and colleagues alternatively consider traumatic coagulopathy to reflect disseminated intravascular coagulation (DIC) with a fibrinolytic phenotype, characterized by activation of the tissue-factor dependent coagulation pathway, insufficient anticoagulant mechanisms and increased fibrinolysis.[81,82] Primary and secondary fibrin(ogen)olysis are considered to be pathologically activated following trauma in response to shock-induced endothelial tPA release[81,83,84] and DIC respectively.[82] Activation of neutrophil elastase-mediated fibrinolytic pathways is additionally believed to contribute to the hyperfibrinolytic state.[85] Subsequently it is proposed that insufficient anticoagulant mechanisms (e.g. low protein C and antithrombin levels) combined with PAI-1-mediated inhibition of fibrinolysis shifts the patient into DIC with a thrombotic phenotype.[81,82,86]

Central to both the aPC and the DIC with thrombotic phenotype hypotheses is the mechanism of shock-induced hyperfibrinolysis. Tissue hypoperfusion in isolation e.g. cardiac

arrest, is capable of initiating hyperfibrinolysis[87,88] and similarly increasing levels of tissue injury will initiate fibrinolysis[10], evidenced on ROTEM by shortened lysis onset time (20% lysis of maximum clot firmness).[87] Sympathoadrenal activation following severe trauma leads to a surge in circulating catecholamines which are hypothesised to contribute to coagulopathy and hyperfibrinolysis by means of endothelial activation and glycocalyx degradation.[89–91] The combination of both tissue injury and haemorrhagic shock in patients suffering major trauma causes a surge in tPA release from the endothelium driving a massive fibrinolytic response.[10,86] Almost 90% of severely injured patients defined as Injury Severity Score (ISS) greater than 15 have plasmin-α2-antiplasmin complex (PAP) levels on admission of at least twice the upper limit of normal.[7] The fibrinolytic response to trauma and shock is a dynamic process which evolves over time and may be exacerbated further by clinical interventions e.g. surgery, resuscitation. The timeframe from injury in which these changes occur, and the specific drivers for any up or down-regulation in the fibrinolytic pathways are not known, in part due to limitations in our ability to measure ongoing fibrinolysis rather than prior activation (e.g. PAP).

1.4 DIAGNOSIS OF HYPERFIBRINOLYSIS IN TRAUMA

Detection of post-injury changes in the fibrinolytic system in a clinically meaningful timeframe (i.e. to guide therapy) is challenging. Diagnostics currently available to quantify fibrinolysis are laboratory measures of fibrin degradation, measurement of individual proteins of the fibrinolytic system and VHA. The Euglobulin Clot Lysis Time (ECLT)[92,93] is a validated assessment of overall fibrinolysis *in vivo,* however, has little clinical utility in trauma due to the prolonged assay time and loss of plasma inhibitors (i.e. antiplasmin) in the acidification process. A further limitation is that ECLT is performed on diluted platelet poor plasma rather than whole blood and it is not capable of assessing the response to

antifibrinolytic therapy since these agents are normally discarded within the supernatant during processing.[94] Excessive fibrin degradation may be indicated by raised D-dimer levels although elevated levels are encountered in most patients following injury[7] and are strongly correlated to injury severity[10]. Kutcher *et al.*[19] found that patients with VHA-detectable hyperfibrinolysis have significantly higher D-dimer levels, but that as a clinical marker on its own, D-dimer was not predictive of hyperfibrinolysis after adjusting for injury severity and shock.

The fibrinolytic response to trauma is characterised by quantification of specific fibrinolytic proteins and complexes which are primarily measured by ELISA.[7,10,28,76,89,95,96] Measurement of tPA and PAP complex levels in combination with fibrinolytic inhibitor levels (PAI-1, α2AP, TAFI) enables a comprehensive, albeit static, assessment of the fibrinolytic system, but is confined to the research setting due to the time it takes to process each assay. A functional, global assay (such as a viscoelastic test or a plasma-based clot lysis assay) may provide better overall evaluation of the status of the fibrinolytic system.[97] Any increase in fibrinolytic activation (PAP>1500 µg/L, twice the upper limit of normal) has been shown to be associated with a 12-fold increase in 28-day mortality and greater transfusion requirements compared to those with 'normal' levels of fibrinolytic activity (PAP < 1500 µg/L).[7] However, measurement of circulating levels of PAP or the downstream D-dimer fragment represents recent plasmin generation (and fibrinolysis) but not necessarily the extent of ongoing fibrinolysis. Development of a point-of-care test capable of rapidly quantifying the extent of fibrinolytic activation e.g. PAP or α2AP may assist in guiding therapy although in isolation would still only represent an assessment of prior fibrinolytic activity. However, in combination with a dynamic assay such as a VHA, both rapid and serial read-outs of fibrinolytic biomarker levels has the potential to better determine the degree of ongoing fibrinolysis.

1.5 ROLE OF VHA IN TRAUMA HAEMORRHAGE & DIAGNOSIS OF HYPERFIBRINOLYSIS

The major advantage of VHAs over other diagnostics is near patient testing and speed, with provision of a comprehensive assessment of clot formation dynamics including fibrinolysis in whole blood (Figure 3). ROTEM and TEG are capable of rapidly diagnosing ATC[20,98,99] and are superior to conventional clotting tests e.g. Prothrombin Time in predicting the need for massive transfusion in trauma.[100] An international panel of trauma researchers has recommended that VHA use should be considered during the early phases of trauma resuscitation and remains the only test capable of diagnosing hyperfibrinolysis in a clinically relevant timeframe.[101] However, the latest National Institute for Health and Care Excellence (NICE) guideline on the management of major trauma[102] concludes that there is currently insufficient evidence to support the superiority of VHA over standard laboratory coagulation tests to target treatment. A recent Cochrane systematic review has suggested that at present in the setting of trauma, VHA should only be used for research purposes.[103] Whilst VHA can provide results rapidly, there is a need for randomised clinical trial data to ascertain any superiority over standard laboratory tests as a tool to guide trauma resuscitation.[104,105] For these reasons European and UK guidelines[102,106] currently recommend early empiric treatment of hyperfibrinolysis in the bleeding trauma patient rather than waiting for VHA-confirmation of increased fibrinolysis.

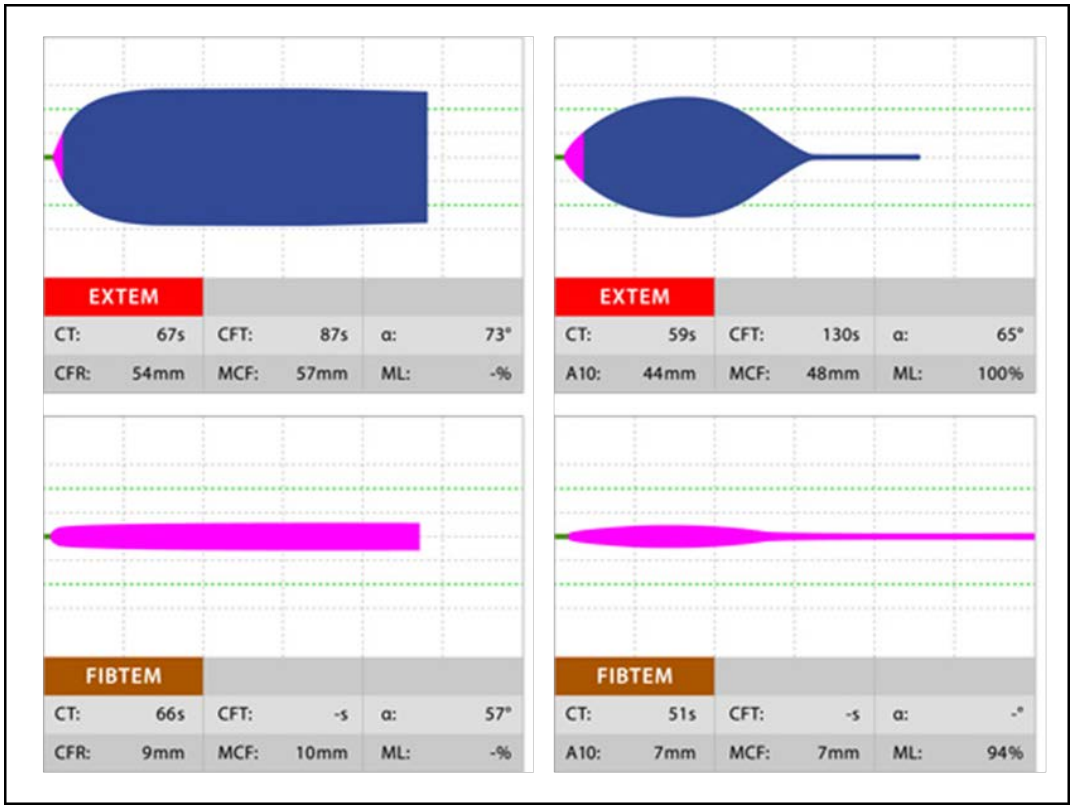


Figure 3 ROTEM graphical output for the EXTEM and FIBTEM assays showing normal coagulation (left) and hyperfibrinolysis (right).

Adapted from [107]

Extrapolation of the incidence and outcomes associated with hyperfibrinolysis in trauma from the literature is confounded by a number of methodological issues: (1) inconsistency in threshold definitions for VHA detected hyperfibrinolysis; (2) lack of standardization of VHAs with consequent lab-to-lab variation; (3) wide variation in patient populations; (4) discrepancies in sampling protocols that vary between minutes of injury to 12 hours; and (5) a lack of clarity between VHA versus biomarker diagnosed hyperfibrinolysis.

VHA detected hyperfibrinolysis is reported in 2 – 20% of trauma patients presenting to the Emergency Department (ED) and is associated with mortality rates up to 100% (Table 1).[15–23] Hyperfibrinolysis is currently defined by ROTEM as a reduction in maximum clot firmness

(MCF) of greater than 15% (ML > 15%), 60 minutes after the onset of clot formation. The continuous ROTEM variable of lysis onset time (LOT) may detect severe hyperfibrinolysis faster[87,108], although this has not been validated in trauma patients. Three distinct temporal patterns of ROTEM-detected hyperfibrinolysis have been described: (1) fulminant lysis described as complete clot lysis (EXTEM ML of 100%) within 30 minutes and associated with the highest mortality; (2) intermediate lysis as that occurring between 30 and 60 minutes; and (3) late lysis occurring beyond 60 minutes.[16] Defining TEG hyperfibrinolysis according to the manufacturer's recommendation of clot lysis exceeding 7.5%, 30 minutes after maximum clot amplitude (LY30 > 7.5%), Cotton *et al*.[22] reported hyperfibrinolysis to be uncommon but highly lethal. Chapman *et al.*[24] subsequently found the lower threshold of 3% to be superior at diagnosing clinically relevant hyperfibrinolysis and predicting both massive transfusion and mortality. Consequently, TEG hyperfibrinolysis is now widely defined as LY30 ≥ 3%.[25,27,109]

1.5.1 Is VHA-diagnosed fibrinolysis an accurate reflection of the status of the fibrinolytic system?

VHAs accurately identify patients with the highest degree of fibrinolysis, but appears relatively insensitive at detecting lower levels of fibrinolytic activation[7], through rapid inhibition of free tPA by PAI-1 following blood draw.[110] Using a composite measure of PAP and VHA on admission to ED, Raza *et al*.[7] have shown fibrinolytic activation following severe trauma to be extremely common. Patients without VHA hyperfibrinolysis (ML < 15%) were categorized as 'normal' fibrinolytic activity if PAP < 1500 µg/L and 'moderate' fibrinolytic activity if PAP >1500 µg/L. Patients with PAP > 1500 µg/L combined with VHA hyperfibrinolysis (ML > 15%) were classified as 'severe'. Whilst only 5% of patients were classified as 'severe', the largest proportion of patients (57%) had 'moderate' fibrinolytic

activation which was not detected by ROTEM. PAP levels were closely related to injury severity, with approximately 90% of patients with ISS>15 demonstrating biomarker confirmed hyperfibrinolysis. In a similar study utilizing PAP and TEG, Cardenas *et al.*[28] found that 45% of patients had PAP>1500 μg/L with an associated 6-fold increase in overall mortality despite there being no evidence of hyperfibrinolysis on TEG (median LY30 1.1 % (0.2 – 2.4) (Table 1).

Differences in ROTEM and TEG methodology and the reagents used (Table 2) alters the sensitivity to fibrinolysis across platforms and individual assays. Harr *et al*.[111] reported that functional fibrinogen TEG (FFTEG) and FIBTEM were comparable in their ability to detect fibrinolysis faster than the other VHA assays. KaolinTEG appears superior in its ability to detect fibrinolysis in a dose-dependent manner across various tPA concentrations whereas RapidTEG detects lysis at high concentrations of tPA only. The more powerful clot activation required to generate faster results by RapidTEG, results in a clot more resistant to tPA-induced fibrinolysis which may result in under-diagnosis of hyperfibrinolysis. VHA and biomarkers of fibrinolysis by definition do not measure the same entity, PAP reflects prior activation of fibrinolysis *in vivo* whereas VHAs quantify coagulation and to some extent fibrinolytic potential. Clarification of the status of the fibrinolytic system and therapeutic requirements of a bleeding trauma patient with grossly elevated PAP or D-dimer levels, but who does not meet the diagnostic threshold for VHA hyperfibrinolysis is clearly a research imperative.

Table 2 Commonly employed VHA assays to measure coagulation and fibrinolysis

VHA Platform	Assay	Reagents used	Description
TEG	KaolinTEG	Re-calcified with calcium chloride and activated with Kaolin	Assessment of clot formation, fibrin polymerisation and fibrinolysis via the intrinsic pathway
TEG	RapidTEG	Re-calcified then activated with Kaolin and tissue factor (RapidTEG Reagent)	Extrinsic pathway assessment of clot formation, fibrin polymerisation and fibrinolysis with faster results than Kaolin TEG.
TEG	Functional Fibrinogen TEG	Re-calcified then activated with lyophilized tissue factor and a platelet inhibitor that binds to glycoprotein-IIb/IIIa receptors (Functional Fibrinogen Reagent)	Assessment of the fibrinogen contribution to clot formation after blocking platelets
ROTEM	EXTEM	Re-calcified with calcium chloride (star-tem) and activated with thromboplastin (tissue factor) derived from rabbit brain (ex-tem)	Assessment of clot formation, fibrin polymerisation and fibrinolysis via the extrinsic pathway
ROTEM	FIBTEM	Re-calcified and platelets inhibited with cytochalasin D (fib-tem) and activated with ex-tem	Assessment of the fibrinogen contribution to clot formation after blocking platelets
ROTEM	APTEM	Re-calcified and fibrinolysis inhibited with aprotinin (ap-tem) and activated with ex-tem	Assessment of clot firmness after blocking hyperfibrinolysis with aprotinin

The circumstances required for VHA to detect 'severe' hyperfibrinolysis have yet to be confirmed. In one study tPA levels of nearly five times normal and α2AP levels below 75% of normal were shown to be required before ROTEM hyperfibrinolysis was visualised.[7] The authors hypothesise that free tPA within the ROTEM cup is required to generate plasmin and that only in the presence of low antiplasmin levels is there reduced inhibition of newly formed plasmin, resulting in ROTEM-detectable hyperfibrinolysis. Platelet dysfunction may additionally influence the ability of VHA to detect hyperfibrinolysis since impairment of ADP-induced platelet activation following trauma is associated with increased sensitivity to tPA-mediated fibrinolysis.[112] Alternatively the pattern of biomarker positive fibrinolysis with negative VHA lysis may represent prior excessive lytic activity which has rapidly reverted to normal or hypofibrinolysis during the early phase response to trauma. A further explanation may lie in the relative availability of promoters or inhibitors of fibrinolysis within the VHA with respect to thrombin generation potential since both clot strength and lysis are products of one another.

1.5.2 VHA-diagnosed hypofibrinolysis

At the opposite end of the spectrum, it is similarly unclear what the implications of low VHA fibrinolysis are for the trauma patient. Whilst the upper boundary for 'normal' VHA fibrinolysis (ML ≤ 15%[113] or LY30 < 3%[24]) is commonly quoted, no lower boundary has been reported and it has recently been suggested that patients with VHA hypofibrinolysis have worse clinical outcomes.[26,27] The concept of "fibrinolytic shutdown" based on VHA results was described by Moore *et al*.[26] in 2014. In a study of 180 patients with an ISS ≥15, patients were categorised into 3 groups according to citrated Kaolin TEG clot lysis (Ly30) assayed from blood obtained within the first 12 hours from injury. Fibrinolysis was categorised as hyperfibrinolysis (Ly30 ≥ 3%), physiological fibrinolysis (Ly30 0.81 – 2.9%) and

shutdown (Ly30 0 – 0.8%). The threshold value of Ly30 0.8% to define the upper limit of the shutdown group was selected as the point with the greatest specificity and sensitivity for mortality based on a receiver operating characteristic curve using patients with Ly30 <3%. The distribution of fibrinolysis was described as: 64% shutdown, 18% physiological and 18% hyperfibrinolysis (Figure 4). Mortality rates were found to have a 'U' shaped distribution, being lowest in the physiological group (5%) compared to the shutdown (26%) and hyperfibrinolytic groups (44%). One of the criticisms of this initial work was the fact that blood samples were taken anytime up to twelve hours from injury during which time temporal changes to the coagulation and fibrinolytic systems occur.

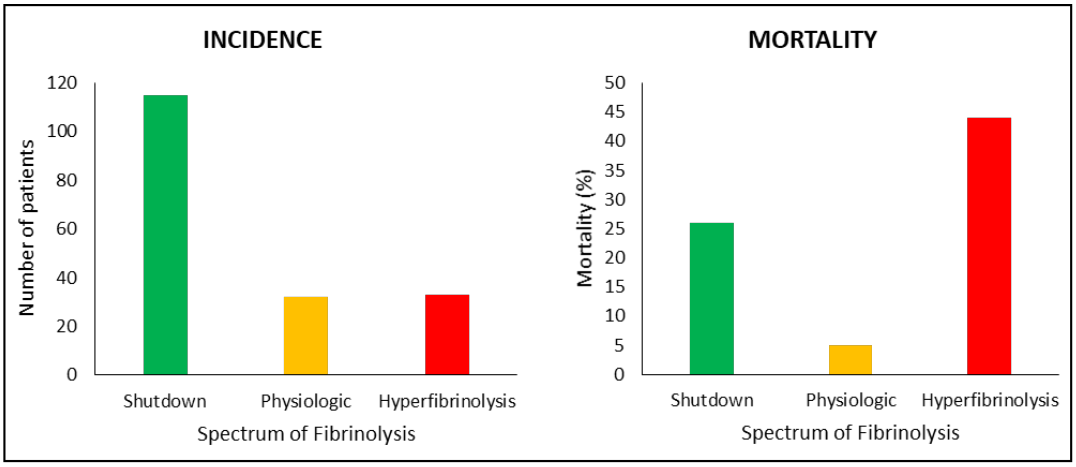


Figure 4 Incidence and associated mortality rates of the three reported fibrinolytic phenotypes

Figure adapted from Moore et al.[26] who studied 180 severely injured trauma patients (ISS ≥ 15).

In a subsequent multicentre study of 2,540 trauma patients[27], with rapid TEG performed on blood drawn within 60 minutes of injury, a similar distribution of patients between the three fibrinolytic phenotypes was described. The mortality rates were similarly high in the shutdown and hyperfibrinolytic phenotypes, with the risk of mortality found to be independent of injury and patient characteristics. The clinical implication of fibrinolytic

shutdown as described by the authors of this work is that further inhibition of fibrinolysis with anti-fibrinolytics in this subgroup of patients would potentially have an adverse effect on survival.[14,114] However, debate exists amongst trauma researchers with suggestion that there is a need for randomised trial data to investigate outcomes for patients with fibrinolytic shutdown who receive antifibrinolytics.[115]

Further investigation is required to phenotype VHA hypofibrinolysis to understand whether all patients with this entity are the same. In particular, what biomarker patterns are associated with VHA hypofibrinolysis, the mechanisms that drive low fibrinolytic activity, the temporal relationship with injury, shock and resuscitation as well as clinical sequela e.g. mortality, VTE, organ failure. Improvements in the sensitivity of existing VHAs or development of diagnostic tools with greater definition to identify and characterise trauma patients with hyper or hypofibrinolysis are urgently required.

1.6 WHO SHOULD RECEIVE ANTIFIBRINOLYTIC TREATMENT?

Treatment of hyperfibrinolysis provides an opportunity to improve upon the poor outcomes associated with TIC, but given the lack of a validated diagnostic tool in trauma haemorrhage, selecting who to treat requires an evidence-based clinical decision. The CRASH-2 trial randomized 20,211 injured patients to receive an antifibrinolytic or placebo based on pragmatic inclusion of all adult trauma patients who were bleeding or were suspected to be bleeding.[116] Patients who received empiric dosing of tranexamic acid (TXA) had a lower overall mortality (14.5% vs 16%) and a lower risk of death due to bleeding (4.9% vs 5.7%). Subgroup analysis from CRASH-2 found the greatest survival benefit to be in those patients with a systolic blood pressure less than 75 mmHg[116], and was confirmed in a single centre

retrospective UK study[117] which additionally reported reduced MOF in shocked patients who received TXA. Similarly, antifibrinolytic therapy administered empirically to military casualties with combat associated traumatic haemorrhage was associated with lower in-hospital mortality.[118] Again, the greatest benefit was observed in patients requiring a massive transfusion with TXA independently associated with survival. Given the current lack of evidence regarding the diagnostic accuracy of VHA[103] and in light of the results from the CRASH-2 trial[116], the evidence supports an empiric based approach to antifibrinolytic therapy in suspected trauma haemorrhage. Some clinicians choose to withhold antifibrinolytic treatment until VHA hyperfibrinolysis is detected due to concern over potentiating thrombotic complications, although this approach is not evidence-based practice.

1.7 HOW SHOULD HYPERFIBRINOLYSIS BE TREATED?

The primary method of targeted reversal of hyperfibrinolysis in trauma is currently with the antifibrinolytic TXA. Important questions remain, however, over the optimal dosing regime, timing, which patient subgroup derives most benefit and later thrombotic events. Early haemorrhage control and reversal of shock may in theory attenuate fibrinolytic activation through improved endothelial oxygenation and reduced tPA generation. Damage control resuscitation with a balanced transfusion strategy including early fresh frozen plasma[119] (a source of α2AP) and platelets may further dampen fibrinolytic activation[52] through increased PAI-1 delivery.

1.7.1 Evidence for the use of Tranexamic Acid in trauma

TXA (trans-4-aminomethylcyclohexane-1-carboxylic acid) (Figure 5) is a synthetic derivative of the amino acid lysine. It exerts an antifibrinolytic effect by competitively blocking the lysine binding sites on plasminogen, thereby preventing the interaction of plasmin(ogen) with fibrin[120] and at higher concentrations is a non-competitive inhibitor of plasmin.[121,122] First described over five decades ago[121,123] TXA has found widespread global clinical application in part due to it being readily available, cheap and having a proven safety profile. It is used routinely in the elective surgical setting, including gynaecological, orthopaedic, cardiac and liver transplant surgery where it has been shown to reduce blood loss and the need for blood transfusion without increased thromboembolic events.[124,125] TXA is considered a relatively old pharmacological agent although is currently being evaluated in international clinical trials of traumatic intracranial bleeding (Clinical Randomization of an Antifibrinolytic in Significant Head Injury; CRASH-3)[126] and non-traumatic gastrointestinal haemorrhage (Haemorrhage Alleviation with Tranexamic acid – Intestinal system; HALT-IT).[127] The randomised controlled WOMAN trial (World Maternal Antifibrinolytic Trial) has recently reported a reduced risk of death due to bleeding from postpartum haemorrhage in women treated with TXA.[128]

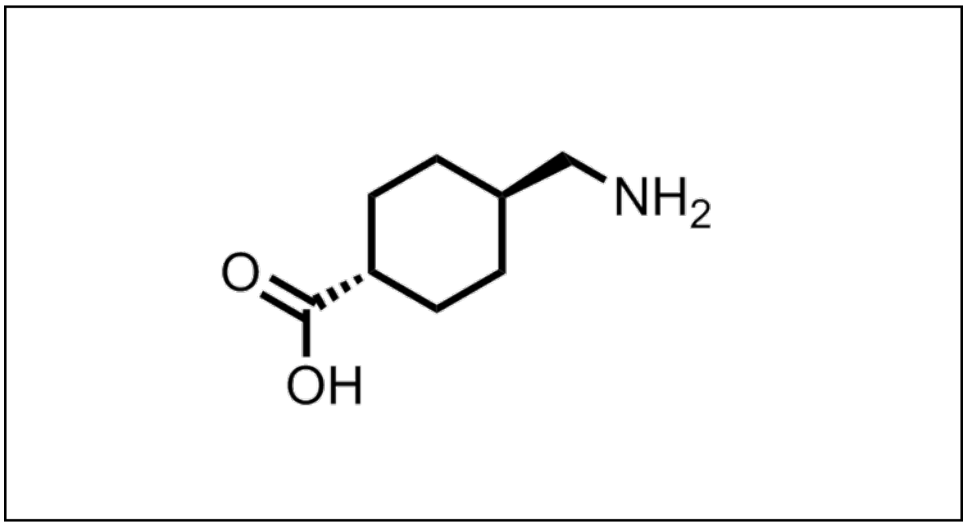


Figure 5 The molecular structure of tranexamic acid

A synthetic amino acid TXA consists of a cyclohexane core, an amine (NH2) and carboxylic acid (CO2H).

The seminal study of TXA use in trauma haemorrhage (CRASH-2) was the first trial to demonstrate improved survival from bleeding with an antifibrinolytic. TXA was administered as a 1g bolus over 10 minutes followed by a second 1g infusion over eight hours. The beneficial effects of early TXA therapy (bolus dose within 3 hours) in reducing all-cause mortality and death due to bleeding did not vary significantly by baseline risk of death.[129] TXA can therefore be administered safely to all patients with traumatic bleeding with no evidence to suggest it should be reserved only for high risk patients with the most severe haemorrhage.[130] Performed in 40 countries, the CRASH-2 results did not identify any effect of geographical location on the efficacy of TXA on reducing death from bleeding.[131] In fact, countries with the most advanced healthcare systems appeared to derive the greatest relative risk reduction. Haemorrhage is the leading cause of preventable death globally from trauma and empiric use of TXA within three hours of injury is likely to save many lives[131] and be highly cost-effective.[132,133]

TXA for the management of haemorrhage following combat injury and was evaluated in the retrospective MATTERs[118] and MATTERs II studies.[134] In study of 896 combat casualties, the MATTERs study concluded that patients receiving TXA (n=293) had a significantly lower overall in-hospital mortality (17.4% vs 23.9%). However, a potential confounding factor is that the TXA cohort received a greater volume of cryoprecipitate. In order to specifically address this, the MATTERs II study examined 1332 patients to investigate the effect of TXA and cryoprecipitate on survival. In-hospital mortality was highest in patients who received neither TXA nor cryoprecipitate (23.6%) and was lowest in patients who received both TXA and cryoprecipitate (11.6%). The individual benefit of TXA and cryoprecipitate therapy was similar; both associated with an odds ratio (OR) of 0.61 and 95% CIs of 0.42 to 0.89 and 0.40 to 0.94 respectively. Combined TXA and cryoprecipitate therapy had an additive rather than a synergistic effect with an OR of 0.34 (95% CI, 0.20 – 0.58). In a recently published multicenter observational study, treatment with TXA and transfusion of high ratios of platelets or plasma to red blood cells (RBC) were independently associated with increased survival and decreased need for massive transfusion.[135]

Based upon this body of evidence, NICE[102], the Cochrane Collaboration[136], the Association of Anaesthetists of Great Britain and Ireland (AAGBI)[137] and the European "STOP the Bleeding Campaign"[106,138] recommend that empiric intravenous TXA be given to all trauma patients with active or suspected active haemorrhage as soon as possible and within three hours of injury. In order to achieve early administration, ideally within the first hour, it is recommended that procedures be in place for delivery of the first dose of TXA pre-hospital at the scene of injury.[106]

The survival benefit from antifibrinolytic therapy is greatest when it is administered early, within the first hour following trauma.[139] In a recent meta-analysis of data from both the CRASH-2 trial and the WOMAN trial, which investigated the effect of TXA in post-partum haemorrhage, the beneficial survival effect of TXA was found to reduce by 10% for every 15 minute delay in instigating TXA treatment.[140] Beyond three hours, no survival benefit was seen. Whether patients with confirmed (biomarker or VHA diagnosed) hyperfibrinolysis derive additional benefit is not known. Furthermore the precise mechanism by which TXA confers survival benefit is unknown with some evidence to suggest it has anti-inflammatory action[117,141,142] in addition to its primary anti-fibrinolytic effects. As a result the relative efficacy of TXA on early bleeding vs late deaths from MOF and sepsis is unclear. Paradoxically, late administration beyond three hours in the CRASH-2 trial was associated with increased risk of death due to bleeding. Possible explanations are that late delivery reflects poorer outcomes associated with delayed trauma care or is secondary to PAI-1-mediated suppression of fibrinolysis with resultant microvascular thromboses.[81] If hyperfibrinolysis transitions rapidly into a hypofibrinolytic state then further blockade of fibrinolysis with delayed antifibrinolytic therapy has the potential to be harmful although the effects of TXA, or other agents on hypofibrinolysis have yet to be characterized. Recently, a novel hypothesis based on data from a murine model of severe Traumatic Brain Injury (TBI) has been proposed.[143] Whilst both tPA and uPA levels in the brain increased following injury, they did so at different rates, with tPA peaking soon after TBI (within three hours) and uPA demonstrating a delayed peak after approximately eight hours. TXA blocks tPA-mediated fibrinolysis, however it actually enhances uPA-mediated fibrinolysis.[144] The delayed and protracted rise in uPA following injury combined with the ability of TXA to enhance uPA-mediated fibrinolysis provides a potential mechanism for the paradox of increased haemorrhage-related mortality with delayed therapy. Further research to evaluate the importance of uPA-mediated fibrinolysis in non-TBI related trauma is required along with the

potential role of therapeutics capable of attenuating both tPA and uPA in bleeding after major injury.

1.7.2 Alternative antifibrinolytic therapy in trauma

The alternate antifibrinolytic agents aprotinin and epsilon aminocaproic acid (EACA) have some unfavourable properties compared to TXA and hence were not selected for clinical trial evaluation in trauma haemorrhage. Compared with TXA, the synthetic lysine analogue EACA is ten times less potent[145] and has not been shown to be associated with reduced transfusion requirements in elective surgery.[146] Aprotinin was withdrawn from the market after it was found to be associated with increased mortality in a randomized trial of patients undergoing cardiac surgery.[147] However, due to methodological deficiencies with this study the conclusions were called into question and the European Medicines Agency have since lifted the suspension.[148] Aprotinin is a potent, long acting antifibrinolytic and future clinical trials should be considered to determine the efficacy in trauma haemorrhage as well as any additional benefits over TXA. In the search for an ideal antifibrinolytic to treat hyperfibrinolysis, a greater understanding of the pathophysiology of fibrinolytic pathways in trauma is required to determine optimal pharmacodynamics and how best to monitor the effect of any drug on fibrinolytic activity.

1.7.3 Benefits of TXA beyond haemostasis

There is a growing appreciation of the interactions between the coagulation, fibrinolytic and immune systems in response to trauma. Plasmin not only degrades fibrin but can additionally activate inflammatory cells such as monocytes, macrophages and dendritic cells, and stimulate the production of cytokines and free radical species, highlighting the

importance of the fibrinolytic system in inflammation, immune response and elimination of infections.[149]

Trauma patients who survive the initial insult of traumatic haemorrhage frequently develop multiple organ dysfunction syndrome (MODS) and sepsis as a consequence of the overwhelming systemic inflammatory response and suppression of the body's ability to fight infections.[150,151] In a prospective cohort study of severely injured patients, empiric administration of TXA was associated with a reduction in MODS and all-cause mortality in shocked patients.[117] The survival benefit and reduction in myocardial infarction rates observed in the CRASH-2 trial may be partly attributable to an anti-inflammatory effect of TXA. Measurement of gene expression in patients undergoing cardiac surgery has shown that antifibrinolytics are associated with less upregulation of pro-inflammatory and more upregulation of anti-inflammatory genes.[152] In an animal model of traumatic haemorrhage, TXA has been shown to attenuate the loss of lung barrier function with the result being reduced levels of inflammatory proteins associated with acute lung injury measured in the lungs of rats resuscitated with TXA.[153]. Future studies to understand the knock-on effect that early massive fibrinolytic activation has on the immune system are additionally warranted and could lead to the development of new therapeutics capable of attenuating both the acute fibrinolytic and inflammatory responses to trauma.

1.8 THROMBOTIC RISK OF ANTIFIBRINOLYTIC THERAPY IN TRAUMA

A principal concern with the use of antifibrinolytics is potentiation of a prothrombotic state, either immediately after trauma during increased thrombin generation, or during the acute phase of recovery from major injury. Without thromboprophylaxis, multi-trauma patients

have a baseline risk of hospital-acquired venous thromboembolism (VTE) exceeding 50%[154], with increasing age an important clinical predictor.[155] As trauma care advances, more patients survive beyond the initial 24 hours from injury and consequently more patients will be at risk of VTE. Some authors are concerned VTE rates are influenced directly by antifibrinolytic therapy[14]; however, TXA has been shown to improve survival and is often administered to those at greatest risk of VTE e.g. major trauma, shock, critical care utilization and invasive procedures. In the surgical setting, a recent meta-analysis concluded that the risk of thromboembolic events with antifibrinolytic use was uncertain.[125] In a retrospective cohort study of 872,416 patients undergoing total hip or knee arthroplasty in the United States, antifibrinolytic therapy was associated with lower rates of blood transfusion without any increase in VTE.[156] CRASH-2 represents the largest randomized trial to evaluate antifibrinolytic use in trauma patients and found no increase in clinically significant vascular occlusive events with TXA compared to placebo (1.7% vs. 2.0%). In fact, patients who received TXA had a lower incidence of myocardial infarction post-injury.[116] Whilst the MATTERs study reported higher unadjusted rates of VTE in patients receiving an antifibrinolytic (TXA vs. No TXA: PE, 2.7% vs. 0.3% and DVT, 2.4% vs. 0.2%), the difference was nonsignificant on multivariate analysis and the investigators attributed the difference to the higher injury burden and degree of shock in the TXA group.[118]

In order to reduce the incidence of VTE in this inherently high-risk group of patients, rather than avoiding early antifibrinolytic therapy, future research should focus on the role of proactive targeted thromboprophylaxis. Development of new methods to monitor fibrinolytic status as the trauma patient transitions from hyperfibrinolysis to a hypofibrinolytic state would permit earlier insertion of retrievable vena cava filters, or use of higher prophylactic doses of anticoagulants. Additionally the role of antiplatelet therapy in prevention of post-traumatic thrombotic events needs greater clarification.

1.9 CONCLUSION

Fibrinolytic activation within the limitations of current assays is presumed almost universal following trauma. Assessing hyperfibrinolysis through biomarker assays (e.g. PAP) remains the gold standard. Rapid evaluation of current or active fibrinolysis remains a challenge and whilst the relative insensitivities of current VHA platforms are acknowledged, they remain the only way to assess fibrinolysis in a clinically relevant time-frame. Current evidence-based guidelines recommend that all bleeding trauma patients (both suspected and confirmed), those that require immediate blood transfusion and all severely injured patients with evidence of haemorrhagic shock receive early empiric antifibrinolytic therapy in the form of TXA.

However, the mechanism by which TXA exerts its beneficial effects on patient outcome has not yet been described. Patients presenting with different fibrinolytic phenotypes e.g. VHA-hypofibrinolysis have still to be fully characterised and the mechanism leading to this pattern of fibrinolysis has yet to be elucidated. These gaps in our knowledge combined with uncertainty surrounding the optimal dosing regimen and timing of TXA treatment in trauma and potential for increased thrombotic events have led to variations in clinical practice globally.

1.10 THESIS AIMS & HYPOTHESES

1. Determine the characteristics of trauma patients with low levels of fibrinolysis as detected by viscoelastic haemostatic assay (VHA) and explore the underlying mechanisms of this subtype.

Hypothesis 1a	Patients with VHA-hypofibrinolysis represent a heterogeneous group of patients and not all low levels of fibrinolysis (as detected by VHA) are pathological
Hypothesis 1b	Patients with VHA-hypofibrinolysis and poor clinical outcomes can be separately identified from those with good outcomes based upon injury and clinical characteristics
Hypothesis 1c	Patients with VHA-hypofibrinolysis and poor clinical outcomes have a non-tPA mediated hyperfibrinolytic biomarker profile.

2. Investigate the effect of early tranexamic acid (TXA) on the coagulation and fibrinolytic systems in patients with traumatic haemorrhage.

Hypothesis 2a	Early TXA induces functional changes on the coagulation and fibrinolytic systems which are detectable by VHA
Hypothesis 2b	Early TXA rapidly arrests fibrinolysis according to plasma protein markers but does not induce an early prothrombotic state

3. Investigate the effect of the TXA infusion and time from injury to TXA treatment on clinical outcomes including thrombotic events

Hypothesis 3a	A single bolus dose of TXA is sufficient to avoid VHA-hyperfibrinolysis during trauma haemorrhage
Hypothesis 3b	Exposure to the TXA infusion is associated with increased incidence of thrombotic events
Hypothesis 3c	Prolonged time to administration of TXA treatment is associated with increased incidence of thrombotic events

Chapter 2

METHODS

2.1 STUDY OVERVIEW

This thesis forms part of a larger ongoing research programme aiming to investigate and better understand the coagulation and immune system changes that occur in the human body in response to traumatic injury. The prospective observational study titled "Activation of Coagulation and Inflammation in Trauma II" (ACIT II; UK CRN ID 5637) provided the ethical framework by which trauma patients were recruited and data obtained for analysis. The ACIT II study was devised by Dr Ross Davenport and Professor Karim Brohi and first received ethical approval in November 2007 from East London and The City Research Ethics Committee (07/Q0603/29) to recruit 500 patients. The study began in 2008 at The Royal London Hospital (RLH), London, with subsequent expansion of recruitment to participating trauma centres across Europe and the United States of America. The participating centres are all members of the International Trauma Research Network (INTRN). In 2013, the ACIT II ethical approval was extended to permit recruitment of 5000 patients across all participating research sites. Six European INTRN partner sites in Amsterdam, Copenhagen, Cologne, Oslo, Oxford and the RLH are currently participating in a European Commission funded project titled "Targeted Action for Curing Trauma Induced Coagulopathy" (TACTIC) under the FP7 HEALTH-Contract No. F3-2013-602771.

2.2 STUDY DESIGN

A prospective observational cohort study of trauma patients admitted to RLH was conducted (Chapters 4 and 5). ACIT II data from patients recruited at five out of the six sites (excluding Oxford) participating in the TACTIC project was analysed in Chapter 3. The Author partook in all aspects of patient screening, recruitment to ACIT II and follow-up as a member of the research team at the RLH between August 2013 and July 2016. The Author was the lead research fellow for the ACIT II study during this time. Between four to eight research fellows

worked together on a shift-basis to cover research recruitment to ACIT II seven days a week. Recruitment hours were between 08:00 to 22:00, extending to 24-hour recruitment from January 2016 onwards. Patients triggering the trauma team activation were screened for eligibility in the resuscitation room (Resus) within the Emergency Department (ED), recruited and followed up until discharge or day 28 of admission.

2.2.1 ACIT II inclusion criteria

All trauma patients triggering a trauma team activation were eligible for inclusion in ACIT II. The trauma team are activated based upon physiological, anatomical and mechanism of injury criteria either pre-hospital or on arrival of the patient in the ED (Table 3). A three-tier trauma team response for adult trauma is in operation at RLH (Table 4). Patients activating the Code Red Trauma Team were of particular interest in order to study fibrinolytic activity and the effects of tranexamic acid during trauma haemorrhage. The RLH major haemorrhage protocol ("Code Red"; Appendix 2) was activated when a patient with suspected traumatic haemorrhage had a systolic blood pressure below 90 mmHg and a poor response to initial fluid resuscitation.

Table 3 Criteria for trauma team activation at The Royal London Hospital

Physiological	Respiratory rate <10 or >29 breaths per minute
	Systolic blood pressure < 90 mmHg
	Glasgow Coma Scale < 14
Anatomical	Chest trauma with altered physiology
	Amputation proximal to wrist or ankle
	Suspected pelvic fracture
	Suspected open or depressed skull fracture
Mechanism	Person hit by train
	Occupant ejected from vehicle
	Fatality in same vehicle as occupant
	Person trapped under vehicle
	Fall from > 2 meters
	Polytrauma with burns
	Penetrating trauma (neck to groin or proximal to elbow / knee)
	Explosions
	Industrial accidents

Table 4 The Royal London Hospital "three-tier trauma team" response

Category	Team	Criteria for activation
Tier 1	Trauma Team	In the absence of anatomical or physiological injury
Tier 2	Advanced Trauma Team	With evidence of anatomical or physiological injury
Tier 3	Code Red Trauma Team	Presence of physiological instability in patients requiring (or likely to require) activation of the massive haemorrhage protocol and damage control surgery or radiological intervention

2.2.2 ACIT II exclusion criteria

- Age < 16 years
- Patients transferred from another hospital
- Patients presenting more than 120 minutes after time of injury
- Patients who have received > 2000mls intravenous fluid prior to ED arrival
- Patients with burns > 5% of their total body surface area
- Patients taking anticoagulant medication (except aspirin)
- Patients with a known bleeding diathesis
- Patients with moderate to severe liver disease (Child's classification B or C)

These exclusion criteria were determined when the ACIT II study was first designed in 2007. The study aims to investigate the trauma patient's early response to traumatic injury and as such those arriving in hospital greater than two hours from injury or those who may have received prior interventions at another hospital are excluded. Those receiving large volumes of crystalloid pre-hospital are excluded as they are likely to have coagulopathy due to dilution. Similarly, the specific high volumes of fluids required by burns patients means they are also at risk of dilutional coagulopathy and are excluded from this work. In an attempt to study those patients developing TIC only, those with a known bleeding diathesis whether inherited, secondary to liver cirrhosis or medication-induced, were excluded.

2.2.3 Consent process

The ACIT II study is designed to examine the immediate coagulation and immune response to trauma. As such, patients must be screened, recruited and initial blood samples obtained at the earliest opportunity after arriving in the ED (within 20 minutes). Most patients (but potentially not all) are incapacitated at the time of ED arrival (e.g. due to critical injury, pain,

sedation or mechanical ventilation). Due to the unplanned nature of trauma, the patient's next of kin are unlikely to be present at the time of ED arrival and if they are present, are likely to be worried and distressed. Discussing a research study in full at this stage would be inappropriate. The standard Good Clinical Practice guidelines for informed consent whereby a study would be discussed with a patient or their next of kin in advance and they are given an opportunity to ask questions and allowed a period of time to consider their decision to partake or not, is not compatible with this type of time-critical research. Therefore, in accordance with the Mental Capacity Act (2005) a hierarchy of consent was used to enable recruitment of trauma patients unable to provide personal consent prior to collection of the initial blood sample.

Initial agreement to enrol a patient in ACIT II was obtained from the Trauma Team Leader (usually an ED Consultant or Registrar who was independent from the research group and familiar with the study and its consenting process) acting as a Professional Consultee. Following signed confirmation of the Professional Consultee agreement, blood samples and data were collected in accordance with the study protocol.

Subsequently, patients were visited on a daily basis to assess whether they had regained the physical and mental capacity to give consent themselves. In the case where they had not yet gained capacity to consent, written agreement was sought from their next of kin (Personal Consultee) as soon as it was deemed appropriate to do so. A written information sheet was provided to the Personal Consultee. The patient continued to be assessed on a daily basis for their capacity to consent and this was sought if appropriate, even when Personal Consultee agreement had previously been obtained. Patients were informed of what study procedures had already been performed and where provided with a written information

sheet. Patients were given the option of withdrawing from the study or continuing to participate. If a patient or Personal Consultee declined to give consent/agreement for continuation with the study at any stage, his/her wishes were respected. The Police assisted in the identification of unidentified patients and in contacting next of kin. All attempts to obtain informed consent and discussions with patients or their next of kin around consent were logged in a secure electronic database. Hard copies of all signed consent forms were filed in the medical records.

2.3 BLOOD SAMPLING

Following Professional Consultee agreement, enrolled patients had a baseline sample of 20ml venous or arterial blood obtained within 20 minutes of ED arrival. A standard panel of blood tests were obtained simultaneously as part of routine trauma care and sent to the hospital laboratory for measurement of: full blood count (FBC), prothrombin time (PT), activated partial thromboplastin time (APTT), International Normalised Ratio (INR), fibrinogen, D-dimer, urea and electrolytes (U&E) and group and save. All patients had an arterial or venous sample taken for blood gas analysis including measurement of base deficit (BD) and lactate as part of routine clinical care.

Patients who required a blood transfusion had a further 20ml research blood sample drawn after transfusion of the 4^{th}, 8^{th} and 12^{th} unit of packed red blood cells (PRBC). A further 20ml of blood was drawn from all patients admitted to hospital at 24 and 72 hours from baseline blood draw.

Research blood samples were collected in specific vacutainers. Blood for rotational thromboelastometry (ROTEM®) analysis, was collected in a 2.7ml citrated vacutainer (0.109 Molar / 3.2% sodium citrate; Becton, Dickinson and Company, Plymouth, UK). Blood for coagulation and fibrinolysis protein assays was collected in two 4.5ml glass citrated vacutainers (0.109 Molar / 3.2% sodium citrate; Becton, Dickinson and Company, Plymouth, UK).

Additional research vacutainers collected alongside the coagulation vacutainers as part of the ACIT II study (e.g. Proteomics, PAXgene RNA tube, TEG and Multiplate samples) are shown in Figure 6 but were not utilised within this thesis and therefore their processing is not described further herein.

2.3.1 Rationale for PRBC sampling interval

One of the primary aims of the ACIT II study is to investigate the coagulation changes that occur following injury and assess the effect resuscitation practices have on the coagulation system during trauma haemorrhage. As such repeated blood samples have to be taken from the patient during trauma haemorrhage. The method of interval sampling chosen in the ACIT II study, which was already well established when I joined the Centre for Trauma Sciences in 2013, was to take samples at baseline and then after every 4th unit PRBC transfused up to the 12th unit. Different factors such as availability of blood products, rate of trauma haemorrhage, timing of definitive haemorrhage control or where haemorrhage stops spontaneously, could all influence the rate and number of PRBC units transfused. In essence the time over which each trauma patient's bleeding episode lasts is unique to that patient. The decision to administer a blood transfusion is a clinical one made by the treating clinicians who are independent to this study process. As such, PRBC transfusions are a surrogate

marker for trauma haemorrhage. A sampling strategy at set PRBC intervals allows for consistent data collection throughout each individual patient's bleeding episode.

An alternative strategy of sampling at set time intervals (e.g. every 15, 30 or 60 minutes during haemorrhage) could have been adopted instead. However, a time-based strategy has the potential to either over sample and take too many samples in the slowly bleeding patient or sample too infrequently in the rapidly bleeding patient. The rapidly exsanguinating trauma patient in RLH can easily be transfused greater than 12 units PRBC within a 60 minute interval. A sampling strategy based upon PRBC intervals allows for data collection at baseline and then again at 3 further points during haemorrhage allowing for temporal changes in coagulation and fibrinolysis to be investigated. A time-based strategy of sampling every 60 minutes in the same rapidly exsanguinating patient would mean that data collection occur only twice and that potentially valuable opportunities to understand pathophysiology and identify when interventions could be beneficial to the patient are missed.

2.3.2 Blood sample processing and storage

All research samples were processed in the Trauma Research Laboratory located within the RLH and frozen at -80°C within two hours of blood draw. Samples were obtained and processed by the Author or else one of the otherwise attending research fellows. A team of four research assistants assisted with the sample processing. All members of the team initially underwent a period of training and shadowing/observation. Sample processing protocols were taught according to a Standard Operating Procedure (SOP). All members of the team were supervised initially by a more senior member of the research team before performing analysis unsupervised in order to ensure consistency and adherence to the SOP. Ongoing supervision was provided by senior members of the research team for quality

control. In 2013, when the ACIT II study was extended to run at European partner institutions, the RLH SOPs were shared with each new site. During the period of expansion of the research programme to partner sites, research fellows from partner institutions attended RLH to observe our recruitment and sample processing procedures and to partake in a period of training. This helped to establish an accurate duplication of the study procedures in their respective centres for the purpose of study standardisation across all sites.

Blood samples were processed as per the ACIT II flow chart (Figure 6). The filled 4.5 ml vacutainers were centrifuged for 10 minutes at 1,750 G in a Clinispin Horizon 853VES Laboratory Centrifuge (Woodley Equipment Company Ltd, Bolton, UK). The top 2/3 of single-spun plasma was removed and then centrifuged for a further 10 minutes at 1,750 G. This double-spun plasma was stored in three 0.5 ml aliquots in separate 0.6 ml Cliklok microcentrifuge tubes (Simport, Jencons, UK). A 0.5 ml aliquot of the bottom 1/3 of single-spun plasma was stored in a separate 0.6 ml Cliklok microcentrifuge tube. The remaining single-spun plasma, "buffy coat" and top layer of red cells were aliquoted into a 1.5 ml Fisherbrand microcentrifuge tube (Fisher Scientific, UK).

All sample aliquots were barcoded and stored in grid labelled cardboard cryoboxes at -80°C in a freezer within the Trauma Research laboratory. Temperature logs were maintained for all fridges and freezers within the laboratory, which were fitted with temperature alarms. At regular intervals, samples were transferred from the research laboratory to the University's long term storage freezers within the Blizard Institute Laboratory, Queen Mary University of London. The long term storage freezers were maintained by the University and had regular internal and external quality control checks. Temperatures were monitored remotely by an

external firm; the Author was one of the contacts initially alerted to and responsible for investigating the alarming of any of our freezers due to their being out with the target temperature range.

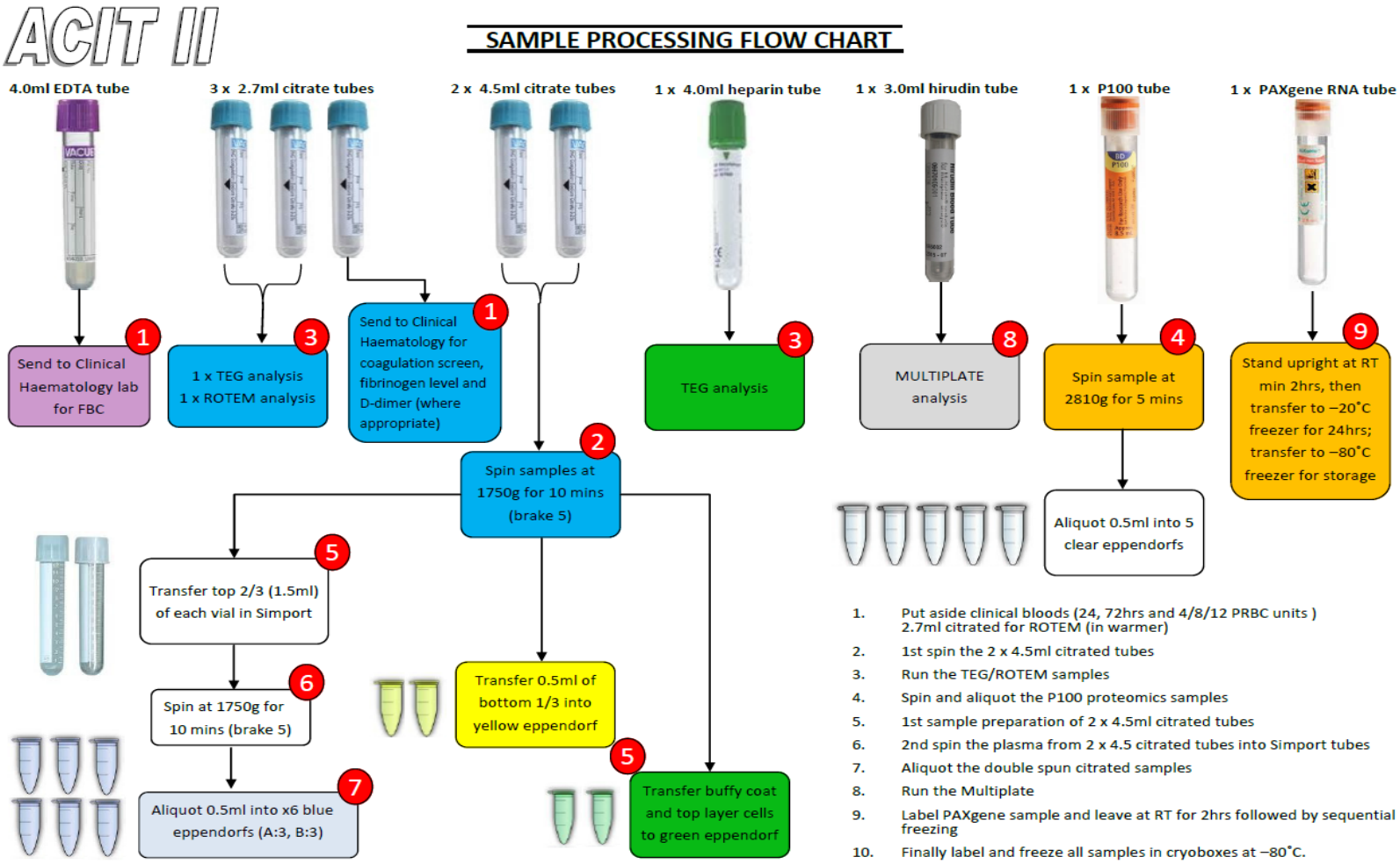


Figure 6 ACIT II blood sample processing flow chart

2.4 ROTATIONAL THROMBOELASTOMETRY (ROTEM®)

A rotational thromboelastometry (ROTEM) device measures the viscoelastic properties of whole blood under low shear conditions to provide a quantitative and qualitative indication of the coagulation state of a whole blood sample. The device provides a graphical output of changes in clot formation and fibrinolysis.

2.4.1 Measurement principle

The ROTEM delta system was used for all analyses. The measuring principle of the ROTEM delta is based upon a fixed cylindrical cup filled with blood and a permanently oscillating vertical axis (Figure 7). The axis is supported by a high precision ball bearing which oscillates left and right through an angle of 4.75 °. A disposable plastic pin is placed over the axis and this is inserted into the fixed cup containing citrated whole blood in addition to specific reagents. The rotation of the pin/axis is detected optically by detection of a light shone at a mirror plate at the upper end of the axis. Movement of the pin is unrestricted when no clot has formed. As a clot forms between the cup surface and pin, movement of the pin is obstructed. Computer software interprets the pin movement and provides the standard ROTEM graphical output (Figure 8) and common measurement parameters (Table 5). Four channels on the device allow for simultaneous measurement of four different ROTEM assays each of which can assess specific aspects of the coagulation system.

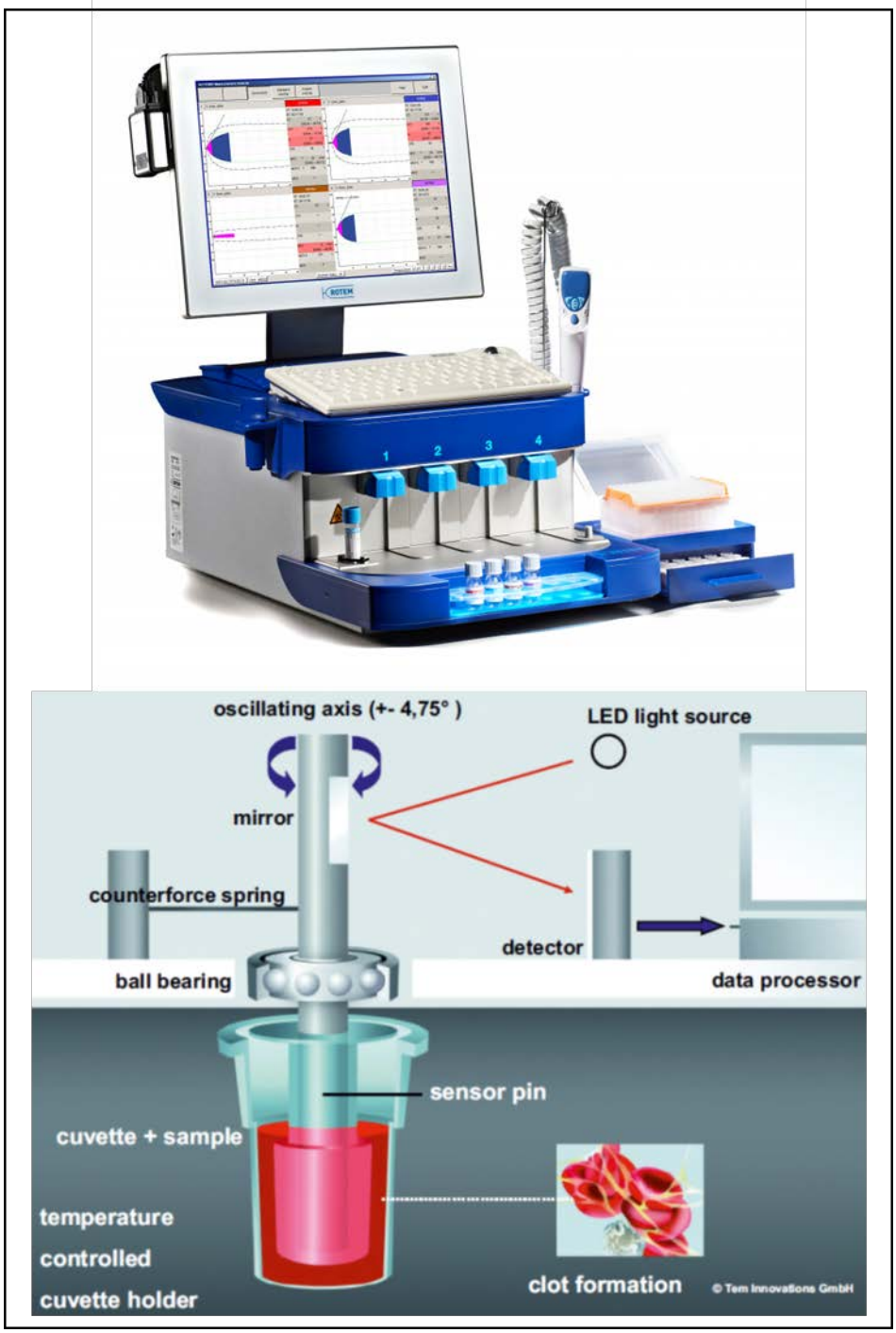


Figure 7 ROTEM (delta) device and detection method [107]

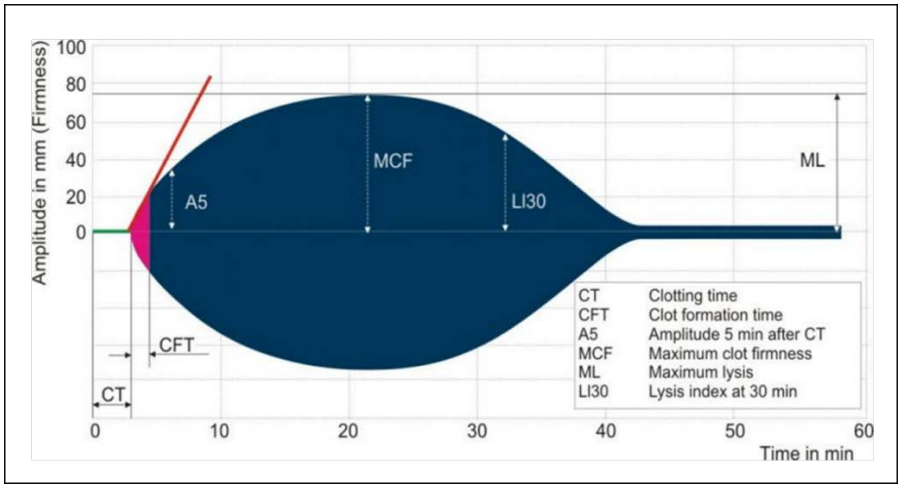


Figure 8 ROTEM output trace with illustrated key parameters [107]

Table 5 Description of measured ROTEM parameters

Parameter	Unit	Description	Interpretation
CT **Clotting Time**	Seconds	Time from beginning of test to detection of first clot (amplitude 2 mm)	**Initiation phase** Lag-phase of thrombin generation (dependent on activity of coagulation factors)
CFT **Clot Formation Time**	Seconds	Time from CT until clot firmness reaches 20 mm	**Amplification phase** Initial clot strengthening by formation of fibrin fibres
α-angle	Degrees	Kinetics of clot development – angle between the horizontal middle axis and tangent to the curve through the 2mm amplitude point	**Thrombin burst** Maximum velocity of clot formation (dependent on platelet function and fibrin polymerisation)
CAx **Clot amplitude at x**	mm	Mechanical clot quality at fixed time point e.g. CA5 at 5 mins	**Propagation phase** Clot strength at fixed time point determined by platelets,, fibrinogen and Factor XIII
MCF **Maximum Clot Firmness**	mm	Mechanical clot quality	Maximal clot strength – determined by balance of platelets, fibrinogen and Factor XIII with fibrinolysis
ML **Maximum lysis**	%	Degree of fibrinolysis relative to MCF at end of measurement (i.e. % clot firmness lost)	**Clot termination** Functional measure of fibrinolysis

2.4.2 Sample processing

The ROTEM assays measured in parallel within ACIT II are:

- EXTEM Activated clotting via extrinsic pathway using tissue factor
- INTEM Activated clotting via intrinsic pathway using ellagic acid
- FIBTEM As EXTEM plus addition of cytochalasin D to inhibit platelets
- APTEM As EXTEM plus addition of aprotinin to inhibit fibrinolysis.

Only EXTEM and FIBTEM assays have been used for this body of work. The reason for selecting these assays is that they have most commonly been used by researchers investigating TIC. The Cochrane Injuries Group identified that the tissue factor-activated assay, EXTEM, was the focus for researchers assessing the accuracy of ROTEM for detection of TIC.[103] A further reason for selecting these assays is that both have direct clinical utility with EXTEM capable of measuring clot formation, fibrin polymerisation and fibrinolysis, whilst FIBTEM allows for an assessment of fibrinogen status with the ability to detect fibrinogen deficiency. Both assays are currently being measured in the iTACTIC randomised control trial assessing the utility of VHA to guide transfusion therapy in trauma haemorrhage[157], further highlighting the clinical relevance of studying these specific assays.

The sampling process for the EXTEM and FIBTEM assays is described below. All pipetting and mixing steps were performed in a standardised manner using the automated electronic pipette program.

The 2.7 ml citrated vacutainer was placed into the well of the warming plate of the ROTEM device to incubate at 37°C for 10 minutes. The ROTEM reagents were removed from the fridge to sit at room temperature for 10 minutes prior to use. The individual reagents were: star-tem® (recalcitrant), ex-tem® (thromboplastin), in-tem® (ellagic acid), fib-tem® (cytochalasin-D) and ap-tem® (aprotinin). The four channels were prepared with a disposable plastic cuvette placed into each test block and a disposable plastic pin positioned on the vertical axis. For the EXTEM assay, 20 µl of 0.2 mmol/l $CaCl_2$ (star-tem®) for recalcification and 20 µl of recombinant tissue factor (ex-tem®) were placed into the test cuvette, after which 300 µl of citrated blood was added. For the FIBTEM assay, 20 µl of fib-tem® (containing cytochalasin D and 0.2 mmol/l $CaCl_2$) and 20 µl ex-tem® were placed into the test cuvette, after which 300 µl of citrated blood was added. Samples were analysed for 60 minutes. Data was extracted from the ROTEM devices on a monthly basis and entered into the ACIT II database. Both continuous internal and routine weekly external quality control testing was performed on all ROTEM machines. The co-efficient of variation for ROTEM provided by the manufacturer is shown in Table 6.

Table 6 ROTEM repeatability and reproducibility (coefficient of variation %)

Precision	CT	CFT	α-angle	CA10	MCF
Intra-assay	6	8	1	2	3
Inter-instrument	7 – 13	5 – 8	2 – 3	2 – 3	1 – 3

2.5 COAGULATION AND FIBRINOLYSIS PROTEIN ASSAYS

The double-spun platelet depleted plasma, prepared and stored as described in section 2.3.1, was subsequently used to measure levels of key coagulation and fibrinolysis protein levels.

Samples were analysed in two batches. The first batch of samples analysed were for the first 324 ACIT II patients recruited to RLH and were performed prior to the Author joining the research team. The second batch of samples included those being analysed as part of the TACTIC programme of work. This much larger second batch included RLH ACIT II patients numbered 325 – 1099 and all of the participating TACTIC partner's ACIT II samples. European sites recruiting to ACIT II shipped frozen plasma samples packaged in dry ice to London, utilising specialist 'same-day' or 'overnight' courier services. Samples were transferred to a -80°C freezer immediately on arrival to RLH. All samples were then processed either in the central RLH Haematology laboratory using a Sysmex automated analyser (where assay capability existed) or manually by the method of enzyme-linked immunosorbent assay (ELISA).

The Author was involved in requesting and managing the transfer of all samples to London for the second batch of analyses. The Author subsequently performed ELISAs on these samples along with three laboratory technicians and had overall responsibility for assimilating the results and managing the dataset. All personnel performing ELISAs were trained in the method by the same senior scientist and underwent regular observation for quality control and standardisation purposes.

2.5.1 Sysmex automated system analyser

Plasma samples for analysis were transferred to the RLH Central Haematology Laboratory -80°C freezer and were subsequently analysed by trained laboratory staff according to the laboratory's Standard Operating Procedures. Frozen plasma aliquots were thawed to 37°C using a water bath prior to all analyses. The number of freeze-thaw cycles was kept as low as practically possible. A Sysmex CS2100i analyser (Sysmex UK Ltd, Milton Keynes, UK) was

used in combination with Siemens reagents (Siemens AG, Munich, Germany). The coagulation assays performed and methods used are detailed in Table 7. There were minor differences in the reference range of some assays used for the first and the second batch of samples analysed, although the variability of each assay remained consistent.

Table 7 Summary of assays performed on the Sysmex CS2100i automated analyser

Parameter	Reference Range RLH 0-324	Reference Range RLH 325+ & All INTRN samples	Units	Reagent	Reagent Supplier	Method	Intra-assay variability (normal sample)	Inter-assay variability (normal sample)
PT	9.4-12.4	9.4-12.2	secs	Siemens Innovin	Sysmex UK	Clotting	1.3%	2.0%
INR	0.9-1.1	0.9-1.1	ratio					
APTT	21-31	21-31	secs	Siemens Actin FS	Sysmex UK	Clotting	4%	4%
APTT ratio	0.8-1.2	0.8-1.2	ratio					
Fibrinogen	1.50-4.50	1.56 – 4.0	g/l	Siemens Thrombin	Sysmex UK	Clauss	5.9%	2.3%
Factor II	78-117	70 – 146	iu/dL	Siemens Innovin and Siemens Factor II deficient plasma	Sysmex UK	Clotting by multi-dilution analysis	2%	7%
Factor V	66-114	62 – 150	iu/dL	Siemens Innovin and Siemens Factor V deficient plasma	Sysmex UK	Clotting by multi-dilution analysis	2%	7%
Factor VII	50-150	67 – 143	iu/dL	Siemens Innovin and Siemens Factor VII deficient plasma	Sysmex UK	Clotting by multi-dilution analysis	2%	7%
Factor VIII	52-153	52 – 153	iu/dL	Siemens Actin FS and Siemens Factor VIII deficient plasma	Sysmex UK	Clotting by multi-dilution analysis	2%	7%
Factor IX	58-138	58 – 138	iu/dL	Siemens Actin FS and Siemens Factor IX deficient plasma	Sysmex UK	Clotting by multi-dilution analysis	2%	7%
Factor X	50-150	65 – 152	iu/dL	Siemens Innovin and Siemens Factor X deficient plasma	Sysmex UK	Clotting by multi-dilution analysis	2%	7%

Factor XI	58-148	58 – 148	iu/dL	Siemens Actin FS and Siemens Factor XI deficient plasma	Sysmex UK	Clotting by multi-dilution analysis	2%	7%
Factor XIII	70-140	55 – 155	iu/dL	Siemens Berichrom F XIII	Sysmex UK	Chromogenic method	5%	6%
Von Willibrand Antigen	50-160	50 – 156	iu/dL	Siemens vWF Ag	Sysmex UK	Latex immunoassay	1.4%	0.9%
Antithrombin	81-119	81 – 119	iu/dL	Siemens Berichrom Antithrombin III (A)	Sysmex UK	Chromogenic	1.3%	4.6%
Protein C	72-162	72 – 162	iu/dL	Siemens Berichrom Protein C	Sysmex UK	Chromogenic	1.3%	1.9%
Free Protein S Antigen	62-120	60 – 139	iu/dL	Stago Liatest Free Protein S	Diagnostica Stago	Latex immunoassay	2.4%	2.6%
α_2-antiplasmin	76-126	68 – 136	iu/dL	Siemens Berichrom α_2-antiplasmin	Sysmex UK	Chromogenic	0.5%	3.2%
Soluble Fibrin Monomer Complexes	<6	0 – 6	µg/mL	Stago Liatest Fibrin Monomer	Diagnostica Stago	Latex immunoassay	2.5%	3.6%
D-Dimer	<550	0 - 440	ng/mL	Siemens Innovance D-Dimer	Sysmex UK	Latex immunoassay	4.1%	4.3%

2.5.2 Enzyme-linked immunosorbent assays (ELISAs)

2.5.2.1 Measurement principles

ELISAs rely upon the reaction between an antibody and an antigen. ELISAs can be performed either in a solution (fluid phase) or on the surface of a plastic (solid phase). Solid phase assays are more sensitive and in this study, the 96-well rigid polystyrene microtiter plate was utilised as the solid phase. Microtiter plates can be coated with either an antigen or an antibody. The ELISAs used in this study are examples of two-site antibody-coated assays, also known as sandwich ELISAs. A sandwich ELISA uses two antibodies to bind an antigen between them (Figure 9). The principle steps in a sandwich ELISA are that the first antibody (the capture antibody) is bound to the microtiter well and it captures the desired antigen. The second antibody (the detection antibody) is labelled with an enzyme conjugate and attaches to another site (or epitope) on the antigen, creating the sandwich. Addition of a substrate which reacts with the enzyme leads to a colour signal being produced. After stopping the reaction, measurement of the absorbance with a spectrophotometer permits quantification of the captured antigen. A sandwich ELISA is a non-competitive process.

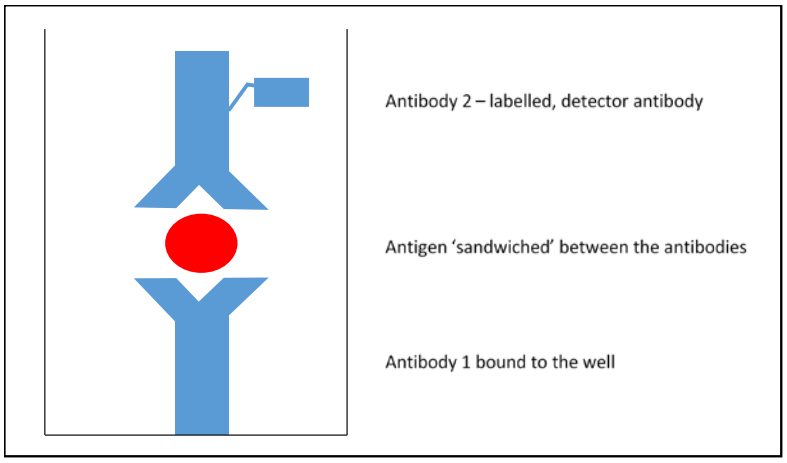


Figure 9 Schematic representation of the sandwich ELISA

2.5.2.2 Measurement process

All ELISAs were performed in the Blizard Institute Laboratory, Queen Mary University of London according to the manufacturer's instructions. Frozen plasma aliquots were thawed to 37°C using a water bath prior to all analyses. The number of freeze-thaw cycles was kept as low as practically possible. A summary of the assays performed by ELISA is detailed in Table 8. ELISA kits from the same manufacturer with the same reference ranges were used for both the initial and the second larger batch of samples analysed. ELISA kits were stored according to the manufacturer's instructions. All plates were analysed with standards, controls and patient samples in duplicate. Where the variation between duplicate results was >20%, the samples were repeated. The mean control sample on each plate was verified to be within the range set by the manufacturer (Figure 10). Given the large number of ELISA plates used, this served as the quality control measure for individual ELISA plate performance and validity. If the measured control value fell out with the specified target range, the samples on this plate were repeated on a new plate where sufficient plasma allowed. In the case the measured protein concentration was higher than that of the highest standard or lower than the lowest standard, where sufficient plasma was available, the sample was re-tested at a lower/higher concentration as appropriate.

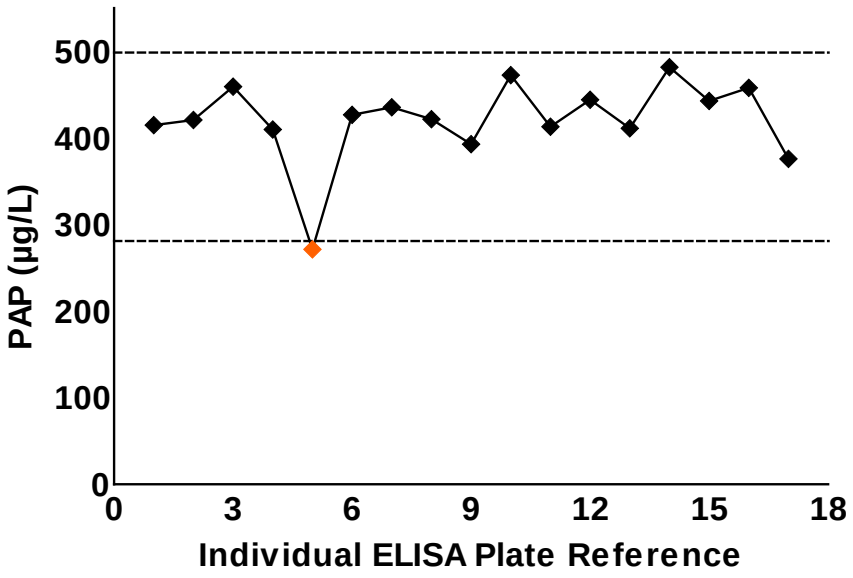


Figure 10 ELISA plate validation - trend in mean PAP control values

Graph demonstrates mean control values from each individual PAP ELISA plate assayed between December 2013 and April 2014. Control value results were confirmed to lie within the expected range (dashed lines) set by the manufacturer, as a quality control measure of plate validity. The control result highlighted in orange measured outside of the range advised by the manufacturer and the samples on this plate were repeated.

All plate washing steps were performed using an auto strip ELx50 automatic plate washer (BioTek, Winooski, USA). The absorbance (OD) of each well was determined at 450 ± 10 nm with a SIAFR Synergy HT plate reader (BioTek, Winooski, USA) using KC4 v3.4 software. The mean absorbance value for each duplicate standard, control and plasma samples was calculated. Standard curves were constructed by plotting mean absorbance for each standard against its concentration and curve fitted by linear regression analysis using Graphpad Prism v5 (Graphpad Software Inc., San Diego, USA) in accordance with the manufacturers' and Graphpad Prism instruction manuals. Using the mean absorbance value for each plasma sample, the corresponding concentration was determined from the standard curve using linear regression, taking into account the dilution factor of the sample.

Table 8 Summary of assays performed by ELISA

Parameter	Reference Range RLH 0-324	Reference Range RLH 325+ & All INTRN samples	Units	Reagent	Reagent Supplier	Method	Intra-assay variability (normal sample)	Inter-assay variability (normal sample)
PAP	120-700	120-700	µg/L	PAP micro	DRG Instruments GmbH, Germany	Immunosorbent assay	2.1 – 6.3%	3.5 – 11%
tPA	2–12	2–12	ng/mL	Asserachrom tPA	Diagnostica Stago SAS, France	Immunosorbent assay	5.1 – 7.3%	3.6 – 4.2%
PF 1+2	69-229	69-229	pmol/L	Enzygnost F 1+2 monoclonal	Siemens Healthcare Diagnostics Products GmbH, Germany	Immunosorbent assay	3.6 – 5.5%	4.4 – 11.2%
PAI-1	4-43	4-43	ng/ml	Asserachrom PAI-1	Diagnostica Stago SAS, France	Immunosorbent assay	4.2 – 5.7%	2.7 – 2.9%
uPA	-	62.5 – 4,000 #	pg/ml	uPA (URK) Human	Abcam, UK	Immunosorbent assay	3.4 – 5.3%	5.4 – 6.8%
S100A10	-	0.156 – 10 #	ng/ml	Human S100A10	Abbexa, UK	Immunosorbent assay	<10%	<12%

PAP, plasmin-α2-antiplasmin complex; tPA, tissue plasminogen activator; PF 1+2, prothrombin fragment F 1+2; PAI-1, plasminogen activator inhibitor-1; uPA, urokinase plasminogen activator. #Expected normal values not given by manufacturer therefore range of the assay is shown.

2.5.2.3 Plasmin-α2-antiplasmin complex assay protocol

All reagents and the 96-well anti-PAP antibody (monoclonal) coated test plate were left at room temperature for 10 minutes prior to use. Each vial of lyophilized standard and control was reconstituted with 1 ml distilled water. Wash solution was made by diluting 30 ml of wash solution concentrate with 1170 ml of deionized water. 200 µl enzyme conjugate concentrate (rabbit anti-human plasminogen antibody conjugated to horseradish peroxidase) was diluted with 11 ml of conjugate diluent. Thawed, double-spun plasma samples were diluted 1:10 by mixing 10 µl plasma with 90 µl assay buffer.

50 µl of assay buffer was dispensed into each well followed by 50 µl of each standard, control or plasma sample in duplicate and mixed. The plate was left to incubate for 15 minutes at room temperature (20 - 25°C). All wells were then aspirated and washed three times using an auto strip ELx50 plate washer (BioTek, Winooski,, USA). 100 µl of enzyme conjugate was dispensed into each well using a multichannel pipette and incubated for 15 minutes at room temperature. All wells were then aspirated and washed three times. 100 µl of tetramethylbenzidine (TMB) substrate solution was added to each well and incubated for 15 minutes at room temperature. The enzymatic reaction was stopped by adding 100 µl of stop solution (0.5 mol/L sulfuric acid) to each well. The absorbance of each well was read with a microtiter plate reader within 30 minutes.

2.5.2.4 Prothrombin fragment F 1+2 assay protocol

The test plate coated with monoclonal mouse antibodies to human F 1+2 and all reagents were left at room temperature for 10 minutes prior to starting. The washing solution was prepared by diluting 100 ml of wash solution concentrate with 1900 ml deionized water. The 250 µl anti-human prothrombin monoclonal mouse antibody conjugated with peroxidase

was mixed with 14 ml conjugate buffer. The control plasma and standard vials were reconstituted by adding 1 ml distilled water to each vial and incubated for 15 minutes at room temperature. 1.5 ml Chromogen TMB was diluted with 15 ml buffer to form the chromogen working solution, which was stored in a closed drawer to protect from light. Thawed double-spun plasma samples were diluted 1:10 with sample buffer.

50 µl sample buffer was dispensed into each well followed by 50 µl of standards, control or plasma samples and mixed. The test plate was covered with adhesive foil and incubated for 30 minutes at 37°C in a water bath. The wells were aspirated and washed twice using the automated plate washer. 100 µl of conjugate solution was dispensed into each well using a multichannel pipette, the plate was covered with adhesive foil and incubated for 15 minutes at 37°C in a water bath. The wells were again aspirated and washed twice using the automated plate washer. 100 µl of chromogen buffer solution was dispensed into each well, plate covered with adhesive foil and then incubated at room temperature protected from light for 15 minutes. Foil was removed and 100 µl stopping solution added to each well. The absorbance of each well was read with a microtiter plate reader within 30 minutes.

2.5.2.5 Plasminogen activator inhibitor-1 assay protocol

A 96-well plate coated with mouse monoclonal anti-human PAI-1 $F(ab')_2$ fragments and all reagents were left for 30 minutes at room temperature before starting. The lyophilized mouse monoclonal anti-human PAI-1 antibody coupled with peroxidase was reconstituted with 8 ml phosphate buffer, left to stand for 30 minutes at room temperature then the vial vortexed before use. The concentrated wash solution was diluted 1:20 with distilled water before use. The standard and control vials of lyophilized human plasma were reconstituted with 0.5 ml distilled water. Vials were left at room temperature for 30 minutes and were

vortexed before use. The reconstituted standard was diluted 1:10 with phosphate buffer to create the starting solution (t) which was diluted t:2, t:4, t:8, t:16 and t:32 to obtain the lower calibrator levels. Plasma samples were diluted 1:10 using phosphate buffer. The control sample was prepared for testing at two dilutions – 1:10 and 1:20.

200 µl of standards, control or diluted double spun plasma were pipetted into each well in duplicate. The plate was covered and incubated for 60 minutes at room temperature. The wells were aspirated and washed five times using the automated plate washer. 200 µl of reconstituted mouse monoclonal anti-human PAI-1 antibody coupled with peroxidase was added to each well. The plate was covered and incubated for 60 minutes at room temperature. The wells were aspirated and washed five times using the automated plate washer. 200 µl of TMB was added to each well and incubated for 5 minutes at room temperature before addition of 50 µl stop solution (1 mol/L sulfuric acid). After 15 minutes, the absorbance was measured with a microtiter plate reader.

2.5.2.6 Tissue plasminogen activator assay protocol

A 96 well plate coated with mouse monoclonal anti-human tPA $F(ab')_2$ fragments and all reagents were left for 30 minutes at room temperature before starting. The lyophilized mouse anti-human tPA antibody coupled with peroxidase was reconstituted with 8 ml phosphate buffer, left to stand for 30 minutes at room temperature, then vortexed. Concentrated washing solution was diluted 1:20 with deionized water before use. The standard and control vials of lyophilized human plasma were reconstituted with 0.5 ml deionized water. Vials were left at room temperature for 30 minutes and were vortexed before use. The reconstituted standard was diluted 1:10 with phosphate buffer to create the starting solution (t) which was diluted t:2, t:4, t:8 and t:16 to obtain the lower calibrator

levels. Plasma samples were diluted 1:10 using phosphate buffer. The control sample was prepared for testing at two dilutions – 1:10 and 1:20.

200 µl of standards, control or diluted double spun plasma were pipetted into each well in duplicate. The plate was covered and incubated for 120 minutes at room temperature. The wells were aspirated and washed five times using the automated plate washer. 200 µl of reconstituted mouse monoclonal anti-human tPA antibody coupled with peroxidase was added to each well. The plate was covered and incubated for 120 minutes at room temperature. The wells were aspirated and washed five times using the automated plate washer. 200 µl of TMB was added to each well and incubated for 5 minutes at room temperature before addition of 50 µl stop solution (1 mol/L sulfuric acid). After 15 minutes, the absorbance was measured with a microtiter plate reader.

2.5.2.7 Urokinase plasminogen activator assay protocol

The uPA ELISA was performed by Paul Vulliamy and Scarlett Gillespie (Centre for Trauma Sciences, QMUL) according to the following protocol: the 96 well anti-human uPA antibody coated microplate and all reagents were brought to room temperature prior to use. The biotinylated anti-human uPA antibody was diluted 1:100 with antibody diluent buffer and mixed thoroughly. The avidin-biotin-peroxidase complex (ABC) was diluted 1:100 with ABC diluent buffer and mixed thoroughly. A 0.01 mol/L solution of PBS was formed by adding 8.5 g NaCl, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to distilled water (200 ml) and adjusting pH to 7.2 – 7.6, before adjusting the total volume to 1000 ml with distilled water. A 10 ng/ml uPA stock standard was prepared by reconstituting the lyophilised recombinant human uPA standard with 1 ml sample diluent buffer and kept at room temperature for 10 minutes. An eight point standard curve was prepared using a 4,000 pg/ml top standard from the 10 ng/ml

stock standard followed by serial 1:2 dilutions using sample diluent buffer. The eighth standard remained a diluent-only blank. Thawed double-spun plasma was diluted 1:2 by adding 100 µl sample to 100 µl sample diluent buffer.

100 µl of prepared standards and diluted plasma samples were added to each well before covering the plate and incubating at 37°C in a water bath for 90 minutes. The seal was removed, contents discarded from each well and plate blotted onto an absorbent paper towel. 100 µl of biotinylated anti-human uPA antibody was added to each well. The plate was re-sealed and incubated for 60 minutes in a water bath at 37°C. The plate was washed three times with 0.01 mol/L PBS using the automated plate washer. 100 µl of ABC working solution was added to each well, the plate sealed, and incubated for 30 minutes in a water bath at 37°C. The plate was washed five times using the automated plate washer. 90 µl TMB was added to each well, plate sealed and incubated at 37°C in a water bath for 20-25 minutes in the dark. 100 µl TMB stop solution was added to each well and the absorbance read by a microtiter plate reader within 30 minutes.

2.5.2.8 S100A10 assay protocol

The S100A10 ELISA was performed by Paul Vulliamy and Scarlett Gillespie (Centre for Trauma Sciences, QMUL) according to the following protocol. All samples and kit were brought to room temperature prior to use. The concentrated wash buffer was diluted 1:30 with deionized water. A six point standard curve was prepared using a 20 ng/ml top standard solution followed by serial 1:2 dilutions using sample diluent buffer. The 120 µl detection reagents A and B were prepared by centrifuging briefly before diluting each 100-fold with 12 ml diluent A and B respectively, and mixing thoroughly.

100 µl of the diluted standards, standard diluent buffer (zero control) and prepared undiluted plasma samples were added to the respective wells. The plate was agitated, covered and incubated for 60 minutes at 37°C in a water bath. The cover was removed, well contents discarded and 100 µl of the detection reagent A working solution added to each well. The plate was re-sealed and incubated for 60 minutes at 37°C in a water bath. The plate was aspirated and washed three times with wash buffer using the automated plate washer. 100 µl of detection reagent B working solution was added to each well, the plate sealed and incubated for 30 minutes at 37°C in a water bath. The solution was discarded and plate washed five times with wash buffer. 90 µl TMB substrate was added to each well, the plate sealed and incubated at 37°C for 10-20 minutes in the dark. 50 µl stop solution was added to each well and the absorbance measured immediately by a microtiter plate reader.

2.6 DATA COLLECTION

In addition to the blood sampling and analysis described, the following data points were recorded for each patient.

2.6.1 Patient and injury characteristics

Patient observations on scene, on ED admission and at the time of all blood sampling, including heart rate, systolic blood pressure (SBP), respiratory rate, temperature and Glasgow coma score (GCS) were recorded alongside timings. Patient demographics including sex and age were documented. Injury characteristics including date, time and mechanism (including blunt vs penetrating) were recorded. Injury severity was collated from the RLH trauma registry (or equivalent service in other European hospitals) as soon as available.

Injuries were scored and classified according to the Abbreviated Injury Score (AIS) to provide a composite overall Injury Severity Score (ISS) based on anatomical injury severity.[158]

2.6.2 Fluid, blood products and haemostatic medications administered

All intravenous fluids and blood products administered over the first 24 hours from the time of injury were recorded. Intravenous fluids were categorised as crystalloids, colloids or hypertonic saline. Blood products included packed red blood cells (PRBC), fresh frozen plasma (FFP), platelets and cryoprecipitate. The timing of blood product delivery during the first 24 hours was recorded. The administration of tranexamic acid and its timing was recorded.

2.6.3 Outcome measures

Patients were observed daily during the first 28 days of their hospital admission or until the point of hospital discharge or death if this occurred earlier. Outcome measures were 28-day survival, hospital length of stay (LOS), critical care LOS (combined total of days in high dependency and intensive care), ventilator free-days (28 minus number of days requiring mechanical ventilation), vasopressor free-days and SOFA organ score for patients whilst in critical care. Occurrence of venous thromboembolism (deep vein thrombosis (DVT) or pulmonary embolism (PE)) or other adverse event (including myocardial infarction, stroke) was documented. We defined these outcomes as a clinical diagnosis supported by a confirmatory test result: duplex ultrasound for DVT; CT pulmonary angiogram for PE; electrocardiogram changes and elevated troponin for MI; and CT head for ischemic stroke.

2.7 STATISTICAL ANALYSIS

Statistical analysis was performed using GraphPad Prism version 5 (GraphPad Software Inc., San Diego, USA), Microsoft Excel 2013 (Microsoft Inc., Redmond, USA) and IBM SPSS Statistics version 25 (IBM, Armonk, United States). Normal quantile plots were used to assess normality. Data without a normal distribution are expressed as median (interquartile range) and analysed using Mann-Whitney U-test or Kruskal-Wallis test with Dunn's multiple comparison test. Normally distributed data are expressed as mean ± 95% confidence intervals and analysed with Student's t test. Percentages were analysed using Chi-squared or Fisher's exact tests, with linear trends over time analysed with Chi-squared test for trend. Correlation between continuous variables was assessed with Spearman's correlation coefficient. Differences in survival were compared with Log-rank (Mantel-Cox) test.

Multivariate analysis was performed using IBM SPSS Statistics. Multivariate logistic regression models were created to investigate the effect on late outcomes, of TXA infusion (binary variable [yes/no]) or time from injury to TXA bolus (continuous variable in minutes) alongside other factors achieving a univariate significance of $p < 0.2$.

A p-value of <0.05 was considered statistically significant throughout unless otherwise stated. Box-whisker plots represent median, interquartile range with 5^{th} and 95^{th} percentiles unless otherwise stated.

The Author performed all statistical analysis with advice from both supervisors.

2.8 Specific Role of the Author within the ACIT II Study

Between August 2013 and July 2016, the Author was a full-time Clinical Research Fellow within The Centre for Trauma Sciences, Queen Mary University of London and an Honorary Clinical Research Fellow at The Royal London Hospital (RLH). During this time, the Author was the lead fellow for the ACIT II research study. The Author worked alongside between four to eight other Fellows on a shift-basis to cover research recruitment to ACIT II seven days a week. Recruitment hours were between 08:00 to 22:00, extending to 24-hour recruitment from January 2016 onwards. The Author partook in all aspects of patient screening, recruitment to ACIT II, sample and data collection and follow-up as a member of the research team at the RLH. The Author and other research fellows performed venepuncture in order to obtain blood samples from trauma patients and then performed sample processing and analysis on for example ROTEM machines themselves as part of the data collection process.

As the lead fellow, I acted as rota master from 2014 to August 2016. The additional responsibilities of the lead fellow role included: day-to-day study oversight within RLH, monitoring patient data collection on a day-to-day basis for completeness and accuracy, training new research fellows and students and acting as a point of contact for any other fellows or research staff with queries. The Author had responsibility for the extraction and "cleaning" of all ROTEM data during this period.

The Author performed a lead role in all ELISA analysis. The Author was trained in the technique of ELISA by a senior scientist within the group. The Author measured four different plasma protein levels each using ELISA for approximately 550 patient samples over a 5 month period. The Author then requested and co-ordinated the sending of plasma samples from

each international INTRN site to London. Two research assistants were employed to carry out the remaining plasma protein analysis on these INTRN samples. The Author assisted in the training of these new team members and performed oversight of all aspects of sample analysis and data processing. The Author was responsible for data validation and "cleaning" of final biomarker data. For those plasma samples analysed within the RLH Central Haematology Laboratory, the Author organised and catalogued all INTRN frozen plasma samples ready for analysis and liaised directly with the senior biomedical scientist performing the analysis, to obtain the results.

Chapter 3

AN OCCULT HYPERFIBRINOLYTIC SUBTYPE PRESENTING WITH EARLY ROTEM HYPOFIBRINOLYSIS FOLLOWING TRAUMATIC INJURY

3.1 CHAPTER ABSTRACT

OBJECTIVE: To determine the characteristics of trauma patients with low levels of fibrinolysis as detected by viscoelastic haemostatic assay (VHA) and explore the underlying mechanisms of this subtype.

BACKGROUND: Hyperfibrinolysis is a central component of acute traumatic coagulopathy but a group of patients present with low levels of VHA-detected fibrinolysis. There is concern that these patients may be at risk of thrombosis if empirically administered an antifibrinolytic agent.

METHODS: A prospective multicentre observational cohort study was conducted at five European major trauma centres. Blood was drawn on arrival, within two hours of injury, for VHA (ROTEM) and fibrinolysis plasma protein analysis including the plasminogen activator S100A10. An outcomes-based threshold for ROTEM hypofibrinolysis was determined and patients grouped by this and by D-dimer (DD) levels.

RESULTS: 914 patients were included in the study. The VHA maximum lysis (ML) lower threshold was determined to be <5%. Heterogeneity existed within the ML_{LOW} group, with survivors sharing similar clinical and injury characteristics to ML_{NORMAL} (5-15%) patients. Those who died were critically injured with a preponderance of traumatic brain injury (TBI) and had a 7-fold higher D-dimer level (died vs survived: 103,170 vs 13,672 ng/ml, p<0.001). The ML_{LOW}+DD_{HIGH} group demonstrated a hyperfibrinolytic biomarker profile, low tPA levels but high plasma levels of S100A10. S100A10 was negatively correlated with %ML (r=-0.26, p<0.001) and caused a significant reduction in %ML when added to whole blood *ex-vivo*.

CONCLUSIONS: Patients presenting with ML_{LOW}+DD_{LOW} have low injury severity and normal outcomes. Patients with ML_{LOW}+DD_{HIGH} are severely injured, functionally coagulopathic and have poor clinical outcomes. These patients have low tPA levels and are not detectable by ROTEM. S100A10 is a cell surface plasminogen receptor which may drive the hyperfibrinolysis at the endothelium in these patients and which when shed artificially lowers %ML *ex-vivo*.

ACKNOWLEDGEMENTS: The research discussed in the following chapter represents a combined output from the International Trauma Research Network (INTRN) to which all of the participating trauma centres are affiliated members. The research leading to these results has received funding from the European Commission under the FP7 HEALTH-Contract No. F3-2013-602771. The work presented represents the collaborative efforts of the author and Paul Vulliamy as the lead research fellows working on this project under the supervision of Mr Ross Davenport and Professor Karim Brohi. The author was the primary investigator for all aspects of the research presented within this chapter with the exception of the uPA and S100A10 protein subgroup analysis. Paul Vulliamy led on the subgroup analysis of these two proteins, the results of which are included within the appendix and discussed in order to present our complete findings. Scarlett Gillespie and Timothy Jones are additionally acknowledged for their assistance in performing biomarker assays.

3.2 INTRODUCTION

Trauma patients rapidly develop an endogenous Acute Traumatic Coagulopathy (ATC) which is associated with poor outcomes including transfusion requirements, critical care utilization and death.[5,9,159] Hyperfibrinolysis is a central characteristic of ATC[10,11] and early empiric antifibrinolytic administration has been shown to improve overall survival.[116,118,134] There is controversy as to whether antifibrinolytics should be administered empirically or given only to patients with diagnostic evidence of hyperfibrinolysis.[14,115] This controversy centres on the potential risks of inducing a thrombotic event in patients without active fibrinolysis and the role of viscoelastic haemostasis assays (VHAs) in the diagnosis of hyperfibrinolysis.[160] This confusion has led to a wide variation in antifibrinolytic practice worldwide.

Our research group has previously shown that a large number of patients with biomarker evidence of hyperfibrinolysis are not detected by current rotational thromboelastometry (ROTEM) viscoelastic devices.[7] Around 60% of trauma patients had an 'occult' hyperfibrinolysis with elevated plasmin-α2-antiplasmin (PAP) and D-dimer (DD) levels, increased mortality and blood product utilization, but no evidence of hyperfibrinolysis on ROTEM viscoelastic assay. Several studies have subsequently identified that high mortality is associated with a hypofibrinolytic pattern on thromboelastography (TEG) and that this represents a group of patients in whom fibrinolysis has been switched off.[27,109] This fibrinolysis 'shutdown' pattern appears to be the most common fibrinolytic phenotype and the implication is that administration of antifibrinolytics to these patients could induce arterial or venous thrombotic events and death. It remains unclear though whether all low VHA detected lysis is abnormal; whether this is a heterogeneous group not differentiated by

VHA; what the underlying mechanisms are that lead to these different patterns of fibrinolysis; and which are associated with poor outcomes.

We hypothesized that not all low levels of fibrinolysis as detected by VHA were pathological. Our first aim was to determine whether low VHA-fibrinolysis represented a homogeneous or heterogeneous group of patients. If heterogeneous, we aimed to determine which patients had poor outcomes and to understand how they might be identified within the whole low VHA-fibrinolysis cohort. We further aimed to characterize the injury characteristics and outcomes of these patients, to shed light on the mechanism of this form of trauma-induced coagulopathy. We conducted an analysis of clinical data and samples from trauma patients prospectively enrolled into our platform study of trauma-induced coagulopathy.

3.3 METHODS

The study was first approved by East London and The City Research Ethics Committee (07/Q0603/29) then subsequently by each local ethics committee and conducted according to the Declaration of Helsinki. Deferred written informed consent was obtained from each patient or their next of kin. Blood samples from healthy volunteers taking no regular medications were obtained after written informed consent (07/Q0702/24).

3.3.1 Study design

A prospective multicentre observational cohort study, the Activation of Coagulation and Inflammation in Trauma (ACIT) study (UK CRN ID 5637) was conducted at five European major trauma centres in London, Oslo, Copenhagen, Amsterdam and Cologne. All centres are members of the International Trauma Research Network (INTRN). Patients were excluded

from ACIT if they arrived >2 hours post injury; were transferred from another hospital; received >2000ml crystalloid pre-hospital; or had sustained burns >5% of their body surface area. Patients were retrospectively excluded if they declined to give consent to the use of their research samples, had severe liver disease, a known pre-existing bleeding diathesis, or were taking anticoagulant medication (excluding aspirin) pre-injury. Of patients in the ACIT study recruited between January 2008 and July 2014, all adults (>15 years) who were either severely injured (ISS ≥ 15), shocked (lactate ≥ 2 or base deficit ≥ 4), coagulopathic (INR > 1.2 or ROTEM EXTEM CA5 ≤ 35 mm) or transfused ≥ 4 packed red blood cell (PRBC) units within the first 12 hours and did not receive tranexamic acid (TXA) had multiple biomarker assays performed as part of the TACTIC program of work and are analysed in this study.[105] These severely injured, shocked, coagulopathic or bleeding inclusion criteria were set in order to select a group of trauma patients expected to have high degrees of fibrinolytic activation, so that low VHA fibrinolysis could be specifically investigated.

3.3.2 Data collection

Research personnel at each centre screened and enrolled patients. Data were collected prospectively and included patient demographics, time of injury, mechanism of injury (blunt or penetrating), Injury Severity Score (ISS), vital signs on-scene and on arrival in the emergency department (ED), total number of blood products and volume of intravenous fluids administered within the first 12 hours from injury. Patients were observed for 28 days from injury for the occurrence of venous thromboembolic events (deep vein thrombosis (DVT) or pulmonary embolism (PE)), and overall mortality (up to 28 days from injury).

3.3.3 Blood Sampling

The baseline research blood sample was drawn within 20 minutes of the patient's arrival in the ED along with standard trauma laboratory tests. A point-of-care arterial blood gas analysis was performed simultaneously for base deficit (BD) calculation. Blood for rotational thromboelastometry (ROTEM®) analysis, was collected in a 2.7ml citrated vacutainer (0.109 Molar / 3.2% sodium citrate; Becton, Dickinson and Company, Plymouth, UK). Blood for coagulation and fibrinolysis protein assays was collected in a 4.5ml glass citrated vacutainer (0.109 Molar / 3.2% sodium citrate; Becton, Dickinson and Company, Plymouth, UK). The filled 4.5ml vacutainer was centrifuged within 1 hour of collection and double-spun plasma subsequently stored at -80°C.

3.3.4 Functional Coagulation Analysis

Functional coagulation analysis was performed within one hour of blood draw at 37°C on a ROTEM® delta instrument (Tem International GmbH, Munich, Germany) using the automated electronic pipette according to the manufacturer's instructions. The methodology and parameters of ROTEM® have been described previously.[161] The EXTEM assay, measuring tissue-factor initiated clotting was run for 60 minutes and the maximum lysis (ML) determined. For spiking experiments, recombinant S100A10 (Abbexa, Cambridge, UK) or vehicle was added to the ROTEM cup prior to the start of the test.

3.3.5 Coagulation and Fibrinolysis Plasma Protein Assays

Plasma stored at -80°C was transferred from each site to London for further protein analysis. Plasma was thawed to 37°C immediately before all analyses. Prothrombin fragment 1+2 (PT Frag 1+2; Enzygnost® F 1+2 (monoclonal); Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany), tissue plasminogen activator (tPA; Asserachrom® tPA, Diagnostica

Stago, Asnières sur Seine, France), plasminogen activator inhibitor-1 (PAI-1; Asserchrom® PAI-1; Diagnostica Stago), plasmin-α2-antiplasmin complex (PAP; PAP micro ELISA; DRG Instruments GmbH, Marburg, Germany), urokinase (uPA, Abcam, UK) and S100A10 (Abbexa, Cambridge, UK) were measured using sandwich enzyme-linked immunosorbent assays (ELISAs). Prothrombin time (and INR) (Siemens Innovin, Sysmex UK), fibrinogen (Siemens Thrombin reagent, Sysmex UK) and α2-antiplasmin levels (Siemens Berichrom α2-antiplasmin, Sysmex UK) were determined in the hospital laboratories with a Sysmex CS2100i automated analyser (Sysmex UK) according to standard protocols. Latex immunoassays were used to quantify the levels of D-dimer (Siemens Innovance D-dimer; Sysmex UK) also with the Sysmex CS2100i automated analyser.

3.3.6 Definitions

Major haemorrhage was defined as administration of at least 4 units of PRBCs within 12 hours of admission, and massive haemorrhage as at least 10 PRBC units in 12 hours. Severe traumatic brain injury was defined as a brain abbreviated injury score (AIS) of 3 or higher. Coagulopathy was defined as an International Normalized Ratio (INR) > 1.2. We defined VHA hyperfibrinolysis as a ML >15%.[7]

3.3.7 Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5 (GraphPad Software Inc., San Diego, USA) and Microsoft Excel 2013 (Microsoft Inc., Redmond, USA). Normal quantile plots were used to assess normality. Data without a normal distribution are expressed as median (interquartile range) and analysed using Mann-Whitney U-test or Kruskal-Wallis test with Dunn's multiple comparison test. Percentages were analysed using Chi-squared or Fisher's exact tests. Correlation between continuous variables was assessed with Spearman's

correlation coefficient. Differences in survival were compared with Log-rank (Mantel-Cox) test. A *p*-value of <0.05 was considered statistically significant. Box-whisker plots represent median, interquartile range with 5th and 95th percentiles unless otherwise stated.

3.4 RESULTS

A total of 2465 subjects were enrolled in the ACIT study during the recruitment period across the five study sites. Of these, 1318 patients met the inclusion criteria and were eligible for enrolment into this study. Twenty-five patients co-enrolled in the CRASH-3 trial were excluded, along with 169 who received TXA, 196 who did not have a baseline ROTEM performed and 14 who did not have a D-dimer measured, leaving 914 patients for analysis.

3.4.1 Determination of ROTEM Maximum Lysis thresholds for hypofibrinolysis.

We first determined our study threshold for low VHA fibrinolysis as this has not been previously defined for ROTEM. The method used to determine the different VHA fibrinolysis groups was in line with the method described by Moore et al.[26] using TEG in order to directly compare the two VHA platforms and then further explore the low VHA fibrinolysis group further. A histogram of mortality for each % maximum lysis (ML) suggested a lower inflection point at less than 5% and below (Figure 11A). As confirmation a receiver operating characteristic curve for mortality was generated using patients with a ML value of ≤15%. The point of ML <5.5% had the optimal performance by the Youden's index with a sensitivity of 61.6% and specificity of 58.4%. Mortality was doubled in patients with ML <5% (15% vs 7.1% for ML 5-15%, p<0.001). We therefore defined ML_{LOW} as ML <5%; ML_{NORMAL} as ML 5-15%; and ML_{HIGH} as ML>15%[7]. These 3 groups of patients showed a U-shaped mortality curve, similar to previous studies of hypofibrinolysis (Figure 4) in trauma[27,109] (Figure 11B). A similar

pattern was identified when incidence of admission coagulopathy (Figure 12) and requirement for red blood cell transfusion (Figure 13) were stratified by admission ML, with ML < 5% again representing a lower inflection point for these outcomes.

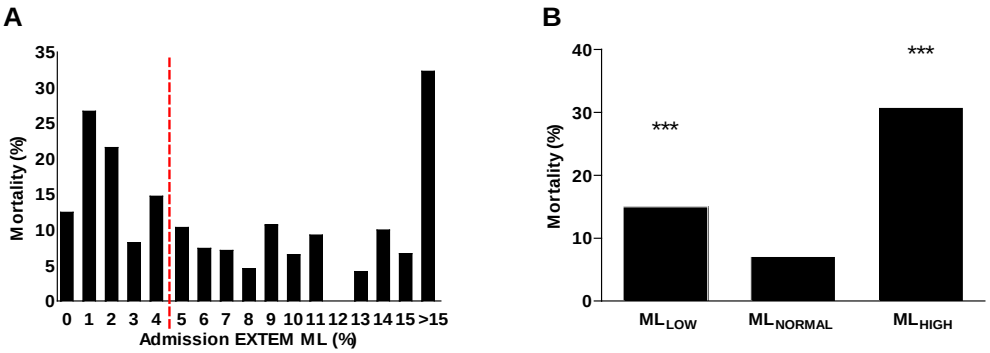


Figure 11 Overall mortality stratified by admission % maximum lysis (ML)

A: Histogram of 28-day mortality for each % maximum lysis (ML) on admission showing a lower inflection point at ML < 5% (dashed line). B: Overall 28-day mortality showed a U-shaped distribution when patients were grouped according to admission ML value.

ML_{LOW} < 5%; ML_{NORMAL} 5 – 15% and ML_{HIGH} >15%. ***p<0.001 vs ML_{NORMAL}.

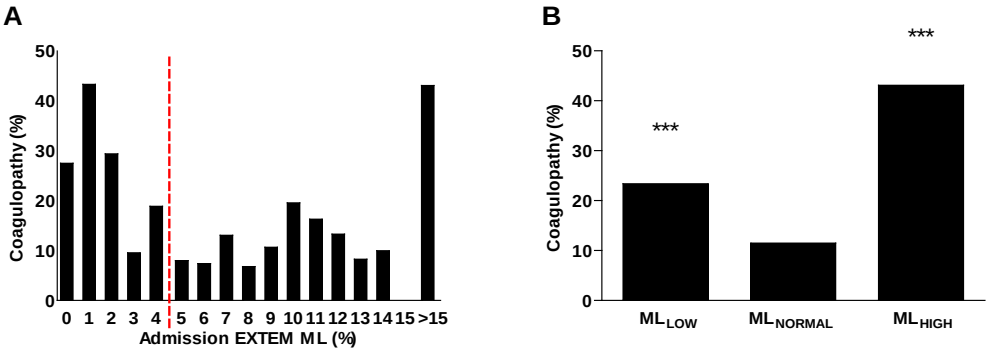


Figure 12 Incidence of coagulopathy on admission stratified by admission % ML

A: Histogram of arrival coagulopathy (INR > 1.2) incidence for each % ML on admission showing a lower inflection point at ML < 5% (dashed line). B: The incidence of coagulopathy demonstrated a U-shaped distribution similar to mortality. ***p<0.001 vs ML_{NORMAL}.

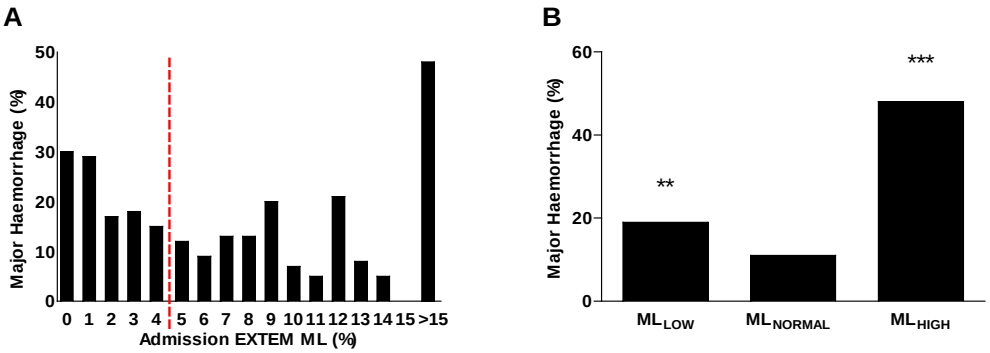


Figure 13 Incidence of major haemorrhage stratified by admission % maximum lysis (ML)

A: Histogram of major haemorrhage (requirement of ≥ 4 red blood cell units in first 12 hours) for each % ML on admission suggesting a similar lower inflection point at ML < 5% (dashed line). B: Presence of major haemorrhage demonstrated a similar U-shaped distribution to mortality and coagulopathy. **p<0.01, ***p<0.001 vs ML_{NORMAL}.

3.4.2 Characterisation of patterns associated with mortality in the ML_{LOW} cohort

To explore the heterogeneity of the ML_{LOW} group, we began by examining the differences between ML_{LOW} patients who lived and those who died. The survivors in this group had very similar admission characteristics, injury patterns and outcomes to ML_{NORMAL} patients. The ML_{LOW} survivors were statistically older, more shocked and more severely injured, but these differences were small (Table 9). Their outcomes in terms of VTE, massive transfusion rates, critical care and hospital lengths of stay were the same as for ML_{NORMAL} patients. In contrast the ML_{LOW} patients who died were much older, with critical injury (median ISS 29), lower GCS than any other cohort (median 7) and 85% of them had a traumatic brain injury (Table 9).

Table 9 Characteristics of the study cohort

	ML_{NORMAL}	ML_{LOW}	ML_{LOW} Survived	ML_{LOW} Died	ML_{HIGH}
Number of Patients	**576**	**273**	**232**	**41**	**65**
Admission characteristics					
Age, years	35 (25 – 51)	42 (31 – 58)	40 (29 – 55)^^	59 (38 – 73)**	43 (24 – 58)
Male, n (%)	464 (81%)	212 (78%)	186 (80%)	26 (63%)*	48 (74%)
SBP, mmHg	133 (112 – 149)	130 (110 – 149)	130 (112 – 148)	130 (95 – 164)	121 (93 – 140)
GCS	15 (12 – 15)	14 (8 – 15)	15 (11 – 15)	7 (3 – 13)**	14 (10 – 15)
BD, mmol/L	1.1 (-0.7 – 3.5)	2.2 (0.4 – 4.8)	2.0 (0.3 – 4.4)^^	3.5 (0.9 – 6.6)*	6.7 (1.4 – 15.2)
INR > 1.2, n (%)	66 (11%)	64 (23%)	38 (16%)	26 (63%)**	28 (43%)
EXTEM ML, %	8 (6 – 10)	3 (2 – 4)	3 (2 – 4)^^	2 (1 – 4)	24 (18 – 100)
D-Dimer, ng/ml	7150 (1320 – 29311)	19313 (3980 – 64988)	13672 (2887 – 47130)^^	103170 (58618 – 182656)**	15608 (3077 – 55939)
Injury to sample time, minutes	75 (50 – 98)	81 (53 – 104)	79 (52 – 103)	92 (68 – 107)	75 (55 – 99)
Injury characteristics					
Blunt, n (%)	464 (81%)	249 (91%)	211 (91%)^^	38 (93%)	55 (85%)
ISS	13 (5 – 25)	20 (9 – 29)	17 (9 – 29)^^	29 (25 – 38)**	17 (4 – 29)
AIS Head & Neck ≥ 3, n (%)	153 (27%)	110 (40%)	75 (32%)	35 (85%)**	17 (26%)
AIS Thorax ≥ 3, n (%)	192 (33%)	116 (42%)	94 (41%)	22 (54%)	21 (32%)
AIS Abdo/pelvis ≥ 3, n (%)	58 (10%)	26 (10%)	25 (11%)	1 (2%)	8 (12%)
AIS Extremity ≥ 3, n (%)	149 (26%)	76 (28%)	69 (30%)	7 (17%)	24 (37%)
Fluid & Blood products					
Pre-baseline crystalloid, ml	0 (0 – 250)	200 (0 – 500)	150 (0 – 500)^^	500 (0 – 1000)*	225 (0 – 1000)
Pre-baseline PRBC, units	0 (0 – 0)	0 (0 – 0)	0 (0 – 0)^	0 (0 – 0)	0 (0 – 0)
12hr Crystalloid, ml	1000 (0 – 2000)	1000 (350 – 2388)	1000 (250 – 2075)	1625 (425 – 2788)	1500 (500 – 3150)
12hr PRBC, units	0 (0 – 0)	0 (0 – 2)	0 (0 – 1)^	0 (0 – 5)*	2 (0 – 8)

Outcomes					
Massive Transfusion, n (%)	8 (1.4%)	6 (2.2%)	4 (1.7%)	2 (4.9%)	13 (20%)
VTE, n (%)	9 (1.6%)	5 (1.8%)	5 (2.2%)	0 (0%)	1 (1.5%)
24hr mortality, n (%)	11 (1.9%)	13 (4.8%)	0 (0%)^	13 (31.7%)**	16 (24.6%)
28-day mortality, n (%)	41 (7.1%)	41 (15.0%)	0 (0%)^^	41 (100%)**	20 (30.8%)

All values are median with interquartile range unless specified. Continuous data compared with Mann-Whitney U-test. Categorical data compared with Fisher's exact test. ^p<0.05, ^^p<0.001, ML_{NORMAL} vs ML_{LOW} Survived. *p<0.05, **p<0.001, ML_{LOW} Survived vs ML_{LOW} + Died. SBP, systolic blood pressure. GCS, Glasgow coma score. BD, base deficit. INR, international normalized ratio. ML, maximum lysis. ISS, injury severity score. AIS, abbreviated injury score. PRBC, packed red blood cells. VTE, venous thromboembolism.

ML_{LOW} patients who died were nearly three times as likely to receive a massive transfusion and were four times as likely to be coagulopathic on admission (63% INR >1.2) than ML_{LOW} patients who survived (Table 9). Despite the low ML, D-dimer levels in patients who died were extremely high (median 103,170 ng/ml), compared to 13,672 ng/ml in survivors ($p<0.001$, Table 9, Figure 14). These D-dimer differences were replicated when analysing coagulopathic vs. non-coagulopathic patients (Figure 15) and red cell transfusion requirements (Figure 16). For further analysis we therefore set a D-dimer threshold at 30,000 ng/ml (approximately the upper quartile in ML_{NORMAL} patients, Table 9) and used this as a biological marker to explore the heterogeneity within the ML_{LOW} patient cohort.

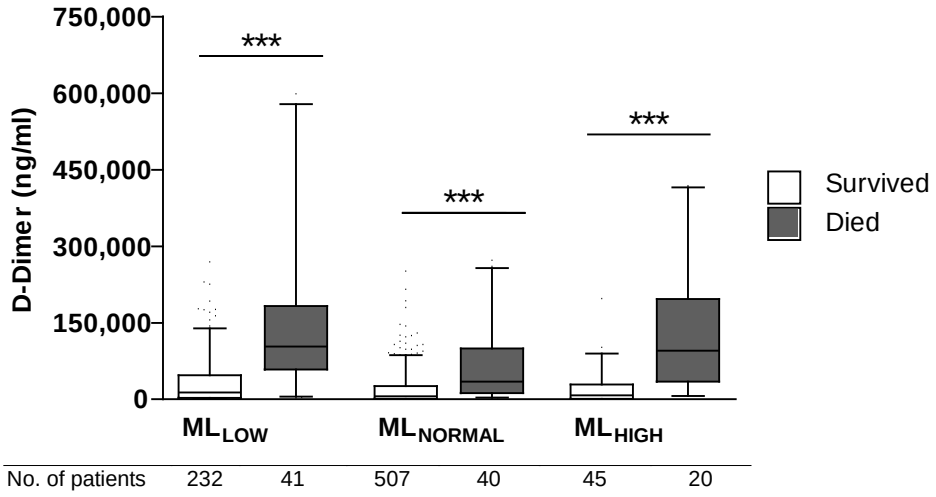


Figure 14 Admission D-dimer levels stratified by ML and 28-day mortality outcome

D-dimer levels were significantly elevated in those patients who died compared to survivors, irrespective of ML group. ***p<0.001 comparing survived vs died within each ML group.

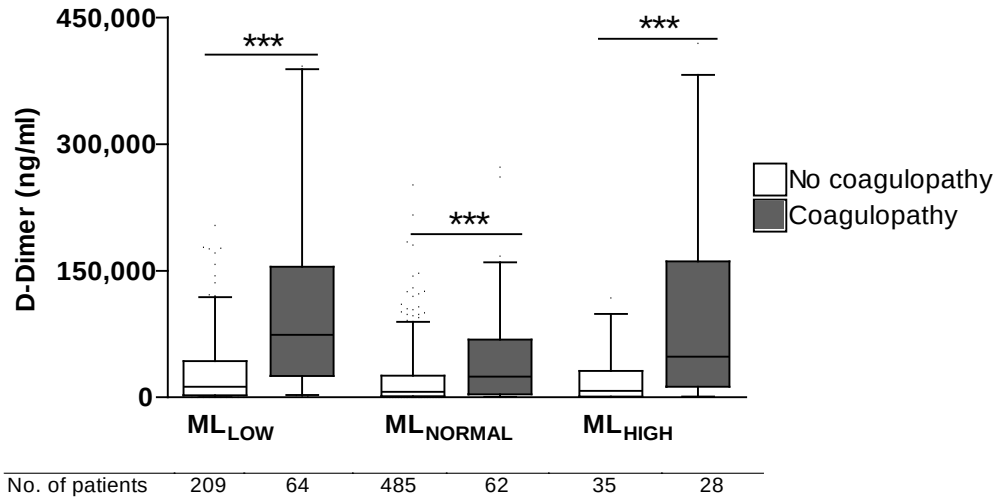


Figure 15 Admission D-dimer levels stratified by ML and presence of coagulopathy

Patients with coagulopathy on admission had significantly higher D-dimer levels compared to patients without coagulopathy, irrespective of admission ML. ***p<0.001 comparing coagulopathy vs no coagulopathy within each ML group.

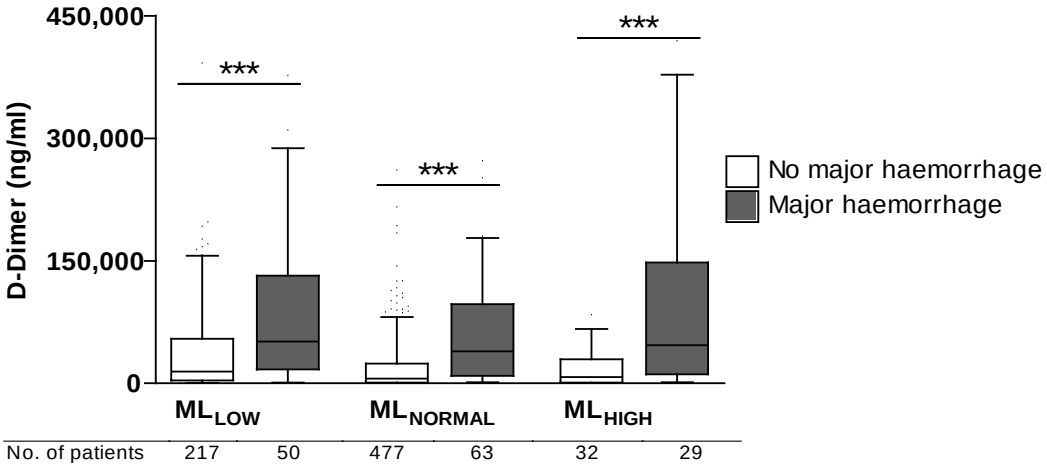


Figure 16 Admission D-dimer levels stratified by ML and presence of major haemorrhage

Patients treated for major haemorrhage had significantly higher D-dimer levels irrespective of admission ML value. ***p<0.001 comparing major haemorrhage vs no major haemorrhage within each ML group.

More than half (58%) of patients with ML_{LOW} on admission had low D-dimer levels. Patients with ML_{LOW} and DD_{LOW} were essentially identical to patients who were ML_{NORMAL} in terms of injuries, admission physiology and outcomes (Table 10). The only significant difference between the two groups was that ML_{LOW} + DD_{LOW} patients were more likely to have received pre-baseline crystalloid (51% vs 37%, p=0.002).

Table 10 Characteristics and outcomes of patients grouped by fibrinolytic profile

	ML_{NORMAL}	ML_{LOW} + DD_{LOW}	ML_{LOW} + DD_{HIGH}	ML_{HIGH} + DD_{LOW}	ML_{HIGH} + DD_{HIGH}
Number of Patients	**576**	**157**	**116**	**38**	**27**
Admission characteristics					
Age, years	35 (25 – 51)	39 (29 – 52)^	48 (34 – 66)*	44 (24 – 53)	41 (24 – 59)
Male, n (%)	464 (81%)	127 (81%)	85 (73%)	29 (76%)	19 (70%)
GCS	15 (12 – 15)	15 (13 – 15)	12 (5 – 15)**	15 (13 – 15)	11 (9 – 14)
SBP, mmHg	133 (112 – 149)	134 (115 – 149)	126 (100 – 148)*	124 (110 – 140)	98 (87 – 128)$^{+}$
BD, mmol/L	1.1 (-0.7 – 3.5)	1.5 (-0.3 – 3.9)	3.3 (1.3 – 6.0)**	3.0 (1.0 – 7.8)	11.4 (6.8 – 21.9)$^{++}$
INR > 1.2, n (%)	66 (11.5%)	18 (11.5%)	46 (40%)**	11 (29%)	17 (63%)$^{+}$
EXTEM ML, %	8 (6 – 10)	3 (2 – 4)^^	2 (1 – 4)*	19 (16 – 36)	98 (25 – 100)$^{++}$
D-Dimer, ng/ml	7150 (1320 – 29311)	5635 (1416 – 13504)^^	75738 (48291 – 131466)**	5056 (906 – 11496)	84286 (46600 – 178106)
Injury to sample time, minutes	75 (50 – 98)	72 (48 – 93)	93 (73 – 114) **	73 (48 – 90)	92 (62 – 105)
Injury characteristics					
Blunt, n (%)	464 (81%)	136 (87%)	113 (97%)*	30 (79%)	25 (93%)
ISS	13 (5 – 25)	13 (5 – 23)	29 (20 – 38)**	9 (2 – 25)	29 (10 – 38)
AIS Head & Neck ≥ 3, n (%)	153 (27%)	41 (26%)	69 (59%)**	7 (18%)	10 (37%)$^{+}$
AIS Thorax ≥ 3, n (%)	192 (33%)	41 (26%)	75 (65%)**	6 (16%)	15 (56%)
AIS Abdo/pelvis ≥ 3, n (%)	58 (10%)	10 (6%)	16 (14%)*	4 (11%)	4 (15%)
AIS Extremity ≥ 3, n (%)	149 (26%)	42 (27%)	34 (29%)	13 (34%)	11 (41%)
Fluid & Blood product totals					
Pre-baseline crystalloid, ml	0 (0 – 250)	100 (0 – 500)^	375 (0 – 750)**	0 (0 – 500)	500 (0 – 1500)
Pre-baseline PRBC, units	0 (0 – 0)	0 (0 – 0)	0 (0 – 0)	0 (0 – 0)	0 (0 – 0.5)$^{+}$
12hr Crystalloid, ml	1000 (0 – 2000)	1000 (0 – 1500)	1632 (600 – 2763)**	1600 (0 – 3000)	1500 (750 – 3900)
12hr PRBC, units	0 (0 – 0)	0 (0 – 0)	0 (0 – 4)**	0 (0 – 4)	6 (1.5 – 16)$^{++}$

Outcomes					
Massive Transfusion, n (%)	8 (1.4%)	1 (0.6%)	5 (4.3%)	5 (13.2%)	8 (29.6%)$^{++}$
VTE, n (%)	9 (1.6%)	0 (0%)	5 (4.3%)*	1 (2.6%)	0 (0%)
Critical Care LOS (survivors)	0 (0 – 3)	0 (0 – 2)	7 (2 – 14)**	5 (1 – 19)	12 (1 – 21)
Hospital LOS (survivors)	7 (2 – 16)	7 (2 – 17)	23 (13 – 40)**	5 (1 – 19)	26 (12 – 38)
24hr mortality, n (%)	11 (1.9%)	4 (2.5%)	9 (7.8%)	2 (5.3%)	14 (51.9%)$^{++}$
28-day mortality, n (%)	41 (7.1%)	6 (3.8%)	35 (30.2%)**	4 (10.5%)	16 (59.3%)$^{+}$
Time to death, days	2 (1 – 7)	1 (0 – 6)	2 (1 – 7)	2 (0 – 7)	0 (0 – 1)$^{++}$

All values are median with interquartile range unless specified. Continuous data compared with Mann-Whitney U-test. Categorical data compared with Fisher's exact test. ^p<0.05, ^^p<0.001, ML_{NORMAL} vs ML_{LOW} + DD_{LOW}. *p<0.05, **p<0.001, ML_{LOW} + DD_{HIGH} vs ML_{LOW} + DD_{LOW}. $^{+}$p<0.05, $^{++}$p<0.001, ML_{HIGH} + DD_{HIGH} vs ML_{LOW} + DD_{HIGH}. GCS, Glasgow coma score. SBP, systolic blood pressure. BD, base deficit. INR, international normalized ratio. ML, maximum lysis. ISS, injury severity score. AIS, abbreviated injury score. PRBC, packed red blood cells. VTE, venous thromboembolism. LOS, length of stay in days.

3.4.3 Characteristics of patients presenting with low ML but high D-dimer levels

Mortality was 30% in the ML_{LOW}+DD_{HIGH} cohort compared to only 3% in the ML_{LOW}+DD_{LOW} group (p<0.001, Table 10). Deaths occurred later in the ML_{LOW}+DD_{HIGH} cohort compared to ML_{HIGH}+DD_{HIGH} patients, who tended to die on the day of admission (Figure 17, Table 10). ML_{LOW}+DD_{HIGH} patients were more severely injured overall (median ISS 29) and were more than twice as likely to have a severe head or torso injury as the ML_{LOW}+DD_{LOW} cohort. ML_{LOW}+DD_{HIGH} patients were also more shocked and coagulopathic (by INR) on admission and had greater fluid and blood product requirements in the first 12 hours than those with ML_{LOW}+DD_{LOW}. Overall ML_{LOW}+DD_{HIGH} patients appeared to be severely injured and bleeding and were more like the ML_{HIGH}+DD_{HIGH} group. The ML_{HIGH}+DD_{HIGH} patients had a greater severity of shock and much higher transfusion requirements, but were less likely to have sustained a severe traumatic brain injury (Table 10).

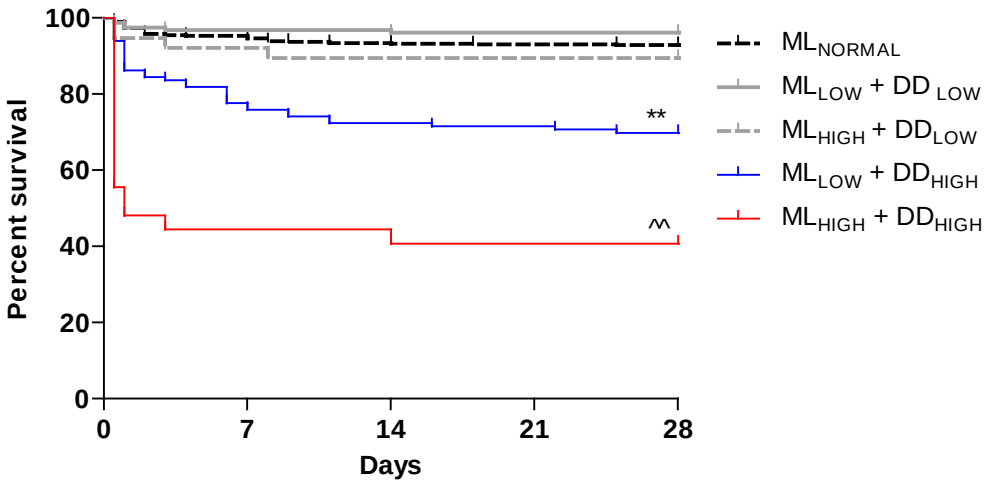


Figure 17 Kaplan-Meier curves for survival in patients grouped by fibrinolytic profile

Overall: Log-rank (Mantel-Cox) test for all groups, p<0.0001.

Comparing individual groups: ML_{LOW} + DD_{LOW} vs ML_{NORMAL}, p=0.15; **ML_{LOW} + DD_{HIGH} vs ML_{LOW} + DD_{LOW}, p<0.0001; ^^ML_{HIGH} + DD_{HIGH} vs ML_{LOW} + DD_{HIGH}, p = 0.0003.

3.4.4 Influence of time from injury on fibrinolytic profile

The time from injury to baseline blood sampling was longer in those patients presenting with DD_{HIGH} (Table 10). The association between time from injury and fibrinolytic profile was analysed by breaking down time from injury into quartiles. There was no association between time to baseline sampling after injury and functional fibrinolysis measured by ML (Figure 18). However, patients sampled 76 minutes or longer after injury (quartiles 3 and 4) were found to have significantly higher levels of D-dimer compared to those sampled within the shortest quartile (<53 minutes; Figure 19).

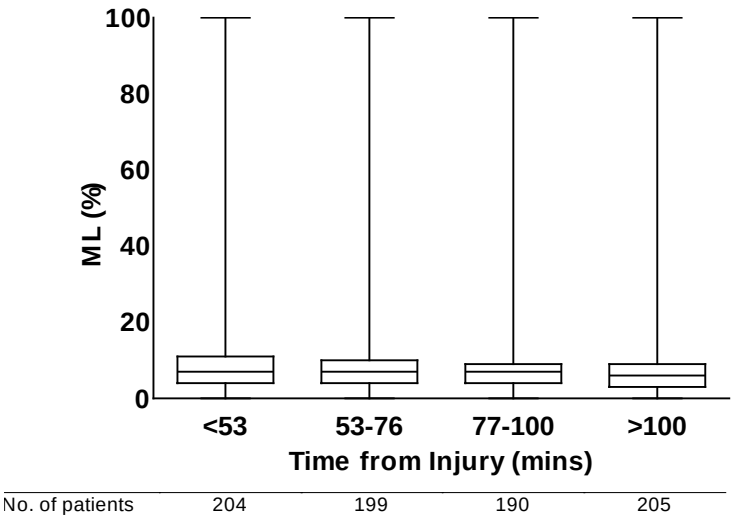


Figure 18 Association between time from injury to baseline sampling and maximum lysis

Whiskers represent minimum and maximum.

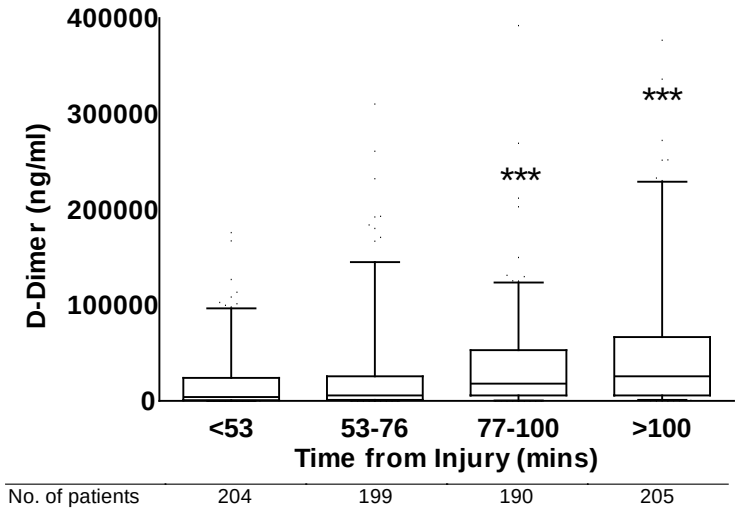


Figure 19 Association between time from injury to baseline sampling and D-dimer level

*****p<0.001 vs <53 mins**

Along with having the highest D-dimer levels, those patients sampled beyond 76 minutes from injury (quartiles 3 and 4), correspondingly were the most severely injured patients with significantly greater injury severity scores (Figure 20). Those patients in the 4th quartile with the longest duration of time from injury to baseline sampling had a greater degree of shock (Figure 21).

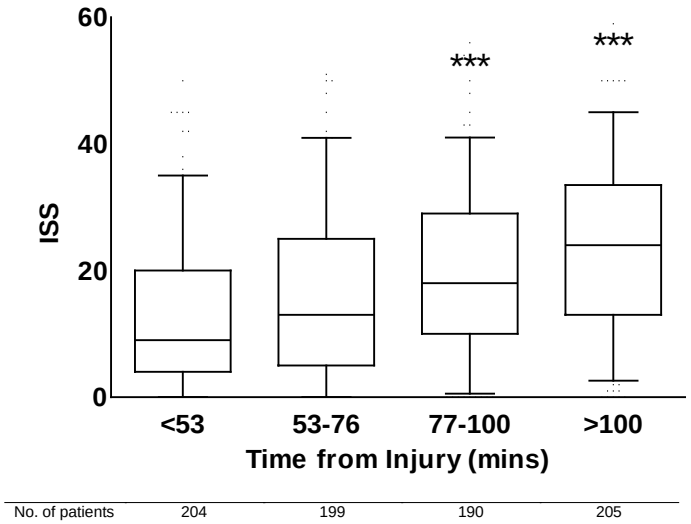


Figure 20 Association between time from injury to baseline sampling and injury severity

*****p<0.001 vs <53 mins**

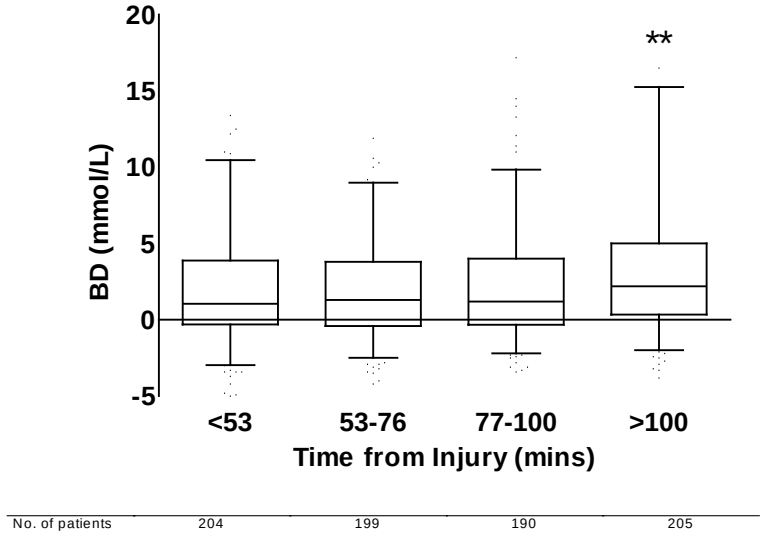


Figure 21 Association between time from injury to baseline sampling and degree of shock

****p<0.01 vs <53 mins**

3.4.5 Coagulation profiles of patients with low ML but high D-dimer levels

We examined the fibrinolytic mechanisms in these patient cohorts (Figure 22 - Figure 27). Across all low D-dimer cohorts, prothrombin fragments (PF1+2), fibrinogen, PAP, tissue plasminogen activator (tPA), plasminogen activator inhibitor (PAI-1) and α2-antiplasmin (α2AP) levels were similar.

Patients with ML_{LOW}+DD_{HIGH} had the highest levels of prothrombin fragments of all cohorts, associated with a reduction in fibrinogen levels (Figure 22 & Figure 23). As expected they also had high PAP levels indicative of increased fibrinolytic activity (Figure 24). Surprisingly tPA levels in patients with ML_{LOW}+DD_{HIGH} were low and comparable to the ML_{NORMAL} and ML_{LOW}+DD_{LOW} cohorts (Figure 25). Plasma levels of PAI-1 were the same across all groups (Figure 26). Antiplasmin levels were low in the ML_{LOW}+DD_{HIGH} group (72 vs ML_{NORMAL} 102 u/dL, $p<0.001$) and even lower in ML_{HIGH}+DD_{HIGH} patients (median 42.0 u/dL, $p<0.001$ vs. ML_{NORMAL}; Figure 27).

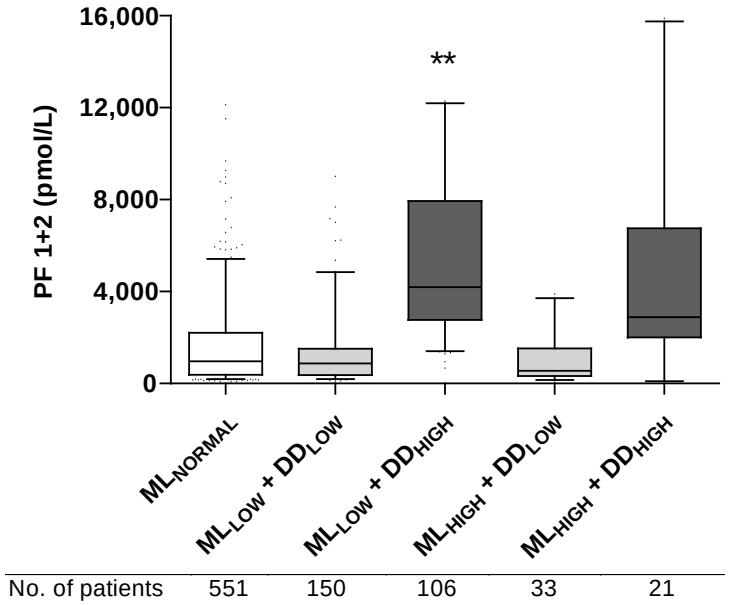


Figure 22 Prothrombin fragment levels in patients grouped by fibrinolytic profile

Prothrombin fragment (PF 1+2) levels across all maximum lysis (ML) and D-dimer (DD) groups with the highest levels seen in the ML_{LOW} + DD_{HIGH} patients. **p<0.001, ML_{LOW} + DD_{LOW} vs ML_{LOW} + DD_{HIGH}

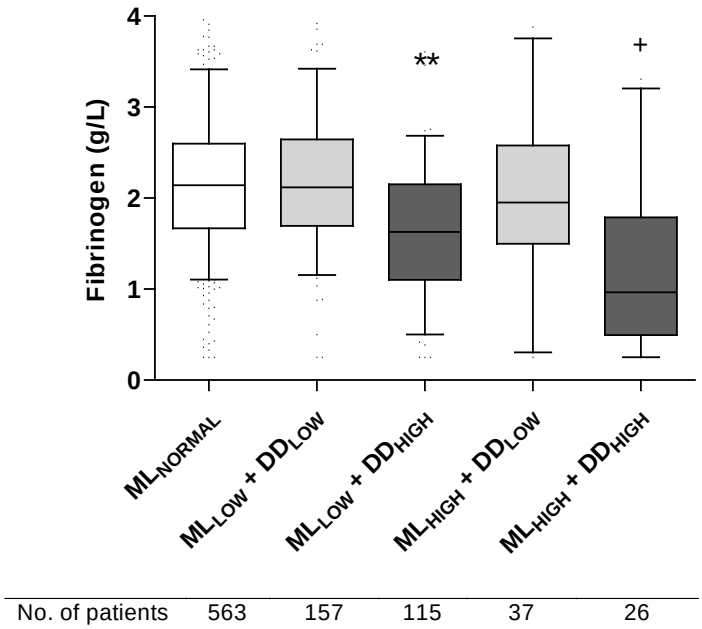


Figure 23 Fibrinogen levels in patients grouped by fibrinolytic profile

**** p<0.001, ML_{LOW} + DD_{LOW} vs ML_{LOW} + DD_{HIGH}; $^{+}$p<0.05, ML_{LOW} + DD_{HIGH} vs ML_{HIGH} + DD_{HIGH}**

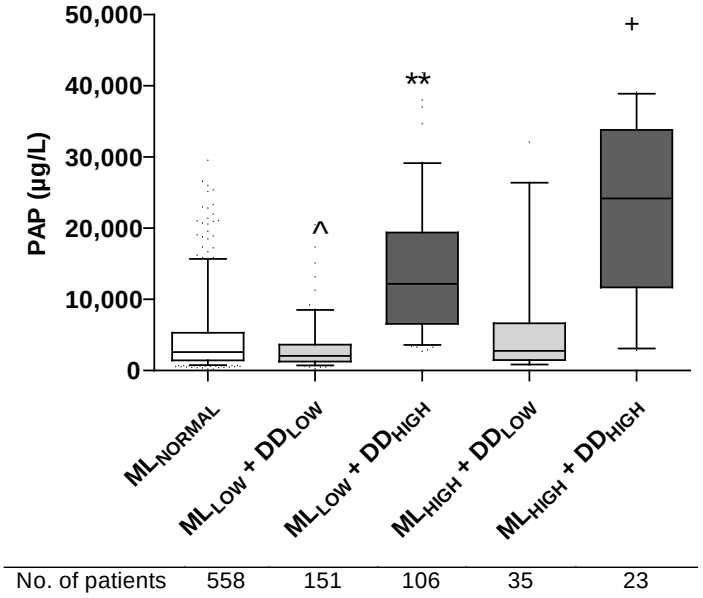


Figure 24 Plasmin-α2-antiplasmin complex in patients grouped by fibrinolytic profile

Plasmin-α2-antiplasmin complex (PAP) across all ML and DD groups indicating high levels of fibrinolytic activity in patients with DD_{HIGH} irrespective of ML. ^p<0.05, ML_{LOW} + DD_{LOW} vs ML_{NORMAL}; **p<0.001, ML_{LOW} + DD_{LOW} vs ML_{LOW} + DD_{HIGH}; $^{+}$p<0.05, ML_{LOW} + DD_{HIGH} vs ML_{HIGH} + DD_{HIGH}

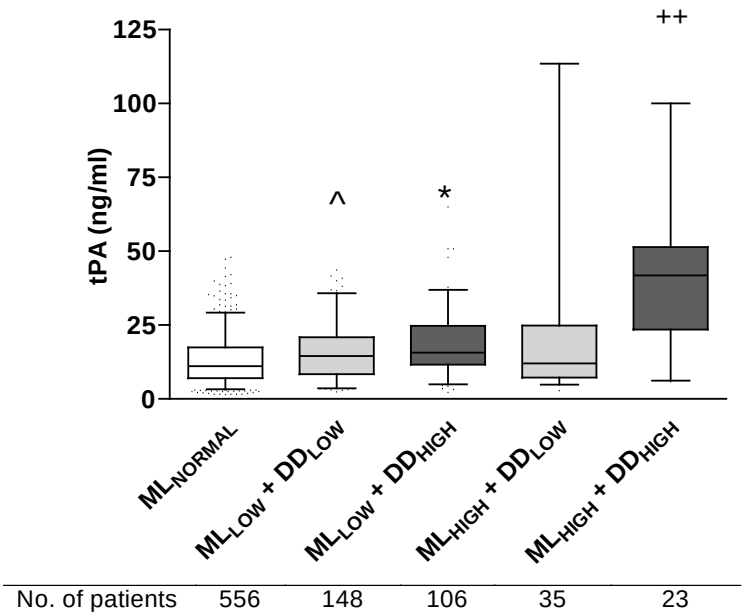


Figure 25 Tissue plasminogen activator (tPA) levels in patients grouped by fibrinolytic profile

Despite an overall hyperfibrinolytic biomarker profile, patients with ML_{LOW} + DD_{HIGH} did not exhibit the rise in tPA seen in those patients with ML_{HIGH} + DD_{HIGH}. ^p<0.05, ML_{LOW} + DD_{LOW} vs ML_{NORMAL}; *p<0.05, ML_{LOW} + DD_{LOW} vs ML_{LOW} + DD_{HIGH}; $^{++}$p<0.001, ML_{LOW} + DD_{HIGH} vs ML_{HIGH} + DD_{HIGH}

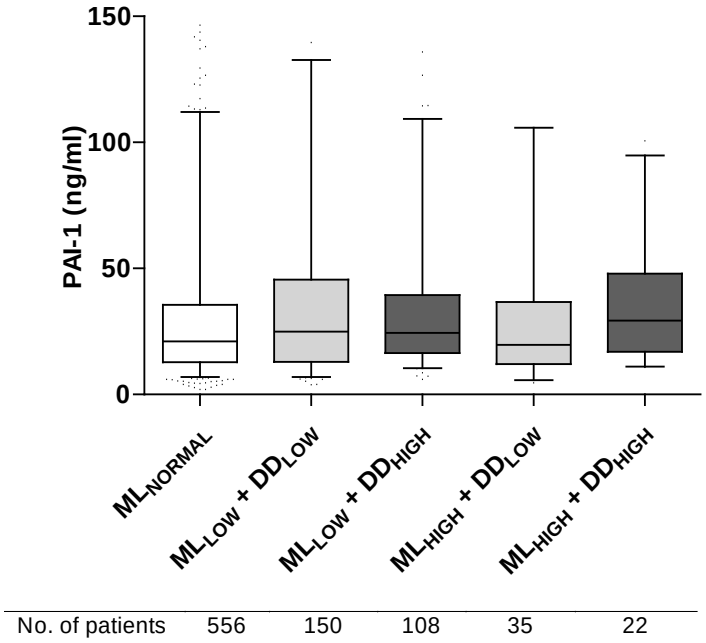


Figure 26 Plasminogen activator inhibitor (PAI-1) levels in patients grouped by fibrinolytic profile

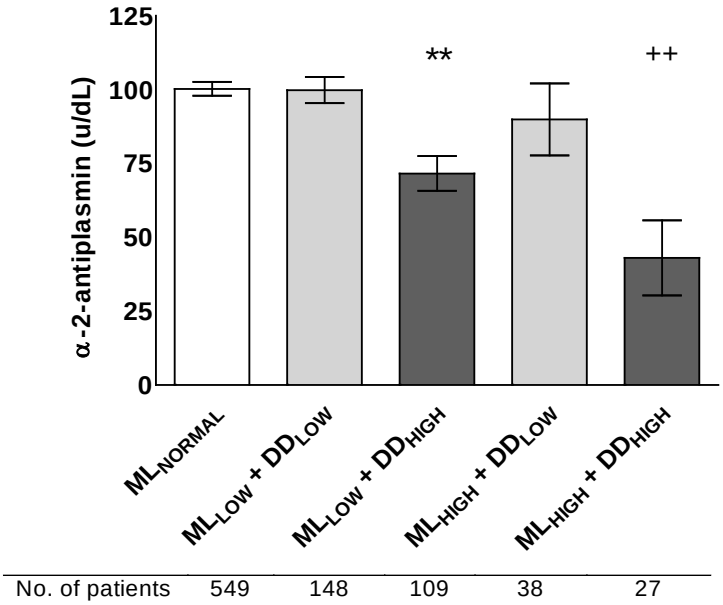


Figure 27 α-2-antiplasmin levels in patients grouped by fibrinolytic profile

Bars represent mean ± 95% CI. **p<0.001, ML_{LOW} + DD_{LOW} vs ML_{LOW} + DD_{HIGH}; $^{++}$p<0.001, ML_{LOW} + DD_{HIGH} vs ML_{HIGH} + DD_{HIGH}

3.4.6 Mechanism of occult hyperfibrinolysis in trauma patients

As ML_{LOW}+DD_{HIGH} patients had high thrombin generation and high fibrinolytic activity but no elevations in the expected plasminogen activators nor increased levels of plasminogen/plasmin inhibitors, we, therefore, proceeded to measure levels of uPA and S100A10, a principally membrane-bound plasminogen receptor and fibrinolysis mediator, in a subgroup of 179 patients.

These analyses were performed by Paul Vulliamy. The results are detailed in Appendix 1 and discussed below.

3.5 DISCUSSION

In this multicentre study of over 900 patients, we have shown that low levels of VHA detected fibrinolysis are physiological in over half of all cases and have very good outcomes, comparable to patients with normal fibrinolysis. Conversely, some patients with low VHA-fibrinolysis are severely injured, functionally coagulopathic, have high transfusion requirements and high mortality. These patients have a hyperfibrinolytic biomarker profile with high levels of thrombin, plasmin and D-dimer generation but have relatively low levels of tPA. We have identified S100A10 as a possible explanation for localised activation of plasmin on the endothelial cell surface in these patients which when shed into the plasma artificially lowers the observed VHA ML% parameter *ex-vivo*. Together these results support the existence of an occult hyperfibrinolysis in severely injured patients, not detectable by existing diagnostic tools and which is associated with very poor outcomes.

S100A10 is a membrane-bound plasminogen receptor which complexes with annexin A2 in a hetero-tetramer formation to bind tPA and plasminogen on the cell surface in close proximity to the uPA receptor (uPAR).[55,162,163] S100A10 is widely expressed in body tissues and particularly in the brain[164] and the complex is upregulated at times of hypoxic stress.[165] S100A10 levels are detectable in the blood but the majority of cellular plasmin is formed on the endothelium with S100A10 catalysing the t-PA mediated activation of plasminogen.[166] The classical presentation of Acute Promyelocytic Leukaemia (APL) is a haemorrhagic and fibrinolytic phenotype characterised by normal levels of tPA but high surface expression of S100A10 on APL cells.[167] Our findings suggest that S100A10 is exposed by tissue injury leading to plasmin generation and hyperfibrinolysis without the need for high levels of circulating tPA.[168] These findings suggest a direct explanation for

the catastrophic coagulopathy often seen with severe traumatic brain injury, the mechanism of which has remained elusive and the severity of which is not apparent on diagnostic tests.

Plasmin has a very short half-life and S100A10 is known to increase plasmin autoproteolysis.[169] *Ex-vivo* detection of fibrinolysis by VHA devices relies on the presence of plasmin to lyse clot forming in the measuring cup. We have shown that the addition of S100A10 to blood reduces VHA-detected lysis. In patients with low tPA levels there is no other available activator of plasminogen and no fibrinolysis will be detected. When tPA levels are high, the effect of S100A10 will be overcome and fibrinolysis will be revealed. These findings are entirely consistent with all observed studies of hyperfibrinolysis in trauma patients.

There has been controversy in the literature as to whether ATC patients with ML_{LOW}+DD_{HIGH} have ongoing hyperfibrinolysis or have had their fibrinolysis shutdown at some time immediately before blood sampling. These patients are severely injured and have large on-going blood product requirements. They have a high incidence of coagulopathy with an underlying biochemistry of high PAP and D-dimer levels. Taken together these features suggest active hyperfibrinolysis. It is difficult to support a fibrinolysis shutdown model which would require explanation of how tPA levels could have suddenly become low given that the active pathological processes of injury, shock and bleeding have not yet been controlled and PAI-1 levels are not elevated. Localised hyperfibrinolysis via a cell bound activator of plasminogen is a more plausible mechanism and matches the observed on-going bleeding and transfusion requirements in these patients. This occult hyperfibrinolysis in ATC does not preclude the likely existence of a later fibrinolytic shutdown in trauma patients due to upregulation of PAI-1 and other control mechanisms.

Tranexamic acid's effective reduction of mortality in trauma patients appears to extend beyond reducing blood loss and death from bleeding to affect all-cause mortality.[116,117] Our results suggest that severe tissue injury can induce hyperfibrinolysis through S100A10 and tranexamic acid may therefore reduce local as well as systemic haemorrhage. In traumatic brain injury this might have a profound effect on outcome and we found S100A10 levels correlated closely with coagulopathy, blood use and mortality in severe TBI patients. S100A10 may provide the mechanistic rationale for the use of tranexamic acid in TBI and the results of on-going clinical trials are anticipated soon.[126,170] Overall, our results confirm an occult hyperfibrinolysis which is prevalent in trauma patients and supports the empiric use of tranexamic acid until new diagnostics are available. Restricting treatment only to patients with VHA detected lysis may exclude 80% of trauma patients with high D-dimer levels who may benefit from tranexamic acid.

Interestingly, patients with longer durations from injury to baseline sampling, were more severely injured, with a greater degree of shock and associated higher D-dimer levels. Time from injury to baseline sampling was not associated with differences in VHA fibrinolysis. Blunt trauma predominated in the two D-dimer high groups and one potential explanation for this association is that the most severely injured patients had longer on-scene times with potential need for extrication, immediate life-saving interventions by the attending HEMS team and longer transit times to RLH. The influence of time on groupings based upon fibrinolytic profile, creates a potential bias in that those sampled quickly after trauma may have a low D-dimer level which had the time interval to sampling been longer could have resulted in a higher D-dimer result and placed the patient within a different phenotype. Further research into the temporal changes in fibrinolysis are required to better understand the influence time has on the fibrinolytic phenotypes described within this body of work.

The inclusion criteria for this study prevent us from determining the absolute incidence of each of these subtypes of TIC. As patients with minor injuries and without shock were excluded, overall rates of hyperfibrinolysis are likely to be overestimates compared to a general trauma population. As the ML_{LOW}+DD_{HIGH} S100A10-driven subtype is essentially exclusive to blunt trauma, its incidence will also vary depending on rates of blunt trauma seen in specific institutions. Our exclusion of patients who had already received tranexamic acid is also likely to have led to an underestimate of the numbers in the ML_{HIGH} subtypes. A survivor bias can also not be excluded from this observational study. Those critically ill patients who die early from haemorrhage will then not be alive to go on to develop thrombotic complications for example and consequently rates reported within the different fibrinolytic phenotypes could be an underestimate.

There are several aspects of this work that require further experimental research. This clinical study cannot definitively state that the ML_{LOW}+DD_{HIGH} patients still have active fibrinolysis after the point of sampling. Our finding of high circulating levels of the primarily tissue-bound fibrinolytic activator S100A10 is circumstantial, but coupled with grossly elevated PAP & D-dimer and no obvious inhibitors is highly suggestive of active lysis, as are subsequent high blood product requirements. However, it will require both experimental evidence of active lysis and clinical trial evidence of the effect of TXA in this subgroup to confirm this. The mechanisms of exposure and release of S100A10 also need exploring, especially in the context of traumatic brain injury. Again, both clinical and experimental studies will be required to understand the role of S100A10 in trauma induced coagulopathy.

3.6 CONCLUSION

We have shown that VHA-detected lysis (ML) is a poor indicator of both the fibrinolytic state and clinical outcomes of patients. Absolute D-dimer levels were more strongly associated with outcomes. Patients with low D-dimer levels had good outcomes regardless of ML. In contrast, a specific phenotype of patients exists with elevated D-dimers but low ML who have poor outcomes. These patients are critically injured and have a high incidence of severe traumatic brain injury. We have identified the tissue-bound S100A10 fibrinolytic mediator as a candidate mechanism for hyperfibrinolysis in these patients that also results in an artificially low ML. S100A10 exposure after critical injury represents a new precision marker and therapeutic opportunity in trauma-induced coagulopathy.

3.7 SPECIFIC CONTRIBUTIONS OF THE AUTHOR

Following the publication of the concept of "Fibrinolysis Shutdown" by the Denver research group, the Author along with supervisors Karim Brohi (KB) and Ross Davenport (RD) conceived the idea of replicating the study using ROTEM data. The Author designed the study in conjunction with KB and RD. Data collection, primary analysis and initial interpretation of results was performed by the Author. Further review of results and data interpretation was performed in conjunction with KB and RD. This written account of our research findings was written by and then re-drafted by the Author following review and comment by RD and KB. The Author was responsible for the creation of all tables and figures within this chapter.

Chapter 4

THE EFFECTS OF EARLY EMPIRIC TRANEXAMIC ACID ON TRAUMA-INDUCED COAGULOPATHY DURING TRAUMA HAEMORRHAGE

4.1 CHAPTER ABSTRACT

BACKGROUND: Tranexamic acid (TXA) is widely used empirically in trauma haemorrhage. However, the effect of early empiric TXA on the coagulation system during haemorrhage has not been described. Theoretically both antifibrinolytic and antifibrinogenolytic, it is unknown where it exerts most of its effect and whether the standard dose is adequate for trauma patients. We aimed to investigate the biochemical and functional coagulation responses of early empiric TXA on the fibrinolytic system in patients during major trauma haemorrhage.

METHODS: Adult trauma patients for whom the major haemorrhage protocol was activated were prospectively recruited. Blood was drawn on admission to the emergency department and during resuscitation (after transfusion of 4, 8, and 12 red blood cell [PRBC] units) for thromboelastometry. Patients who received TXA before admission blood draw (TXA group) were compared with patients who did not (No TXA group).

RESULTS: 222 patients were included (113 No TXA group, 109 TXA group) for functional coagulation analysis and of these 162 patients (102 No TXA, 60 TXA) had biomarker analysis. No patients receiving early empiric TXA (1g) exhibited functional hyperfibrinolysis on admission (No TXA vs TXA 20% vs 0%, p<0·0001) or during haemorrhage, despite an equally high degree of initial fibrinolytic system activation (plasmin-α2-antiplasmin complex: 10595 vs 8798µg/L, p=0.41). Reduced fibrinolysis following massive haemorrhage was evidenced by lower D-dimer levels in the TXA group (after 12 PRBC: 30.4 vs 12.1µg/ml, p=0.005). Following massive blood transfusion, clot strength was maintained in the TXA group compared with the no TXA group (maximum clot firmness after 12 RBC: 44 vs 53mm, p=0·008). TXA was associated with a degree of protection from trauma-induced coagulopathy (TIC) during ongoing haemorrhage (TIC after 12 RBC: 100% vs 67%, p=0·21).

CONCLUSIONS: Early empiric TXA avoids functional hyperfibrinolysis during trauma haemorrhage and protects against the development of TIC after massive transfusion.

ACKNOWLEDGEMENTS: Scarlett Gillespie and Timothy Jones are duly acknowledged for their assistance in performing biomarker assays.

4.2 INTRODUCTION

Tranexamic acid (TXA) is an antifibrinolytic agent which appears to improve outcomes in patients with traumatic haemorrhage. Bleeding kills patients within hours following injury and contributes to 40% of all trauma related deaths.[3] Evidence from the civilian and military literature suggests that TXA can improve survival and reduce organ failure in trauma patients with suspected active haemorrhage.[116–118] While the empirical administration of TXA has been adopted into guidelines[102,106] and incorporated into institutional practices, its uptake worldwide remains variable[171–173], in part because its effects on the coagulation system during haemorrhage have not been described.

Trauma-induced coagulopathy (TIC) exacerbates haemorrhage and is associated with greater transfusion requirements, higher levels of organ dysfunction and a four-fold increase in mortality.[5,6,159] Fibrinolytic activation is almost universal after severe injury, with hyperfibrinolysis now recognized as a key component of TIC.[6,7] Administration of an antifibrinolytic to directly inhibit fibrinolysis is therefore based on a sound biological principle with the potential to improve clotting and reduce bleeding. The effect of TXA on the key stages and components of coagulation e.g. initiation, clot formation, fibrinogen and fibrinolysis has not been described during active bleeding in trauma. It is unknown whether if dosed empirically (1g) as described in the CRASH-2 trial, TXA fully reverses post-injury fibrinolysis and how long this effect persists for in the bleeding patient.[116] Understanding the effect of TXA on coagulation during trauma haemorrhage may provide insights into its mechanisms of action and clarify its role in the management of bleeding trauma patients.

The overall objective of this study was to investigate the effect of early TXA on the coagulation system in patients with suspected traumatic haemorrhage. Our first aim was to

describe the effect of early TXA on the incidence of hyperfibrinolysis and coagulopathy upon arrival in the emergency department (ED) and during trauma haemorrhage. The second aim was to characterize the effect of early TXA on functional coagulation tests. Third, we aimed to investigate the effect of early TXA on plasma markers of fibrinolysis and coagulation. Finally, we wished to describe the effect of early TXA on thrombosis risk and overall mortality in this cohort. We analysed data from a prospective cohort study of patients with suspected traumatic haemorrhage, presenting to an urban major trauma centre.

4.3 METHODS

All adult patients (≥16 years) who meet the local criteria for trauma team activation were consecutively screened (between 8:00am – 8:00pm daily) for inclusion in the Activation of Coagulation and Inflammation in Trauma study (ACIT II; UK CRN ID 5637). Deferred informed written consent was obtained from the patient or their next-of-kin. The study was approved by East London and The City Research Ethics Committee (07/Q0603/29). Patients were excluded if they arrived >2 hours post injury; were transferred from another hospital; received >2000ml crystalloid pre-hospital; or had sustained burns >5% of their body surface area. Patients were retrospectively excluded if they declined to give consent to the use of their research samples, had severe liver disease, a known pre-existing bleeding diathesis, or were taking anticoagulant medication (excluding aspirin) pre-injury.

The empiric administration of TXA at our institution is restricted to patients who activate our Major Hemorrhage Protocol (MHP). The MHP is activated either pre-hospital by the physician-led London Air Ambulance (LAA) or in-hospital by the trauma team leader, when a patient with suspected traumatic haemorrhage, has a systolic blood pressure below 90

mmHg and a poor response to initial fluid resuscitation. The MHP was implemented in September 2008 and updated to formally include TXA in February 2011 (Appendix 2). Prior to February 2011, TXA was administered at the discretion of the trauma team leader within the hospital setting when haemorrhage was suspected or diagnosed. Following its formal inclusion within the protocol, TXA is delivered to trauma patients activating the MHP either as part of pre-hospital care or in the ED within three hours of injury, as a 1g bolus infused over 10 minutes. In hospital, this is followed by a further 1 g of TXA infused over eight hours. All patients in the TXA group received the 1 g bolus dose of TXA only, prior to baseline blood sampling on ED arrival. Pre-hospital blood transfusions are delivered by LAA as part of the resuscitation of bleeding trauma patients and were initiated in our trauma system in January 2014. For this study we analysed consecutive ACIT II patients who activated the MHP from January 2009 up to June 2015.

4.3.1 Data Collection

Data were collected prospectively and included patient demographics, time of injury, mechanism of injury (blunt or penetrating), Injury Severity Score (ISS), vital signs on-scene and on arrival in ED, total number of blood products and volume of intravenous fluids administered within the first 12 hours from injury. The administration of TXA and its timing relative to baseline blood sampling was documented.

Patients were observed for 28 days from injury for the occurrence of venous thromboembolic events (deep vein thrombosis (DVT) or pulmonary embolism (PE)), other thrombotic events (including myocardial infarction (MI) and ischemic stroke) and overall mortality. We defined these outcomes as a clinical diagnosis supported by a confirmatory

test result: duplex ultrasound for DVT; CT pulmonary angiogram for PE; electrocardiogram changes and elevated troponin for MI; and CT head for ischemic stroke.

4.3.2 Blood Sampling

The baseline research blood sample was drawn within 20 minutes of the patient's arrival in the ED along with standard trauma laboratory tests. A full blood count and conventional coagulation screen was performed as part of the standard trauma laboratory tests for determination of haemoglobin, platelet count and prothrombin time (PT) respectively. A point-of-care arterial blood gas analysis was performed simultaneously for base deficit (BD) and lactate concentration. Subsequent blood samples were obtained during the active haemorrhage phase: following the transfusion of 4 units of packed red blood cells (4PRBC), 8 units (8PRBC) and 12 units (12PRBC) from baseline sampling; and at 24 hours.

Blood for rotational thromboelastometry (ROTEM®) analysis, was collected in a 2.7ml citrated vacutainer (0.109 Molar / 3.2% sodium citrate; Becton, Dickinson and Company, Plymouth, UK). Blood for coagulation and fibrinolysis protein assays was collected in a 4.5ml glass citrated vacutainer (0.109 Molar / 3.2% sodium citrate; Becton, Dickinson and Company, Plymouth, UK). The filled 4.5ml vacutainer was centrifuged for 10 minutes at 1,750 G within 1 hour of collection. Plasma was removed and then centrifuged for a further 10 minutes at 1,750 G. The double-spun plasma was subsequently stored at -80°C.

4.3.3 Functional Coagulation Analysis

Functional coagulation analysis was performed within one hour of blood draw at 37°C on a ROTEM® delta instrument (Tem International GmbH, Munich, Germany) using the

automated electronic pipette according to the manufacturer's instructions. The methodology and parameters of ROTEM® have been described previously.[161] Two separate assays were performed on each blood sample: EXTEM, measuring tissue-factor initiated clotting and FIBTEM, for qualitative assessment of fibrinogen levels via the addition of the platelet inhibitor, cytochalasin D. Samples were run for 60 minutes and treating clinicians were blinded to the results. We defined coagulopathy as an EXTEM clot amplitude at 5 minutes (CA5) of ≤ 35mm. This cut-off has been shown to identify ATC and predicts transfusion requirements.[100] Hyperfibrinolysis was defined as an EXTEM maximum lysis (ML) of > 15% after a 60 minute assay time.[7]

4.3.4 Coagulation and Fibrinolysis Plasma Protein Assays

As part of the on-going ACIT II study, the first 1099 patients recruited (up to 15 April 2014) had coagulation and fibrinolysis protein assays performed. 102 patients from the No TXA group and 60 patients from the TXA group were contained within this cohort. Plasma stored at -80°C was thawed to 37°C immediately before all analyses. Prothrombin fragment 1+2 (PT Frag 1+2; Enzygnost® F 1+2 (monoclonal); Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany), tissue plasminogen activator (tPA; Asserachrom® tPA, Diagnostica Stago, Asnières sur Seine, France), plasminogen activator inhibitor-1 (PAI-1; Asserchrom® PAI-1; Diagnostica Stago), and plasmin-α2-antiplasmin complex (PAP; PAP micro ELISA; DRG Instruments GmbH, Marburg, Germany) were measured using sandwich enzyme-linked immunosorbent assays (ELISAs). Coagulation factors II (Siemens Innovin and Siemens Factor II deficient plasma, Sysmex UK, Milton Keynes, UK) and XIII (Siemens Berichrom Factor XIII, Sysmex UK), fibrinogen levels (Siemens Thrombin reagent, Sysmex UK) and α2-antiplasmin (Siemens Berichrom α2-antiplasmin; Sysmex UK) were determined in the hospital laboratories with a Sysmex CS2100i automated analyzer (Sysmex UK) according to standard

protocols. Latex immunoassays were used to quantify the levels of D-dimer (Siemens Innovance D-dimer; Sysmex UK) and soluble fibrin monomer complexes (sFMC; Stago Liatest Fibrin Monomer, Diagnostica Stago) also with the Sysmex CS2100i automated analyser.

4.3.5 Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 5 (GraphPad Software Inc., San Diego, USA) and Microsoft Excel 2013 (Microsoft Inc., Redmond, USA). Normal quantile plots were used to assess normality. Data without a normal distribution were expressed as median (interquartile range) and analysed using Mann-Whitney U-test. Normally distributed data are expressed as mean ± 95% confidence intervals and analysed with Student's t test. Percentages were analysed using Chi-squared or Fisher's exact tests, with linear trends over time analysed with Chi-squared test for trend. A p value of <0.05 was considered statistically significant. Where multiple time points were analysed (i.e. at baseline, 4PRBC, 8PRBC and 12PRBC), the two groups at each time point were compared individually and an adjusted p value of <0.0125 considered statistically significant to take account of multiple repeated tests performed. Box and whisker plots show median, interquartile range, maximum and minimum values unless otherwise stated.

4.4 RESULTS

A total of 284 patients activated the MHP and were recruited to ACIT II during the study period (Figure 28). Patients were excluded for the following reasons: 21 did not have a baseline ROTEM® performed; 14 in whom the timing of TXA administration was not documented; three were recruited into the CRASH-3 trial[126] and 24 either refused consent or were retrospectively excluded as they did not fulfil ACIT II inclusion criteria. The exclusion

of patients without a baseline ROTEM was necessary since the premise of this study was to use ROTEM to evaluate the effects of early TXA on TIC. The CRASH-3 trial randomised patients with traumatic brain injury to receive either TXA or placebo in a blinded manner. Patients co-recruited into this trial were excluded as it would not have been known whether they had received TXA or not.

A total of 222 patients were available for study analysis - 113 did not receive TXA pre-baseline sampling (No TXA group) vs 109 patients who did receive TXA pre-baseline sampling (TXA group). In the No TXA group, 23 patients received TXA post-baseline sampling but within three hours of injury. These patients were included in the No TXA group for the baseline analysis but were excluded from all subsequent PRBC, 24-hour and outcome analyses.

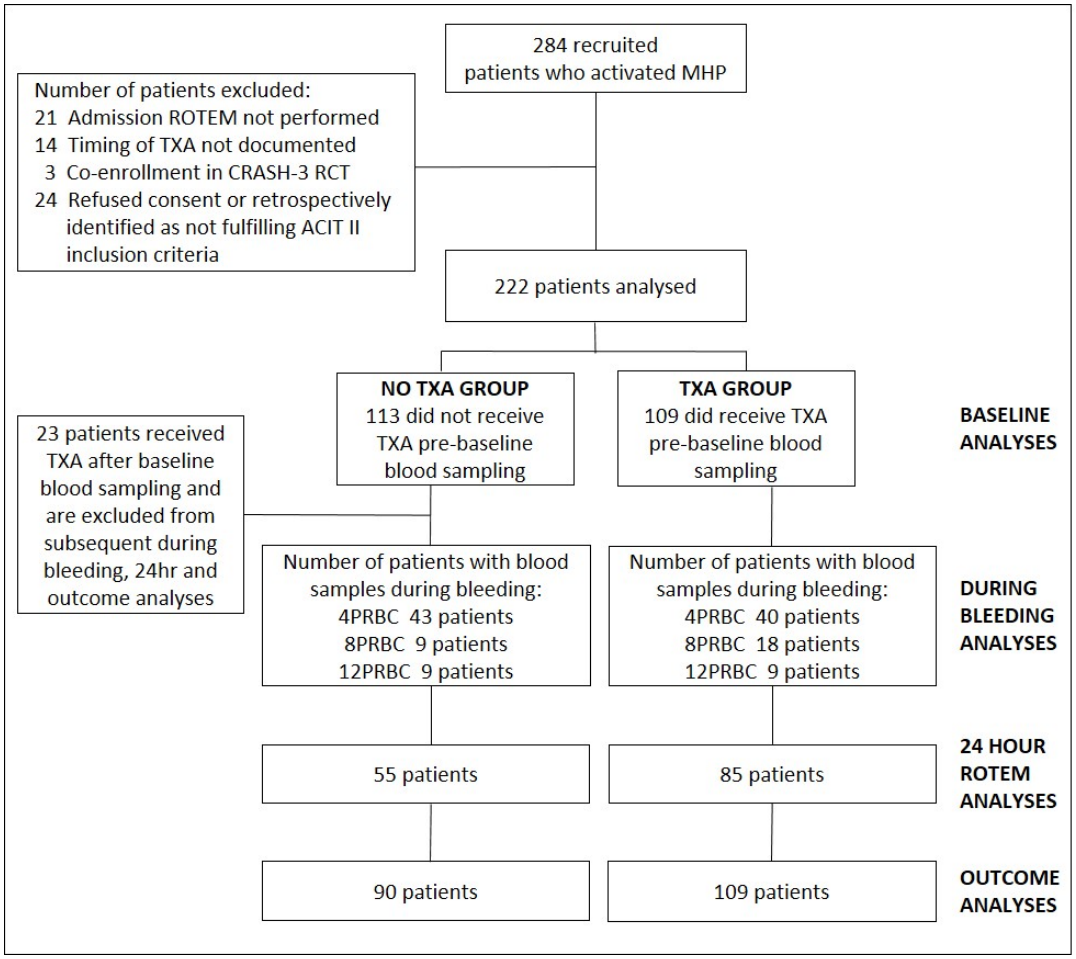


Figure 28 Flow diagram of study groups and number of patients analysed at each time point

MHP, major haemorrhage protocol; RCT, randomised control trial; PRBC, packed red blood cells

4.4.1 Patient characteristics

Baseline demographics and clinical characteristics of the patients are shown in Table 11. The median time from injury to ED arrival in the No TXA group vs TXA group was 90 (70 – 107) vs 85 (70 – 104) minutes, p=0.19. We observed a clear temporal difference in TXA administration during the study period. No patients received TXA in 2009 or 2010 compared with an annual average of 75% of patients who received TXA (pre-baseline blood sampling) each subsequent year from 2011 – 2015. All patients in the TXA group received a 1g bolus of TXA pre-baseline (86% administered pre-hospital, 14% in ED). Data on the administration

of the second 1g infusion dose of TXA was only available in 74 cases, with 37 (50%) of these patients found to have received the second 1g infusion of TXA. Patients treated with TXA had a greater degree of shock, with a lower on-scene systolic blood pressure and higher lactate on ED arrival (Table 11). Glasgow Coma Score (GCS) was lower in the TXA cohort but with no significant difference in Head Abbreviated Injury Scale (AIS) scores between the two groups (median 0). Sex, age, injury severity and vital signs were similar between the two groups of patients who had further plasma assay studies. As in the overall cohort, the TXA patients received a greater number of PRBC pre-baseline (median pre-baseline PRBC: No TXA vs TXA 0 vs 1 unit, $p<0.001$).

Blood product and intravenous fluid administration during the first 12 hours post injury are detailed in Table 12. The median time interval from baseline to 4, 8 and 12PRBC sampling was 51, 89 and 87 minutes respectively, with no significant difference observed between groups. More patients in the TXA group received a transfusion of at least one unit of PRBC pre-baseline (No TXA vs TXA: 26% vs 66%, $p<0.0001$) (Table 12). No patients received colloids, fresh frozen plasma (FFP), platelets or cryoprecipitate pre-baseline. During resuscitation, blood product and fluid administration was similar between the two groups up to the 8 PRBC time point. Between 8 and 12 units of PRBC, median FFP use was the same, however, the TXA group received a higher ratio of FFP with more platelets and cryoprecipitate (Table 12). Massive transfusion rates (≥10 PRBC in 12 hours) were similar between the two groups (No TXA vs TXA: 21% vs 22%, $p=0.89$). There was no statistical difference in the total number of individual blood components transfused to either group at 12 hours following injury (Table 12).

Table 11 Patient characteristics and outcomes

	No TXA	TXA	*p**
Patient and Injury Characteristics			
Number of patients	113	109	
Age, years	38 (24 – 53)	33 (24 – 53)	0.27
Male	90 (80)	73 (67)	0.03
Blunt injury	84 (74)	80 (73)	0.87
ISS	29 (18 – 37)	29 (20 – 43)	0.21
ISS > 15	97 (86)	92 (84)	0.76
On Scene Parameters			
Scene HR, bpm	113 (94 – 132)	120 (101 – 140)	0.16
Scene SBP, mmHg	110 (90 – 130)	97 (76 – 115)	0.01
Scene GCS	14 (8 – 15)	11 (4 – 14)	0.02
ED Admission Parameters			
ED HR, bpm	120 (96 – 138)	120 (102 – 135)	0.88
ED SBP, mmHg	102 (85 – 127)	102 (76 – 124)	0.50
ED GCS	13 (8 – 14)	10 (4 – 14)	0.03
Base deficit, mmol/L	6.4 (3.5 – 11.1)	8.3 (4.8 – 16.2)	0.06
Lactate, mmol/L	3.4 (2.2 – 7.4)	5 (3.0 – 8.4)	0.03
Hemoglobin, g/dL	12.6 (11.0 – 14.0)	13.3 (11.8 – 14.6)	0.02
Platelets, x10^9/L	211 (158 – 253)	207 (166 – 252)	0.89
Functional coagulation at 24 hours			
Number of patients	55	85	
Extem CT at 24hrs, s	61 (53 – 75)	65 (51 - 80)	0.35
Extem CA5 at 24hrs, mm	40 (33 – 46)	40 (35 – 47)	0.32
Extem MCF at 24hrs, mm	59 (53 – 63)	62 (58 – 67)	0.01
Extem ML at 24hrs, %	8 (5 – 10)	4 (1 – 6)	<0.0001
Outcomes			
Number of patients	90	109	
Venous thromboembolic events	0	5 (5)	0.07#
Other thrombotic events	0	5 (5)	0.07#
24hr Mortality	21 (23)	8 (7)	0.002
28 Day Mortality	31 (34)	30 (28)	0.29

Values are median (IQR) or number (%). *χ2 test for categorical variables unless otherwise indicated and Mann Whitney U test for continuous variables; # Fisher's exact test. ISS, Injury Severity Score; HR, heart rate; SBP, systolic blood pressure; GCS, Glasgow Coma Score; ED, Emergency Department; CT, clotting time; CA5, clot amplitude at 5 minutes; MCF, maximum clot firmness; ML, maximum lysis.

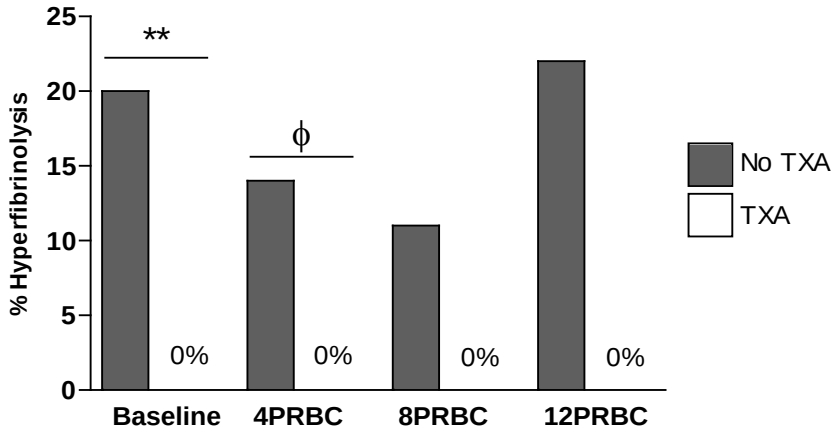
Table 12 Blood products and fluids administered during bleeding and for those patients surviving to 12 hours

	Pre-baseline			Baseline – 4PRBC			4PRBC – 8PRBC			8PRBC – 12PRBC			Total from Injury – Hour 12		
	No TXA	**TXA**	*p*	**No TXA**	**TXA**	*p*	**No TXA**	**TXA**	*p*	**No TXA**	**TXA**	*p*	**No TXA**	**TXA**	*p*
No. of patients	113	109		43	40		9	18		9	9		74	102	
PRBC:FFP:Platelet Ratio	0 : 0 : 0	1 : 0 : 0		1 : 0.75 : 0	1 : 0.75 : 0		1 : 0.75 : 0	1 : 1 : 0.75		1 : 1 : 0	1 : 1 : 1.5		1 : 0.8 : 0.6	1 : 0.7 : 1	
PRBC	0 (0 – 1)	1 (0 – 3)	<0.0001	-	-	-	-	-	-	-	-	-	5 (3 – 8)	6 (4 – 9)	0.14
FFP	0 (0 – 0)	0 (0 – 0)	0.39	3 (0 – 4)	3 (0 – 4)	0.97	3 (1 – 4)	4 (3 – 4)	0.46	4 (0.5 – 4)	4 (4 – 5)	0.04	4 (2 – 6)	4 (3.75 – 8)	0.06
PLTS	-	-	-	0 (0 – 0)	0 (0 – 0)	0.85	0 (0 – 1)	0.5 (0 – 1)	0.73	0 (0 – 0.5)	1 (1 – 1)	0.006	0.5 (0 – 1)	1 (0 – 1)	0.16
CRYO	-	-	-	0 (0 – 0)	0 (0 – 0)	0.40	1 (0 – 2)	0 (0 – 1)	0.13	1 (0 – 2)	2 (2 – 3.5)	0.03	0 (0 – 2)	2 (0 – 2)	0.06
Colloid, ml	-	-	-	0 (0 – 0)	0 (0 – 0)	0.59	0 (0 – 0)	0 (0 – 125)	0.10	333 (0 – 750)	0 (0 – 0)	0.09	1000 (0 – 1500)	0 (0 – 500)	<0.0001
Crystalloid, ml	500 (0 – 1000)	250 (0 – 675)	0.01	0 (0 – 1000)	0 (0 – 438)	0.21	500 (0 – 1250)	0 (0 – 625)	0.34	500 (0 – 1667)	0 (0 – 400)	0.19	2889 (1375 – 4000)	2200 (1100 – 3463)	0.04

Values are median (IQR) and analysed by Mann Whitney U test. PRBC, packed red blood cell units; FFP, fresh frozen plasma units; PLTS, platelet pools; CRYO, cryoprecipitate pools.

4.4.2 Incidence of hyperfibrinolysis and coagulopathy

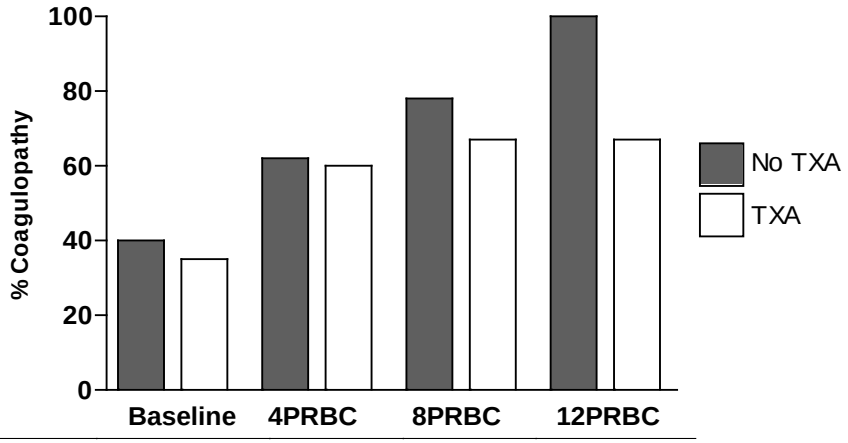
No patients who received early TXA exhibited hyperfibrinolysis on ROTEM® at any point during the acute bleeding phase (Figure 29). In contrast, 20% of patients who did not receive early TXA had hyperfibrinolysis on their admission ROTEM® ($p<0.0001$ vs TXA group). During on-going haemorrhage, TXA appeared to protect against the development of TIC (Figure 30). Despite similar incidence of coagulopathy on admission in both groups (No TXA vs TXA: 40% vs 35%, $p=0.45$), by 8 units more patients in the No TXA group were coagulopathic. After transfusion of 12 units of PRBCs, 100% of patients in the No TXA group were coagulopathic compared to 67% of patients in the TXA group ($p=0.21$). At the 12 PRBC time point, patients in the TXA group were less coagulopathic as defined by both EXTEM CA5 and PT ratio (Figure 31 & Figure 32). During massive haemorrhage, early empiric TXA avoids functional hyperfibrinolysis and is associated with protection against TIC.



	Baseline	4PRBC	8PRBC	12PRBC
# No TXA	23 / 113	6 / 42	1 / 9	2 / 9
# TXA	0 / 109	0 / 40	0 / 18	0 / 9

Figure 29 Incidence of hyperfibrinolysis during trauma haemorrhage

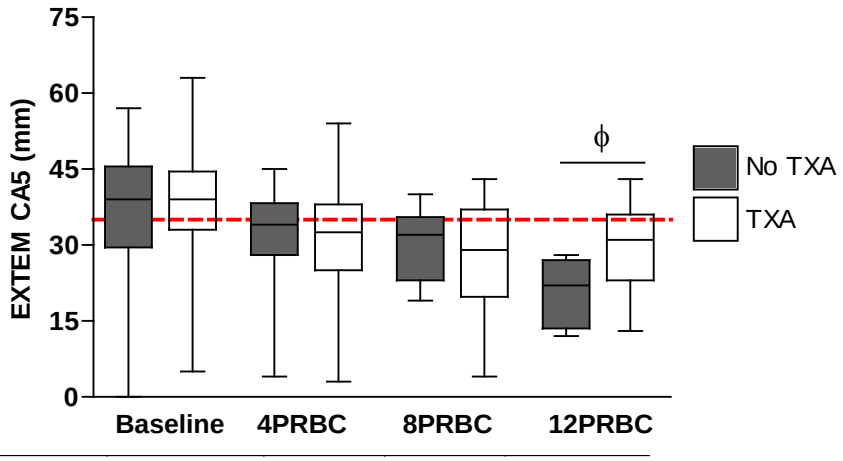
No patients receiving TXA exhibited hyperfibrinolysis on arrival to the ED (No TXA vs TXA 20 vs 0%, *p*<0.0001) or during ongoing haemorrhage. $^{\Phi}$ *p*<0.05, ** *p*<0.001



	Baseline	4PRBC	8PRBC	12PRBC
# No TXA	45 / 113	26 / 42	7 / 9	9 / 9
# TXA	38 / 109	24 / 40	12 / 18	6 / 9

Figure 30 Incidence of coagulopathy during trauma haemorrhage

There was a linear trend over time of increasing coagulopathy in both the No TXA (Chi-squared test for trend, p<0.0001) and TXA groups (p=0.0008). After transfusion of 12 units of PRBCs, 100% of patients in the No TXA group were coagulopathic compared to 67% of patients in the TXA group (p=0.21).



	Baseline	4PRBC	8PRBC	12PRBC
# No TXA	113	42	9	9
# TXA	109	40	18	9

Figure 31 Clot amplitude at 5 minutes (CA5) during trauma haemorrhage

CA5 is relatively maintained during bleeding with TXA. EXTEM CA5 after 12 units: No TXA vs TXA 22 vs 31mm, p=0.02. Dotted line represents cut-off of coagulopathy (CA5≤35mm). ϕp<0.05.

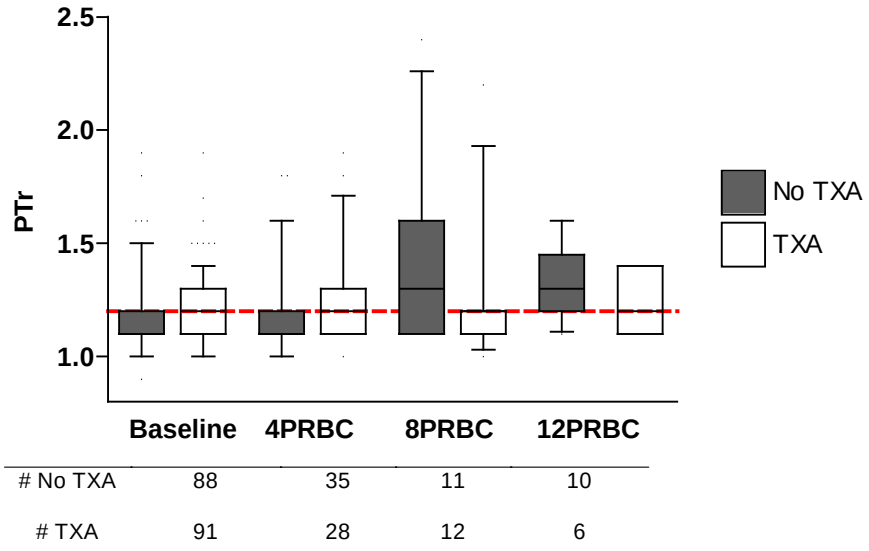
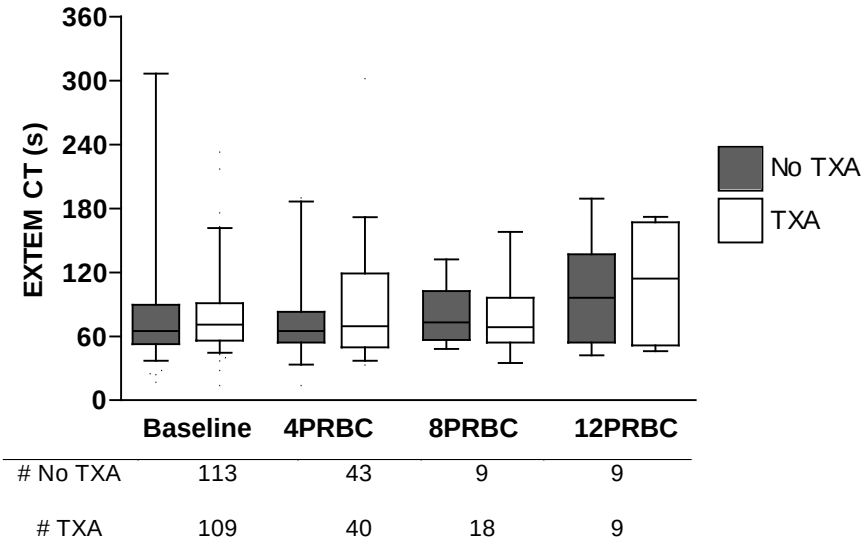


Figure 32 Prothrombin time ratio (PTr) during trauma haemorrhage

No statistical difference in prothrombin time ratio (PTr) was identified between the TXA and No TXA groups during haemorrhage. After 12PRBC, 60% of No TXA vs 33% of TXA patients (p=0.30) had a prothrombin time ratio (PTr) greater than 1.2. Whiskers represent 10th and 90th percentiles. Dotted line represents threshold for coagulopathy based upon conventional laboratory coagulation tests (PTr > 1.2).

4.4.3 Association between early TXA and functional coagulation assays

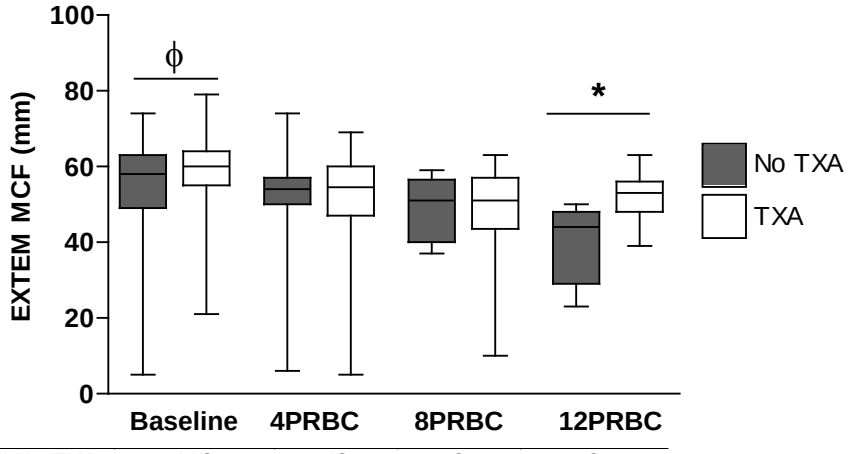
Early TXA administration was associated with stronger clot formation and reduced clot breakdown. There was no difference in the ROTEM® time to clot initiation (CT) (Figure 33) but the TXA group showed increased clot firmness both upon ED arrival (EXTEM MCF: No TXA vs TXA, 58 vs 60mm, p=0.03) and after a massive transfusion (EXTEM MCF after 12 units: No TXA vs TXA, 44 vs 53mm, p=0.008) (Figure 34 and Appendix 3 - Figure A66). No significant differences in functional fibrinogen or fibrin polymerisation were identified with TXA (Figure 35).



	Baseline	4PRBC	8PRBC	12PRBC
# No TXA	113	43	9	9
# TXA	109	40	18	9

Figure 33 Functional measure of clot initiation during haemorrhage

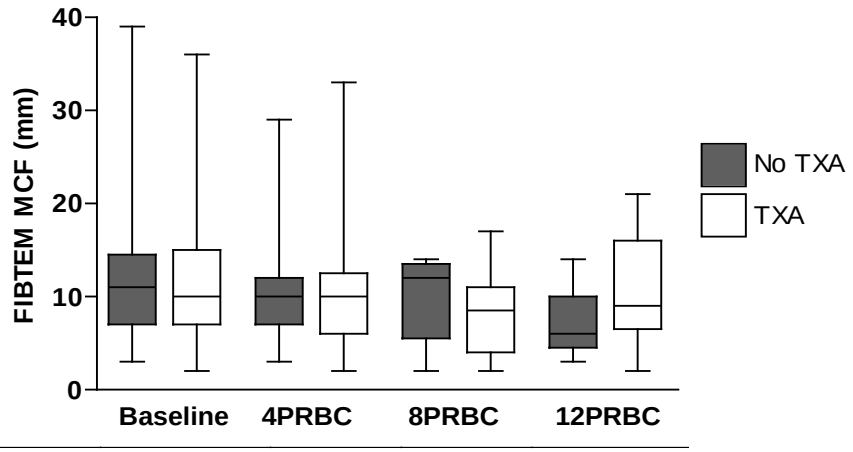
No significant differences in clotting time (EXTEM CT), a measure of clot initiation, were identified with TXA. Whiskers represent 5th and 95th percentiles.



	Baseline	4PRBC	8PRBC	12PRBC
# No TXA	113	43	9	9
# TXA	107	40	18	9

Figure 34 Functional measure of clot strength during haemorrhage

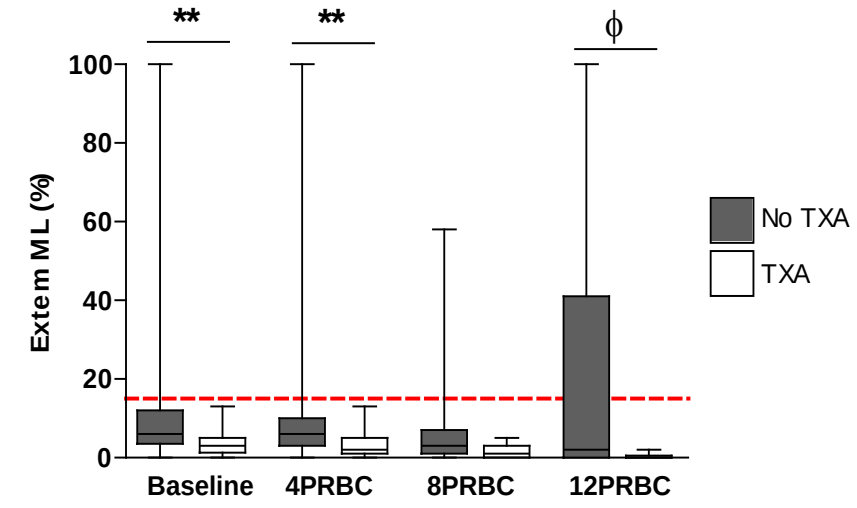
Maximum clot firmness (EXTEM MCF) was greater with TXA on admission (No TXA vs TXA 58 vs 60mm, p=0.03) and significantly enhanced after 12PRBC (No TXA vs TXA 44 vs 53mm, p=0.008). ϕ *p*<0.05, **p*<0.0125.



	Baseline	4PRBC	8PRBC	12PRBC
# No TXA	101	38	9	9
# TXA	106	37	18	9

Figure 35 Functional measure of fibrinogen level and fibrin polymerisation during haemorrhage

After treatment with TXA, functional fibrinolysis was significantly lower on ED arrival (ML: No TXA vs TXA 6 vs 3%, p<0.0001) and throughout active haemorrhage (Figure 36 and Figure A67). There was no significant difference on admission or during bleeding in either EXTEM MCF or ML between patients in the TXA group who received the bolus dose only and those who received both bolus and infusion doses of TXA. Overall patients who received TXA had increased clot strength and significantly reduced functional lysis by viscoelastic evaluation.



	Baseline	4PRBC	8PRBC	12PRBC
# No TXA	113	43	9	9
# TXA	108	40	18	9

Figure 36 Functional measure of fibrinolysis during haemorrhage

Maximum lysis (EXTEM ML) was reduced with TXA on admission (ML: No TXA vs TXA 6 vs 3%, p<0.0001) and throughout bleeding. ϕ *p*<0.05, ***p*<0.001. Dotted line represents threshold for hyperfibrinolysis (ML>15%).

4.4.4 Association between early TXA and plasma markers of fibrinolysis

There was a marked elevation in PAP levels on admission to the ED demonstrating an initial high degree of fibrinolytic system activation post injury in both the No TXA and TXA groups (Figure 37 and Figure A68).

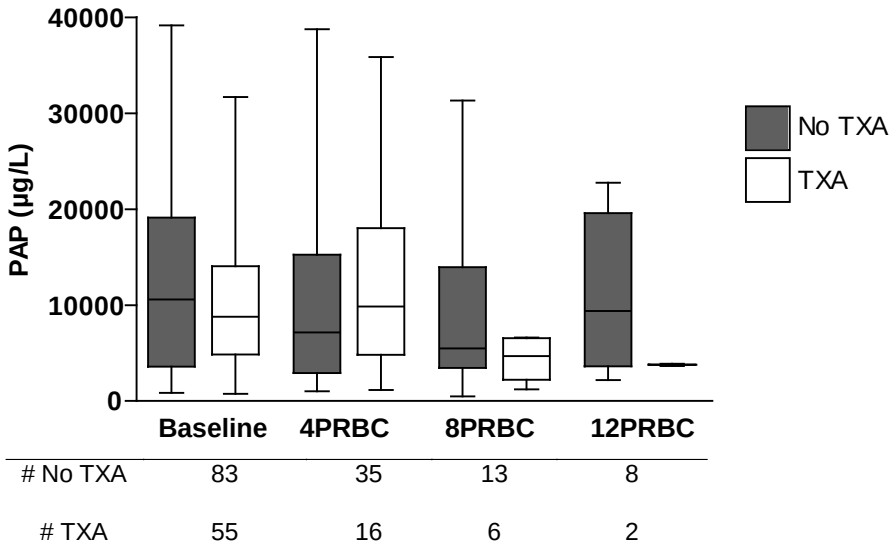


Figure 37 Plasmin-α2-antiplasmin complex (PAP) levels during haemorrhage

Significant fibrinolytic activation was seen in both No TXA and TXA groups on admission as measured by plasmin-α2-antiplasmin complex (PAP) levels (No TXA vs TXA 10595 vs 8798 µg/L, p=0.41).

TXA reduced functional lysis to below the diagnostic threshold of hyperfibrinolysis irrespective of the level of plasmin generation (Figure 38).

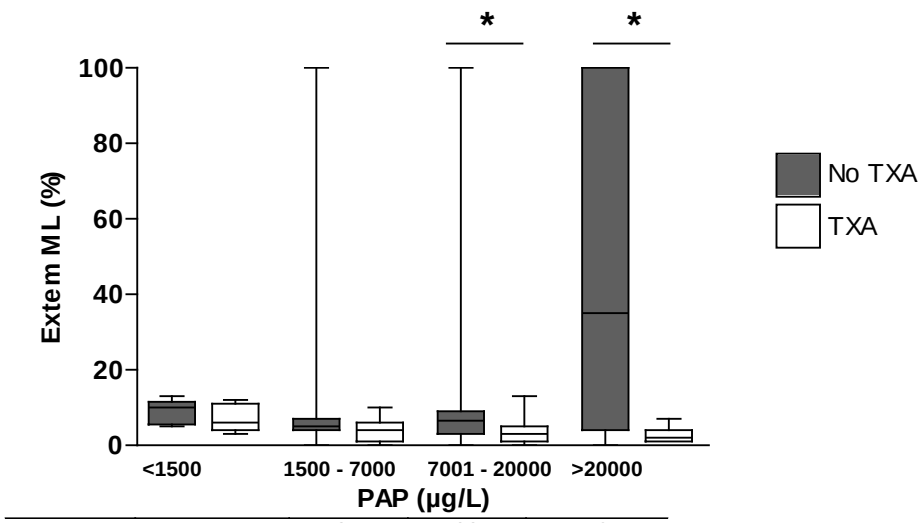
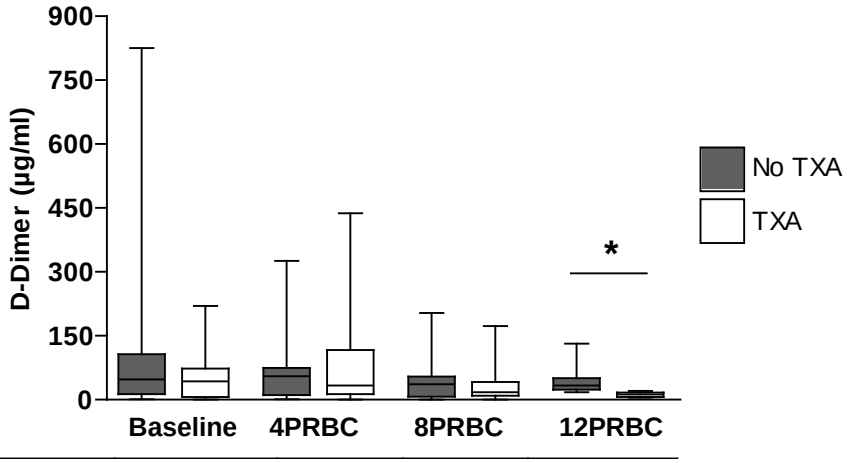


Figure 38 Functional fibrinolysis on admission stratified by PAP level

TXA reduced functional fibrinolysis even in those patients with the highest degree of fibrinolytic activation measured by PAP (ML in patients with PAP 7001-20000: No TXA vs TXA 7 vs 3%, p=0.01; ML in patients with PAP >20000: No TXA vs TXA 35 vs 2%, p=0.009). *p<0.0125.

The anti-fibrinolytic action of TXA in traumatic haemorrhage was evident by the observation that TXA patients generated fewer D-dimers after massive haemorrhage compared to the No TXA patients (Figure 39 and Figure A69). Complete data on D-dimer and PAP levels, to compare TXA group patients who received bolus dose only vs bolus + infusion doses was only available at baseline and 4 RBC with no significant difference observed.

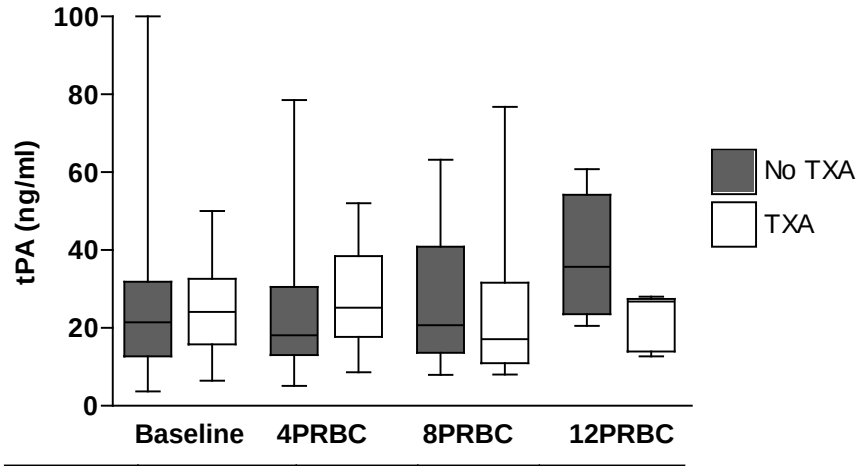


	Baseline	4PRBC	8PRBC	12PRBC
# No TXA	94	43	9	8
# TXA	58	20	11	6

Figure 39 D-dimer levels during trauma haemorrhage

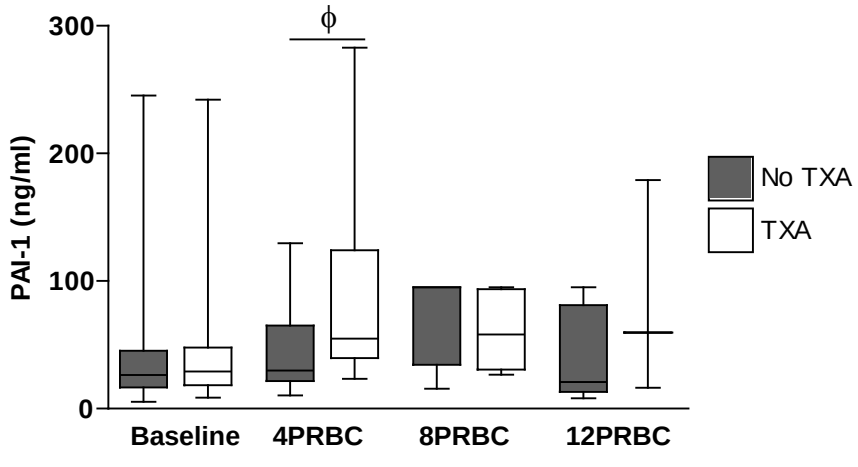
Patients treated with TXA had significantly lower D-dimer levels after massive haemorrhage (D-dimer after 12 units: No TXA vs TXA 33.1 vs 12.4µg/ml, *p=0.0013).

Measurement of the primary fibrinolytic pathway control proteases during haemorrhage, demonstrated no statistical difference in levels of tPA (Figure 40), PAI-1 or α-2-antiplasmin with TXA (Figure 41 & Figure 42) at baseline or during haemorrhage other than higher PAI-1 levels in the TXA group at the 4PRBC time point.



# No TXA	79	42	13	6
# TXA	57	19	11	5

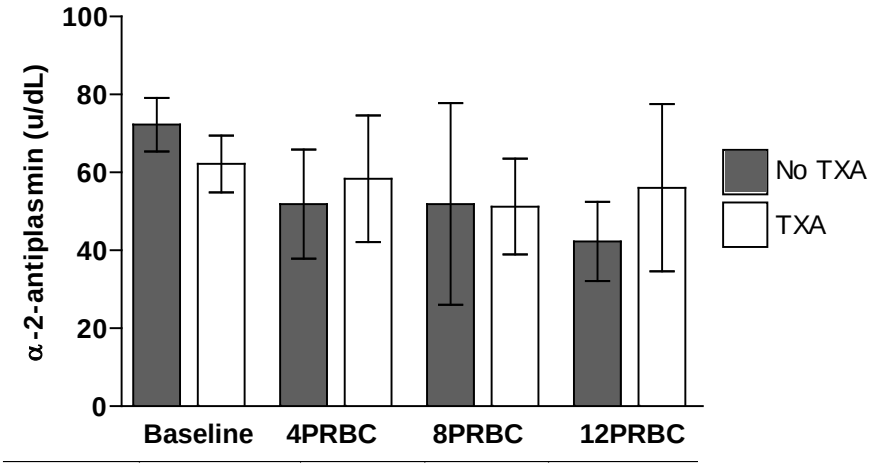
Figure 40 Tissue plasminogen activator (tPA) levels during haemorrhage



# No TXA	80	41	13	8
# TXA	56	16	6	3

Figure 41 Plasminogen activator inhibitor-1 (PAI-1) levels during trauma haemorrhage

PAI-1 levels were similar on admission and after 8 and 12PRBC. Higher PAI-1 levels were noted with TXA after 4PRBC (No TXA vs TXA 28.1 vs 54.9ng/ml, p=0.014). $^{\Phi}p<0.05$



	Baseline	4PRBC	8PRBC	12PRBC
# No TXA	92	16	6	2
# TXA	59	20	11	6

Figure 42 α2-antiplasmin levels during haemorrhage

Bars represent mean ± 95% CI.

4.4.5 Association between early TXA and plasma markers of coagulation

TXA had no discernible effect on plasma fibrinogen levels measured on admission and during haemorrhage (Figure 43). Controlling for α-2-antiplasmin, admission fibrinogen levels fell in both No TXA and TXA groups, with no protective effect of TXA on fibrinogen identified (α-2-antiplasmin lowest quartile (<48u/dL): No TXA vs TXA, fibrinogen 0.89 g/L vs 1.03 g/L, p=0.40).

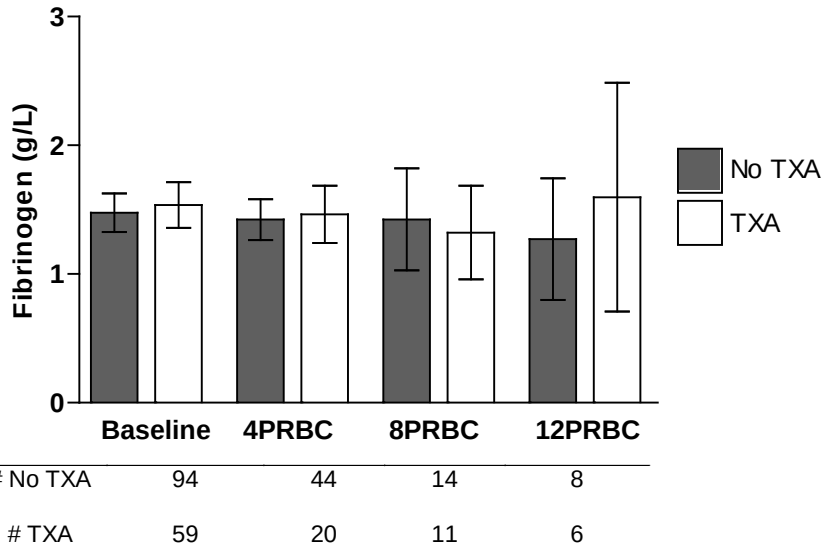
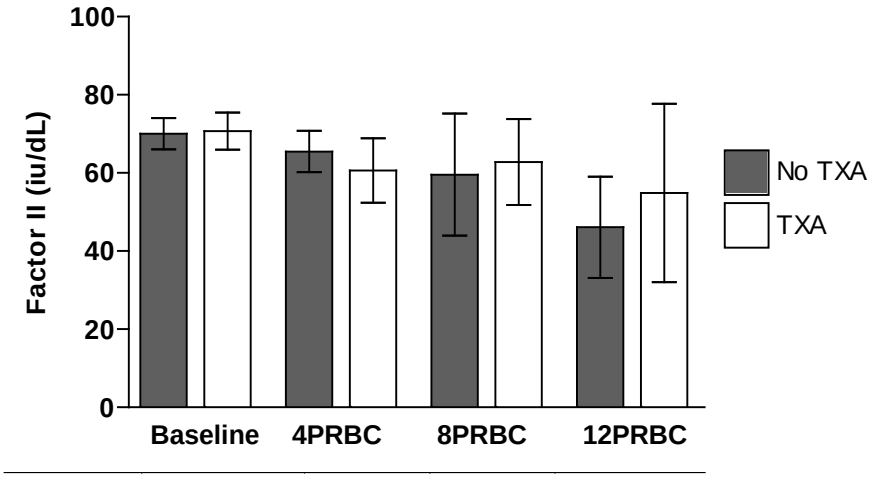


Figure 43 Fibrinogen levels during haemorrhage

Fibrinogen levels were found to be low during haemorrhage but were maintained at a relatively constant level irrespective of TXA administration. Bars represent mean ± 95% CI.

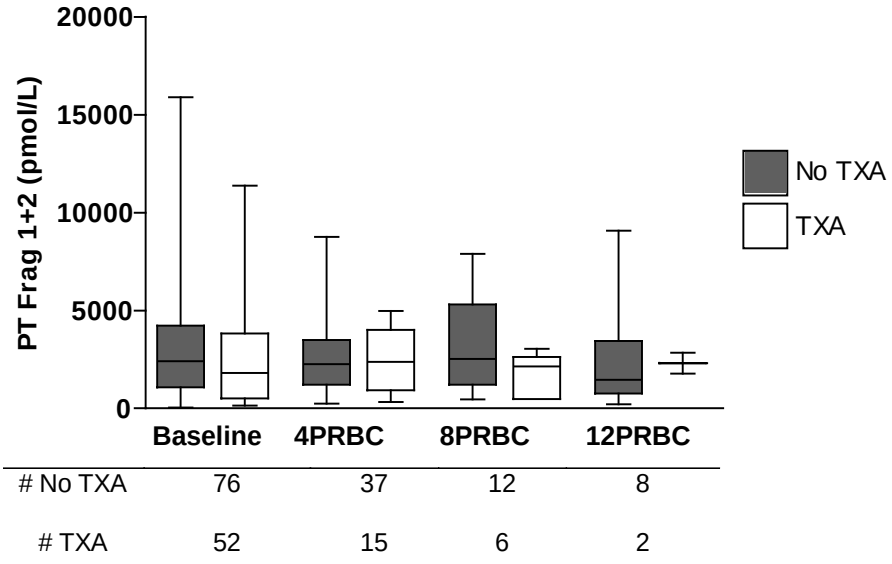
Levels of prothrombin (Figure 44) and prothrombin fragment 1+2 levels (Figure 45) were similar between TXA and No TXA groups. Soluble fibrin monomer complex (sFMC) was significantly higher in the No TXA group at baseline (No TXA vs TXA: 238.0 µg/ml vs 199.4µg/ml, p=0.003). Patients who received TXA appeared to have preservation of Factor XIII levels following a massive transfusion although this did not reach statistical significance (Figure 46). Overall, TXA appeared to ameliorate fibrinolysis throughout the bleeding phase with minimal effects on clot generation.



# No TXA	96	39	7	7
# TXA	59	20	11	6

Figure 44 Prothrombin (Factor II) levels during haemorrhage

Prothrombin levels fell during haemorrhage with no significant difference between the two groups. Bars represent mean ± 95% CI.



	Baseline	4PRBC	8PRBC	12PRBC
# No TXA	76	37	12	8
# TXA	52	15	6	2

Figure 45 Prothrombin fragment 1+2 (PT Frag 1+2) levels during haemorrhage

No statistically significant difference in levels of prothrombin fragments 1+2 (PT Frag 1+2) between the two groups was identified during haemorrhage.

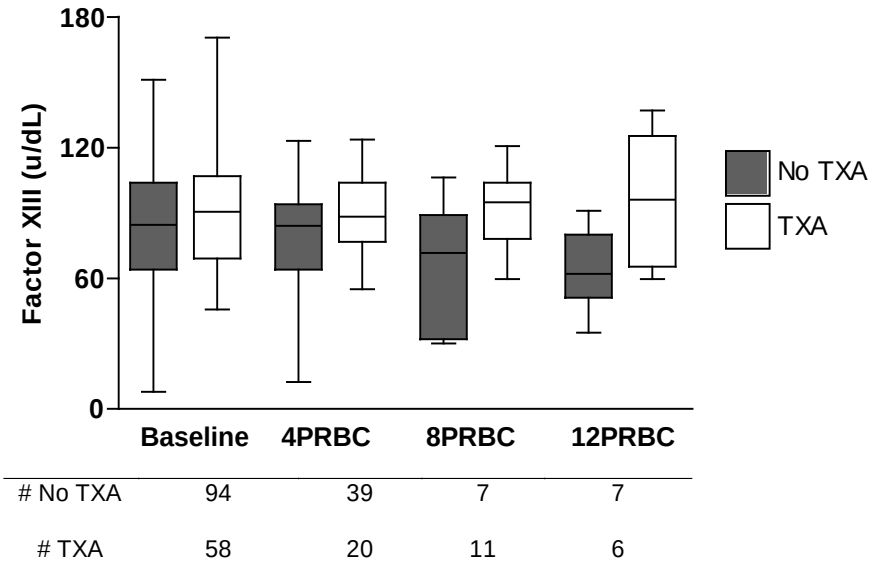


Figure 46 Factor XIII levels during haemorrhage

Maintained factor XIII levels were observed with TXA compared to falling levels in the No TXA group during haemorrhage, although these differences did not reach statistical significance. Factor XIII after 12 PRBC units: No TXA vs TXA 62 (51 – 80) vs 96 (65 – 125) u/dL, p=0.10).

4.4.6 Mortality and thrombotic events

There was no evidence of functional hypercoagulability at 24-hours following TXA administration (Table 11), although we observed a small difference in MCF between the two groups in addition to a persistent reduction in ML at 24 hours in the TXA cohort. For patients in the TXA group, there was no difference in clot strength (MCF: 62mm vs 65mm, p=0.09) or maximum lysis (ML: 4.5 vs 5%, p=0.95) at 24 hours between those who received a bolus dose only vs bolus and infusion.

Thrombotic complications were observed in the TXA group (Table 11). Of 109 TXA patients, four patients developed a PE and one patient developed an isolated lower limb DVT. Other thrombotic complications in the TXA group were: two ischemic strokes (one stroke patient had a cardiac thrombus associated with an incidental finding of a patent foramen ovale), one internal jugular vein thrombosis, one MI and one of the patients with bilateral PE also required a femoral embolectomy for an acutely ischemic lower limb post arterial line removal. No venous or arterial thrombotic events were identified in patients who did not receive TXA. The 23 patients from the No TXA group who later received TXA post baseline blood sampling are excluded from the outcome data in Table 11. Two patients who received late TXA (post-baseline) developed a PE and an ischemic stroke respectively. Patients in the early TXA group had an improved overall 24-hour survival (24-hour mortality: No TXA vs TXA 23 vs 7%, p=0.002) (Table 11). All-cause mortality at 28-days was lower in the early TXA group but did not reach statistical significance.

4.5 DISCUSSION

This study has assessed the impact of early TXA on measures of functional coagulation and fibrinolysis during trauma haemorrhage. Patients who received TXA prior to, or immediately in, the ED had no functional hyperfibrinolysis as measured by ROTEM®. This effect persisted throughout the bleeding episode. TXA was associated with improved clot strength, lower maximum lysis and a degree of protection against TIC during ongoing haemorrhage. Consistent with the CRASH-2 trial[116] and large military studies in bleeding trauma patients[118,134], TXA was associated with a significant reduction in early deaths. Survivors receiving TXA had a higher unadjusted incidence of arterial and venous thrombotic events. 28-day mortality in the TXA group was 6% lower than in controls although this did not reach statistical significance. Early TXA prevents the development of functional hyperfibrinolysis

during active haemorrhage and provides some protection against resuscitation associated TIC.

Both groups in this study had evidence of initial fibrinolytic system activation (PAP >12x upper limit of normal) on arrival. In patients who had received TXA, this was not associated with functional hyperfibrinolysis. TXA was associated with a degree of protection from TIC during bleeding and improved clot strength without evidence of enhanced clot generation. TXA is postulated to also reduce fibrinogenolysis, especially when antiplasmin levels fall below 60%.[174] However, we did not observe any protective effect of TXA on fibrinogen levels regardless of antiplasmin levels. The effect of TXA on the coagulation system therefore appears to be primarily related to its anti-fibrinolytic action. This effect persisted throughout the acute bleeding episode despite many patients receiving only a single dose of TXA. TXA is regarded as a relatively weak antifibrinolytic and the recommended dose of 1g is at the lower end of those used in elective procedures such as cardiac surgery.[175] Whilst further studies are required to determine the optimal dose-response in trauma, the currently recommended dose of TXA appears effective at preventing functional hyperfibrinolysis during haemorrhage, at least up to the 12th unit of RBC transfusions and as measured by this viscoelastic assay.

Thrombotic complications were observed more frequently in those patients treated with TXA and of similar incidence to those reported in the MATTERs study.[118] Severely injured bleeding patients have a high baseline risk of developing thrombotic complications.[176,177] In this study, those treated with TXA were both more severely shocked and more likely to survive beyond 24-hours, both of which may explain the increased rate of thrombotic events. At 24-hours there was no evidence of functional hypercoagulability in those patients who

received TXA except a small and likely clinically insignificant difference in maximum clot strength. However, we did observe a 50% lower maximum lysis in the TXA group at 24-hours and further studies to correlate this finding with biochemical markers of fibrinolysis, transfusion products and dosing of TXA is required to ascertain the significance of this observation.

This study has several limitations. A temporal change in resuscitation practice of bleeding trauma patients was observed during the study period with TXA included in the MHP from 2011. However, from 2011-2015 an average of 25% of patients did not receive early TXA and therefore the No TXA group are not simply a historic control group. Data on administration of the second TXA infusion dose was only available for 68% in the TXA group. In those patients for whom data was available, only 50% received both the bolus + infusion doses of TXA, reflecting the real-life challenge of administering drugs to the acutely bleeding trauma patient. It does suggest, however, that for many patients, a single 1g bolus of TXA may be sufficient to ameliorate functional hyperfibrinolysis although further studies on optimal dosing are required.

The TXA group in this study were more likely to receive transfusion of PRBC pre-baseline blood draw and between 8-12 units of PRBC, received higher ratios of FFP, cryoprecipitate and platelets, likely reflecting temporal differences between the groups. Whilst the TXA group were more shocked on admission, pre-hospital blood transfusion[178] and cryoprecipitate[59] have in pre-clinical studies been shown capable of modulating TIC. The potential effects of TXA seen in this study should therefore be interpreted with these group differences in mind. As highlighted in the military setting, it is likely that TXA combined with cryoprecipitate as part of a balanced transfusion strategy, exhibits a synergistic effect on

survival.[134] Cause of death was not ascertained in this study. Although 24-hour mortality was lower in patients receiving early TXA, we are unable to confirm whether this was due to fewer people bleeding to death as a direct result of TXA. Finally, we have focused this paper on a description of the effect of TXA on the coagulation system, and we have only presented crude outcome rates, without adjustment or survivorship analysis. Our crude outcome rates are broadly consistent with our previous study of outcomes associated with TXA use in a whole civilian trauma cohort.[117]

4.6 Conclusion

In this prospective cohort study of bleeding trauma patients, we have characterised the effect of early TXA on the coagulation system. Early TXA at the currently recommended dose avoids functional hyperfibrinolysis and appears to provide a degree of protection against the development of TIC during massive haemorrhage. These benefits are likely greatest when early and empiric TXA is combined with a balanced transfusion strategy as part of damage control resuscitation.

4.7 Specific Contributions of the Author

The Author was guided towards investigating the effect of TXA in trauma haemorrhage by KB. The Author designed the study and planned the analysis included within this chapter under the guidance of RD and KB. The Author collated the data and performed all initial analyses. Data interpretation was further discussed during meetings with KB and RD. The Author wrote the first draft of this manuscript summarising our findings. RD and KB edited the manuscript. The Author prepared all tables and figures.

Chapter 5

AN INVESTIGATION INTO THE EFFECT OF THE TRANEXAMIC ACID INFUSION AND TIMING OF TREATMENT ON CLINICAL OUTCOMES INCLUDING THROMBOTIC EVENTS FOLLOWING TRAUMA HAEMORRHAGE

5.1 CHAPTER ABSTRACT

BACKGROUND: Despite evidence of an outcome benefit associated with Tranexamic acid (TXA) therapy in trauma haemorrhage, there is a disparity in its use worldwide. The resistance to using it centres on a concern of precipitating thrombotic complications. The aim of this study was to explore the effect of the TXA infusion and timing of TXA treatment on clinical outcomes including thrombotic complications.

METHODS: A prospective observational cohort study was conducted at a single major trauma centre. Patients activating the major haemorrhage protocol and receiving TXA were included. Blood was drawn on arrival, during bleeding and at 24 and 72-hours from injury for thromboelastometry and D-dimer quantification. Patients were observed for 28-days or until death or discharge for occurrence of thrombotic events.

RESULTS: 215 patients were included of whom 134 received a single TXA bolus only, 76 received a TXA bolus + infusion and 5 received a double TXA bolus. There was no difference in clot strength or functional fibrinolysis (ML) between the single bolus vs bolus + infusion groups throughout bleeding and including the 24-hour sampling point. At 72-hours those receiving bolus + infusion had increased clot strength (70 vs 68 mm, p=0.03) and associated reduced ML (5 vs 7 %, p=0.007) compared with single bolus. On univariate analysis there was a non-statistically significant increase in thrombotic events in the bolus + infusion group (18 vs 11% in single bolus, p=0.15) and a linear increase in thrombotic events with later TXA treatment after injury in those receiving a TXA infusion. However, on multivariate analysis, neither the TXA infusion nor the time from injury to administration of TXA bolus were associated with development of thromboses, MODS or 28-day mortality.

CONCLUSIONS: Thrombotic events increased in a linear fashion over time in patients receiving a TXA infusion, although, on multivariate analysis the effect of the TXA infusion and time to TXA bolus were not associated with development of thrombotic events in this cohort. A single TXA bolus may provide a lower effective therapeutic dose in trauma haemorrhage with potential for reduced thrombotic complications.

ACKNOWLEDGEMENTS: Paul Vulliamy and Scarlett Gillespie are kindly acknowledged for their assistance with data collection for this study.

5.2 INTRODUCTION

Seven years following the landmark publication of the CRASH-2 trial[116] showing improved outcomes in trauma haemorrhage associated with the use of Tranexamic acid (TXA), debate regarding its role in trauma resuscitation persists and as a result there is disparity in its use worldwide. The primary issue raised regarding the use of the antifibrinolytic agent is over concern that treatment could cause harm by potentiating later thrombotic complications. Trauma patients have a high baseline risk of developing thromboses even with the use of modern thromboprophylaxis techniques.[154,179] Despite existence of evidence to suggest that TXA is safe in both trauma[116,118] and the elective surgical setting[156], the temporal changes that occur in the coagulation and fibrinolytic systems after severe injury have yet to be fully characterised. The mechanism by which tranexamic acid administered beyond three hours of injury appears to have a deleterious effect on outcomes is not known.[139] In this study of bleeding trauma patients, we aimed to explore the effect of the TXA infusion and timing of TXA treatment on clinical outcomes including thrombotic complications.

In Chapter 4, early empiric TXA was found to be effective at avoiding functional fibrinolysis on admission and throughout bleeding despite many patients receiving the 1g bolus dose only. In this study, thrombotic complications were only encountered in patients receiving TXA although potentially explainable by the fact patients receiving TXA were more severely shocked and more likely to survive beyond 24-hours than those who received no TXA. However, a single 1g bolus may provide a lower effective therapeutic dose compared to the bolus + infusion regimen. The effects of the TXA infusion over a single TXA bolus on functional measures of coagulation and on outcomes following trauma haemorrhage have not previously been investigated.

Analysis of the CRASH-2 trial dataset identified the importance of early treatment with TXA.[139] The risk of death due to bleeding was found to be significantly reduced if TXA was given early, within the first hour from injury with a lesser but still beneficial effect in treatment up to three hours. TXA administered after three hours was paradoxically found to increase the risk of death due to bleeding. The reason for this time-effect has yet to be fully explained. One hypothesis from an experimental model of traumatic brain injury suggests that temporal differences in levels of the plasminogen activators tPA and uPA could provide the explanation.[143,144] TXA blocks early tPA-mediated lysis but later uPA-mediated lysis could be potentiated by TXA with resultant worsening of bleeding. Whilst the first TXA bolus appears beneficial in reversing hyperfibrinolysis, it remains unclear whether the infusion dose provides additional benefit or whether prolonged exposure to TXA with an 8-hour infusion may potentially cause harm as the trauma patient evolves from the initial hypocoagulable to a hypercoagulable state.[63,64,66]

The hypothesis of this study was that a single bolus of TXA would be as effective as a bolus + infusion in trauma haemorrhage and that later exposure to TXA was associated with increased thrombotic complications. Our first aim was to assess the effect of two different dosing regimens of TXA on functional measures of coagulation and fibrinolysis and on outcomes. The second aim was to investigate the effect of the TXA infusion and time from injury of TXA treatment on clinical outcomes including thrombotic complications. Finally, we wished to characterise patients developing a thrombotic complication and determine any effect of TXA therapy in this cohort. We conducted a prospective observational cohort study of trauma patients activating the Major Haemorrhage Protocol within a single Major Trauma Centre.

5.3 METHODS

All adult patients (≥16 years) who meet the local criteria for trauma team activation, were consecutively screened (between 8:00am – 8:00pm daily until January 2016 after which screening was 24-hours) for inclusion in the Activation of Coagulation and Inflammation in Trauma study (ACIT II; UK CRN ID 5637). Deferred informed written consent was obtained from the patient or their next-of-kin. The study was approved by East London and The City Research Ethics Committee (07/Q0603/29). The inclusion and exclusion criteria of the ACIT II study are detailed in sections 2.2.1 and 2.2.2.

For this study we analysed consecutive ACIT II patients enrolled at The Royal London Hospital who activated the Major Hemorrhage Protocol (MHP) from January 2013 to 31 July 2017. The MHP is activated either pre-hospital by the physician-led London Air Ambulance (LAA) or in-hospital by the trauma team leader, when a patient with suspected traumatic haemorrhage, has a systolic blood pressure below 90 mmHg and a poor response to initial fluid resuscitation. The empiric administration of TXA at our institution is restricted to patients who activate our MHP. As detailed in section 4.3, TXA was formally introduced into the MHP in February 2011 (Figure A64) as a 1g IV bolus over 10 minutes (within three hours of injury) followed by a 1g IV infusion over eight hours. The MHP protocol was further revised in August 2016 (Figure A65) and the 1g infusion of TXA over 8-hours removed based on expert consensus of opinion. Therefore, from August 2016, patients activating the MHP received a single bolus of 1g IV TXA over 10 minutes (within three hours of injury).

5.3.1 Data Collection

ACIT II data collection was carried out as detailed in section 4.3.1.

The timing of TXA administration from injury was documented. Patients were observed for 28 days from injury or until hospital discharge for the occurrence of thrombotic events or death. Length of either critical care or overall hospital stay was calculated for patients surviving to hospital discharge or day 28 from injury only. Cause of death was determined by retrospective review of case notes, trauma department morbidity and mortality meeting outcome reports and post mortem reports where applicable.

5.3.2 Blood Sampling

Research blood samples were obtained alongside clinical laboratory tests at baseline as detailed previously in section 4.3.2. Further blood samples were obtained during haemorrhage after transfusion of 4, 8 and 12 units packed red blood cells (PRBC) and at 24 and 72 hours following injury.

The D-dimer concentration was determined by the RLH central Haematology laboratory using a Sysmex CS2100i automated analyser (Sysmex, UK). For the D-dimer assay, concentrations above the assay upper measurement limit of 80.00 mg/L FEU [fibrin equivalent units] are reported as 80.00 mg/L FEU.

5.3.3 Functional Coagulation Analysis

Functional coagulation analysis was performed within one hour of blood draw at 37°C on either a ROTEM® delta instrument (Tem International GmbH, Munich, Germany) using the automated electronic pipette or a ROTEM Sigma instrument (Tem International GmbH, Munich, Germany) according to the manufacturer's instructions. Patients co-enrolled in the iTACTIC randomised control trial[157] had samples analysed on the automated ROTEM

Sigma instrument. The manufacturer states that the two platforms are comparable since the technical method used in each is identical. Two separate 60-minute assays were performed on each blood sample: EXTEM, measuring tissue-factor initiated clotting and FIBTEM, for qualitative assessment of fibrinogen levels via the addition of the platelet inhibitor, cytochalasin D.

5.3.4 Definitions

Major haemorrhage and massive haemorrhage were defined as transfusion within the first 24-hours of injury of at least 4 PRBC or at least 10 PRBC respectively.[180] Multiple Organ Dysfunction Syndrome (MODS) was determined for those surviving at least 24-hours and admitted to a critical care setting (either High Dependency or Intensive Care) and defined as the occurrence of a total SOFA score greater than 5 during the first 7 days of admission.[151] Thrombotic events included deep vein thrombosis (DVT), pulmonary embolism (PE), arterial thrombosis, myocardial infarction (MI) and ischemic stroke. Thrombotic events were defined as a clinical diagnosis supported by a confirmatory test result: duplex ultrasound for DVT; CT pulmonary angiogram for PE; arterial thrombosis requiring surgical intervention e.g. embolectomy; electrocardiogram changes and elevated troponin for MI; and CT head for ischaemic stroke. Univariate and multivariate analysis of the effect of TXA on late outcomes (28-day mortality, thrombotic events and MODS) included those patients surviving beyond 24 hours from injury only.

5.3.5 Statistical Analysis

Statistical analysis was performed using GraphPad Prism version 5 (GraphPad Software Inc., San Diego, USA) and Microsoft Excel 2013 (Microsoft Inc., Redmond, USA). Normal quantile plots were used to assess normality. Data without a normal distribution were expressed as

median (interquartile range) and analysed using Mann-Whitney U-test or Kruskal-Wallis test with Dunn's multiple comparison. Percentages were analysed using Chi-squared or Fisher's exact tests, with linear trends over time analysed with Chi-squared test for trend. Multivariate analysis was performed using IBM SPSS Statistics version 25 (IBM, Armonk, United States). Multivariate logistic regression models were created to investigate the effect on late outcomes, of TXA infusion (binary variable [yes/no]) or time from injury to TXA bolus (continuous variable in minutes) alongside other factors achieving a univariate significance of $p < 0.2$. A p value of <0.05 was considered statistically significant. Box-whisker plots represent median, interquartile range, 5^{th} – 95^{th} percentiles unless otherwise stated.

5.4 RESULTS

A total of 281 patients activated the major haemorrhage protocol and were enrolled in the ACIT II study during the study period. Patients were excluded for the following reasons: 14 patients or their next-of-kin withdrew consent; 7 found retrospectively not to have met the ACIT II inclusion criteria; 20 did not receive any TXA; 6 in whom it was unknown if they had received a TXA bolus and/or infusion; 1 recruited into the CRASH-3 trial and 18 with no ROTEM performed at baseline. A total of 215 patients were included within the analysis, of whom 82% were severely injured (ISS > 15) and 93% were transfused at least one unit of red blood cells.

5.4.1 Patient characteristics and unadjusted clinical outcomes

Three TXA dosing regimens were identified: a single 1g bolus (single bolus group); initial 1g bolus followed by a later 2^{nd} 1g bolus (double bolus group) and 1g bolus followed by a 1g infusion over 8 hours (bolus + infusion group). Patient and injury characteristics stratified by

TXA dosage regimen are shown in Table 13. Patients receiving a single TXA bolus and those receiving a bolus + infusion were similar in terms of sex, injury severity, mechanism of injury and time to administration of first TXA dose following injury. The bolus + infusion patients differed from the single bolus group in that they were younger, with a slightly greater degree of shock on admission.

Rates of major haemorrhage and total 24 hour PRBC transfusion requirements were greater in the bolus + infusion group compared to the single bolus group (Table 13). Massive haemorrhage and MODS rates were found to be similar. The incidence of thrombotic events occurring in the bolus + infusion group was greater although not statistically significant (18% vs 11% in single bolus, p=0.15). Overall 24-hour mortality was lower in the bolus + infusion group, although this did not reach statistical significance compared to the single bolus group (7.9% vs 17.9%, p=0.06) and by 28-days, overall mortality rates were similar. There were no significant differences in cause of death between the single bolus and bolus + infusion groups and in particular, no statistically significant difference in deaths secondary to haemorrhage (9.0% vs 5.3%, p=0.42; Table 14).

The five patients who received a double bolus of TXA suffered from predominantly penetrating injury (80%), with evidence of severe shock (median BD 22.2 mmol/L) on admission and the greatest blood transfusion requirement of any group, with 80% suffering massive haemorrhage. By day 28, four out of these five patients had died and there were no recorded thrombotic events in this group of patients.

Table 13 Patient characteristics and unadjusted outcomes stratified by TXA dosing regimen

	Single bolus	Double bolus	Bolus + infusion
Number of Patients	134	5	76
Timings			
Injury - ED admission, minutes	79 (61 – 100)	80 (74 – 99)	84 (56 – 104)
1st TXA bolus pre-hospital (%)	102 (76%)	2 (40%)	55 (72%)
Injury - 1st TXA bolus, minutes	69 (52 – 90)	84 (73 – 106)	74 (51 – 94)
Injury - 2nd TXA bolus or infusion, minutes	-	151 (135 – 714)	158 (116 – 220)
Injury & patient admission characteristics			
Age, years	36 (26 – 52)	50 (27 – 55)	27 (22 – 49)*
Male, n (%)	104 (78%)	5 (100%)	54 (71%)
Blunt, n (%)	95 (71%)	1 (20%)^	51 (67%)
Injury Severity Score (ISS)	27 (17 – 39)	21 (11 – 25)	29 (22 – 43)
Base Deficit (BD), mmol/L	7.4 (3.8 – 14.2)	22.2 (7.9 – 63.6)	9.2 (6.2 – 17)*
EXTEM CA5, mm	35 (30 – 43)	36 (16 – 41)	37 (29 – 44)
EXTEM ML, %	3 (1 – 6)	11 (1 – 94)	3 (1 – 5)
Blood product totals			
24hr PRBC, units	5 (2 – 8)	19 (5 – 37)^	7 (4 – 10)*
Outcomes			
Major haemorrhage, n (%)	83 (62%)	4 (80%)	59 (78%)*
Massive haemorrhage, n (%)	24 (18%)	4 (80%)^	21 (28%)
Incidence of MODS, n (%)	n = 95 77 (81%)	n = 3 3 (100%)	n = 65 58 (89%)
All thrombotic events, n (%)	15 (11.2%)	0	14 (18.4%)
24hr mortality, n (%)	24 (17.9%)	2 (40%)	6 (7.9%)
28 day mortality, n (%)	37 (27.6%)	4 (80%)^	20 (26.3%)

^p<0.05, Double TXA bolus vs single TXA bolus; *p<0.05, TXA bolus + infusion vs single TXA bolus. MODS, multiple organ dysfunction syndrome: includes patients surviving beyond 24 hours and admitted to a critical care unit.

Table 14 Cause of death stratified by TXA dosing regimen

	Single bolus	Double bolus	Bolus + infusion
Number of patients	134	5	76
Haemorrhage	12 (9.0%)	1 (20%)	4 (5.3%)
Head injury	15 (11.2%)	0	6 (7.9%)
Multi-organ failure/sepsis	2 (1.5%)	1 (20%)	5 (6.6%)
Multiple injuries	4 (3.0%)	0	1 (1.3%)
Hypoxic brain injury	0	1 (20%)^	2 (2.6%)
Thrombotic event	1 (0.7%)	0	0
Unknown	3 (2.2%)	1 (20%)	2 (2.6%)

Values are n (%). ^p<0.05, Double TXA bolus vs single TXA bolus.

5.4.2 Effect of TXA infusion on functional coagulation and D-dimer levels

There was no significant difference on admission or during bleeding (up to the 12th PRBC sampling time point) in clotting time (Figure 47), clot strength (Figure 48 and Figure 49) or maximum lysis (Figure 50) between patients who received a bolus + infusion vs single bolus of TXA. At 24 hours, again no difference in functional coagulation was identified, according to administered TXA dosing regimen. At 72 hours, clotting time was similar, however, EXTEM clot strength (70 vs 68 mm, p=0.03) and FIBTEM clot strength (34 vs 30 mm, p=0.006) were increased with a correspondingly lower maximum lysis (5 vs 7 %, p=0.007) detected in those patients receiving TXA bolus + infusion vs single bolus only. D-dimer levels were similarly elevated in both the bolus + infusion and single bolus groups on admission, with levels found to have reduced by the 24-hour and again by the 72-hour time points, with no significant difference identified between the two groups (Figure 51).

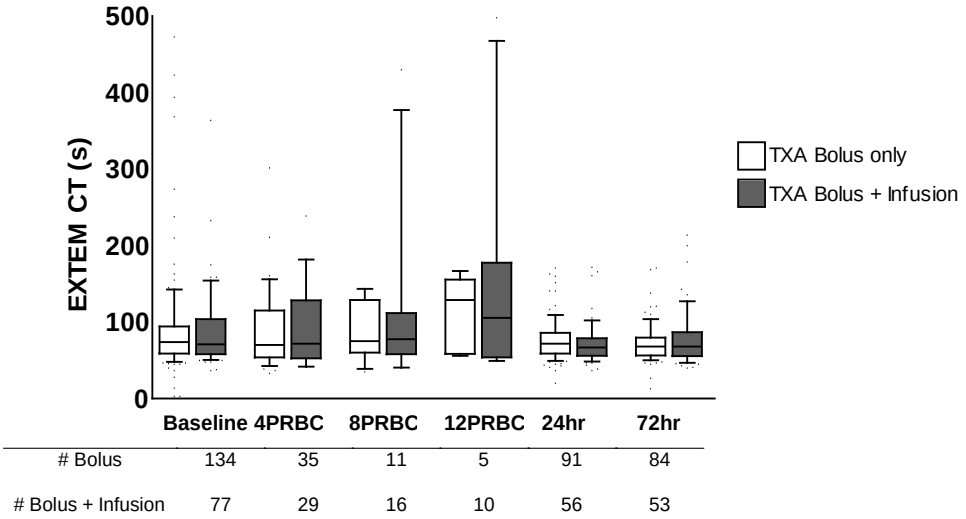
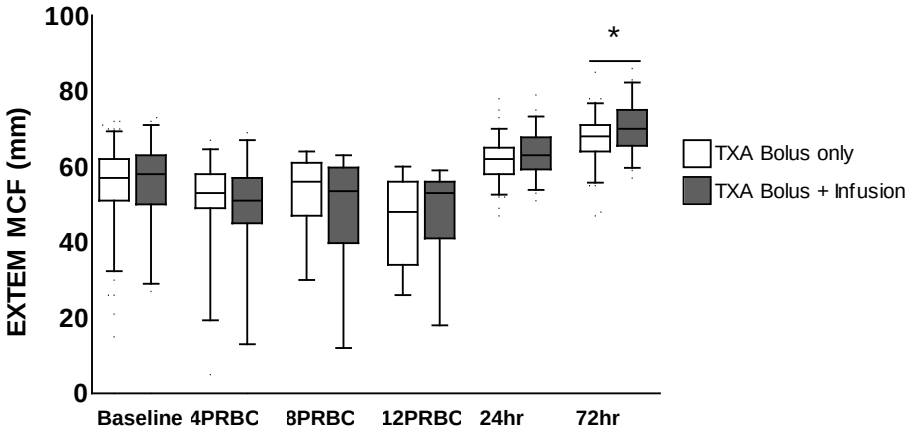


Figure 47 Clotting time (EXTEM CT) on admission, during bleeding and at 24 and 72 hours comparing patients who received TXA bolus vs bolus + infusion

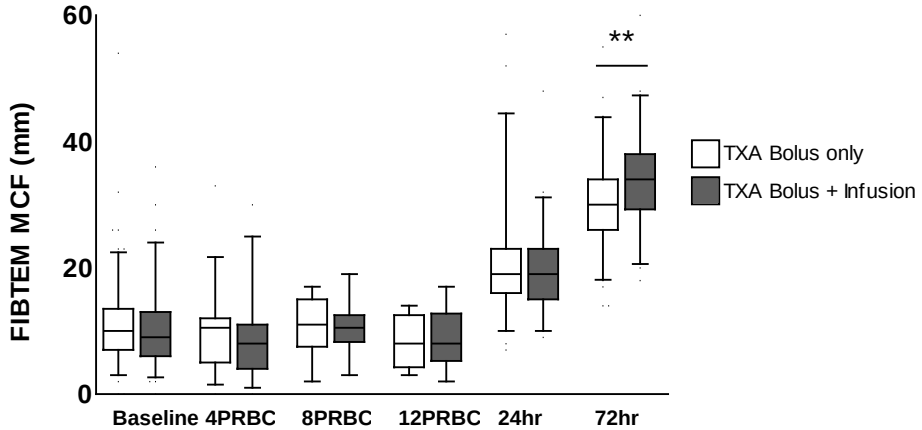
Whiskers represent 10 – 90th percentiles. PRBC, packed red blood cells.



	Baseline	4PRBC	8PRBC	12PRBC	24hr	72hr
# Bolus	131	35	11	5	91	84
# Bolus + Infusion	75	29	16	10	56	53

Figure 48 Maximum clot firmness (EXTEM MCF) on admission, during bleeding and at 24 and 72 hours comparing patients who received TXA bolus vs bolus + infusion

***p=0.03**



	Baseline	4PRBC	8PRBC	12PRBC	24hr	72hr
# Bolus	129	34	9	4	89	81
# Bolus + Infusion	72	29	14	10	55	52

Figure 49 FIBTEM maximum clot firmness (MCF) on admission, during bleeding and at 24 and 72 hours comparing patients who received TXA bolus vs bolus + infusion

****p=0.006**

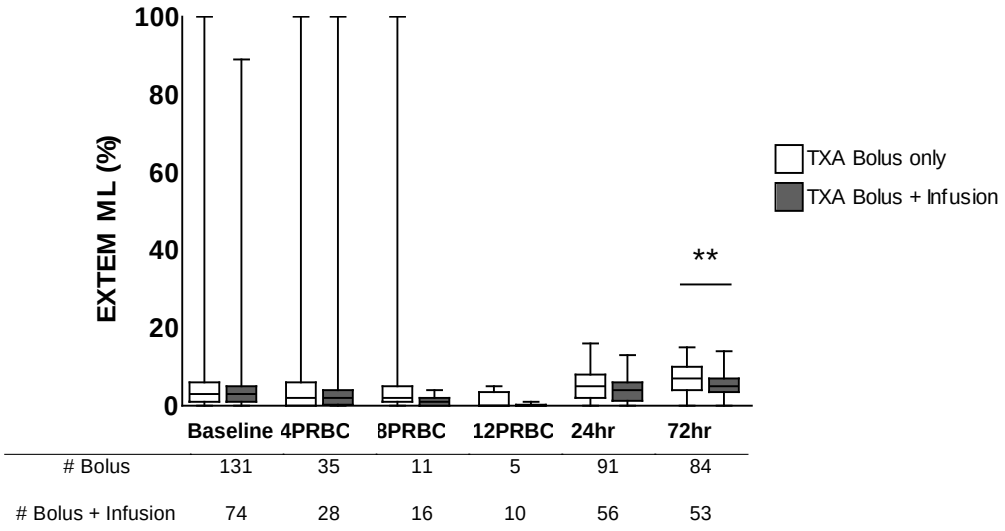
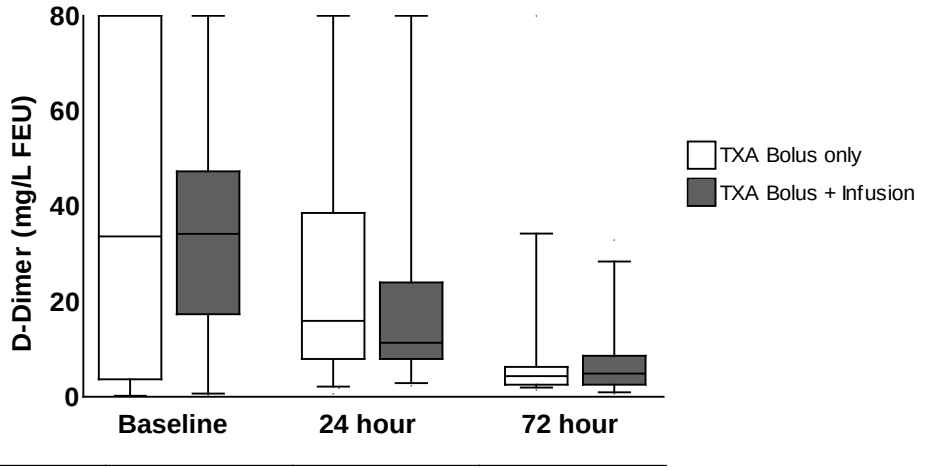


Figure 50 Functional fibrinolysis (EXTEM ML) on admission, during bleeding and at 24 and 72 hours comparing patients who received TXA bolus vs bolus + infusion

Whiskers represent minimum and maximum values. **p=0.007



# Bolus	46	43	35
# Bolus + Infusion	34	35	32

Figure 51 D-dimer levels on admission, at 24 and 72 hours comparing patients who received TXA bolus vs bolus + infusion

5.4.3 Effect of timing of TXA administration on unadjusted outcomes

The majority (92%) of patients started to receive a first bolus dose of TXA within 120 minutes of injury with only one patient's bolus starting beyond the three hour mark at 190 minutes from injury (Figure 52). Patients in the double bolus group received their second bolus of TXA a median of 151 minutes from injury (Table 13), with the latest dose administered over 16 hours from injury. Patients in the bolus + infusion group started to receive the infusion after a median of 158 minutes from injury (Table 13), with 36% of patients beginning their infusion greater than three hours following injury. The maximum length of time from injury to a TXA infusion starting was 11.5 hours.

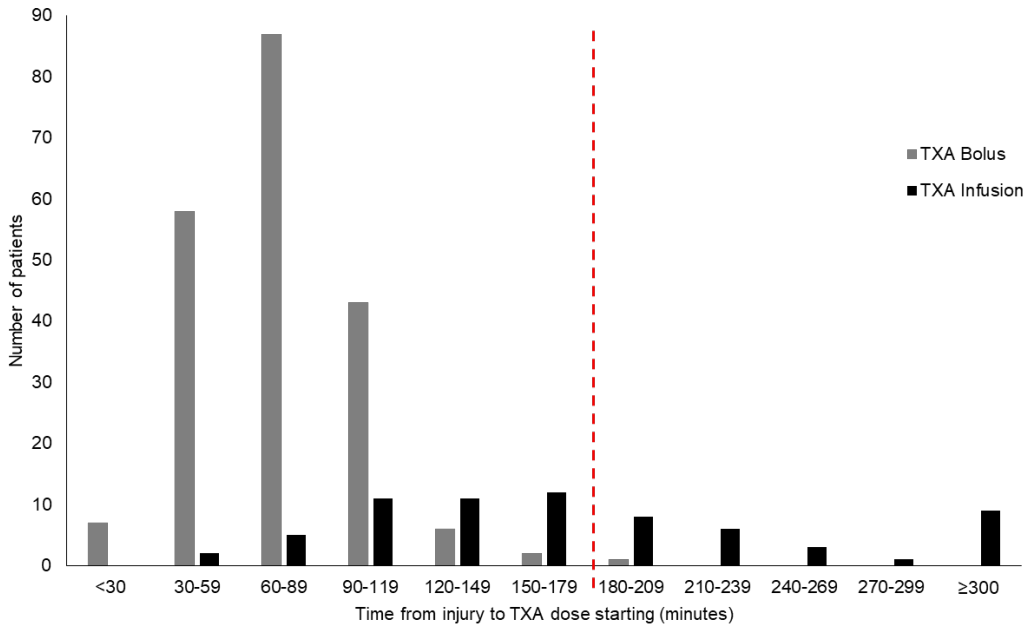


Figure 52 Histogram illustrating time from injury to start of TXA administration for patients in single bolus and bolus + infusion groups

Red dashed line represents three hours from injury

To explore the effect of timing of TXA therapy on patient outcomes, patients were stratified into 30-minute time windows based upon when their TXA therapy (either bolus or infusion) finished. Patient and injury characteristics are demonstrated in Table 15 and Table 16 along with clinical outcomes.

In the single bolus group, patients completing TXA therapy within 60-minutes of injury were significantly younger than those in the 90-119 minute window (29 vs 45 years, $p<0.01$; Table 15) and had a higher incidence of penetrating injury (50% vs 13%, $p=0.003$). There was no difference in injury severity or degree of shock across the 30-minute time windows. No significant differences in incidence of major or massive haemorrhage, thrombotic events (Figure 53), critical care or overall hospital LOS associated with timing of TXA bolus therapy were identified. Mortality at 24-hours and 28-days was greatest in those completing treatment between 60 – 89 minutes from injury.

No significant differences in patient characteristics, severity of injury or degree of shock were identified when assessing the bolus + infusion group according to time of infusion completion (Table 16). Incidence of major or massive haemorrhage did not vary significantly over time. Patients who started the TXA infusion later and therefore were exposed to TXA for a longer duration after injury, had an associated increase in subsequent thrombotic events (Chi-square test for trend $p=0.0007$; Figure 54). Patients completing the TXA bolus + infusion within 10.5 hours of injury were found to have a low incidence of thrombotic events, whilst those completing therapy beyond 11 hours had a 6-fold higher incidence of thromboses. Later TXA therapy was also associated with an increased overall hospital LOS (Table 16).

Table 15 Characteristics and outcomes for the single bolus group, stratified by time from injury to TXA 10-minute bolus finishing

	Time from injury to TXA finishing (minutes)				
	< 60	**60-89**	**90-119**	**≥ 120**	*p*
Number of survivors at time window	130	130	126	122	
Number completing TXA bolus during time window	32	56	30	12	
Age, years	29 (24 – 37)	39 (26 – 55)	45 (31 – 59)	36 (25 – 54)	0.008
Male, n (%)	26 (81%)	45 (80%)	20 (67%)	9 (75%)	0.47
Blunt, n (%)	16 (50%)	43 (77%)	26 (87%)	9 (75%)	0.009
Injury Severity Score	25 (16 – 43)	31 (18 – 43)	27 (19 – 36)	22 (18 – 34)	0.83
Base Deficit, mmol/L	9.4 (4.3 – 20.0)	6.6 (3.1 – 15.2)	7.3 (3.5 – 11.9)	5.6 (2.5 – 7.9)	0.16
Major haemorrhage, n (%)	20 (63%)	34 (61%)	17 (57%)	9 (75%)	0.74
Massive haemorrhage, n (%)	8 (25%)	13 (23%)	1 (3%)	2 (17%)	0.10
24hr mortality, n (%)	5 (3.8%)	13 (10%)	4 (3.2%)	1 (0.82%)	0.004
28-day mortality, n (%)	7 (5.4%)	17 (13.1%)	9 (7.1%)	3 (2.5%)	0.009
Time to death, hours	4 (2 – 36)	5 (3 – 25)	57 (4 – 130)	55 (12 – 208)	0.13
MODS, n (%)	16 (17%)	31 (33%)	20 (21%)	8 (8%)	0.0004
Thrombotic events, n (%)	3 (2.3%)	5 (3.8%)	6 (4.8%)	1 (0.8%)	0.27
Critical Care LOS, days	8 (1 – 18)	11 (4 – 21)	9 (6 – 22)	5 (1 – 11)	0.17
Hospital LOS, days	18 (7 – 32)	28 (13 – 49)	39 (15 – 58)	24 (11 – 61)	0.055

Includes data from n=130/134 patients with known timing of TXA administration. Mortality and thrombotic events calculated as % of survivors at each time window. % MODS based upon 95 patients surviving beyond 24hrs and admitted to Critical Care. Length of stay (LOS) for survivors only.

Table 16 Characteristics and outcomes for the bolus + infusion group, stratified by time from injury to TXA 8-hour infusion finishing

	Time from injury to TXA infusion finishing (minutes)					
	< 570	**570-599**	**600-629**	**630-659**	**≥ 660**	*p*
Total number of survivors to this time window	63	63	63	63	63	
Number completing TXA bolus during time window	7	11	11	12	27	
Age, years	24 (19 – 40)	27 (22 – 58)	27 (21 – 34)	35 (19 – 46)	32 (24 – 58)	0.34
Male, n (%)	7 (100%)	8 (73%)	5 (45%)	9 (75%)	19 (70%)	0.17
Blunt, n (%)	3 (43%)	6 (55%)	7 (64%)	9 (75%)	21 (78%)	0.35
ISS	26 (25 – 54)	22 (17 – 38)	35 (16 – 58)	32 (19 – 43)	33 (22 – 45)	0.45
BD, mmol/L	10.2 (8.8 – 22.4)	9.2 (4.4 – 16.0)	12.0 (10.1 – 21.8)	8.8 (5.7 – 18.7)	7.6 (5.0 – 12.2)	0.09
Major haemorrhage, n (%)	6 (86%)	8 (73%)	9 (82%)	9 (75%)	21 (78%)	0.58
Massive haemorrhage, n (%)	2 (29%)	4 (36%)	4 (36%)	4 (33%)	5 (19%)	0.71
24hr mortality, n (%)	0 (0%)	2 (3.2%)	2 (3.2%)	1 (1.6%)	1 (1.6%)	0.67
28-day mortality, n (%)	3 (4.8%)	3 (4.8%)	5 (7.9%)	2 (3.2%)	6 (9.5%)	0.55
Time to death, hours	35 (29 – 93)	8 (4 – 60)	27 (4 – 76)	36 (6 – 66)	97 (22 – 199)	0.36
MODS, n (%)	6 (9%)	6 (9%)	7 (11%)	10 (15%)	22 (34%)	0.0003
Thrombotic events, n (%)	0 (0%)	1 (1.6%)	1 (1.6%)	4 (6.3%)	7 (11.1%)	0.01
Critical Care LOS, days	4 (1 – 10)	6 (4 – 12)	9 (3 – 16)	18 (11 – 32)	10 (5 – 24)	0.11
Hospital LOS, days	8 (3 – 47)	17 (8 – 38)	14 (9 – 36)	51 (24 – 75)	42 (22 – 60)	0.03

Includes data from n=68/76 patients with known timing of TXA infusion administration. Mortality and thrombotic events calculated as % of survivors at each time window. % MODS based upon 65 patients surviving beyond 24hrs and admitted to Critical Care. Length of stay (LOS) for survivors only.

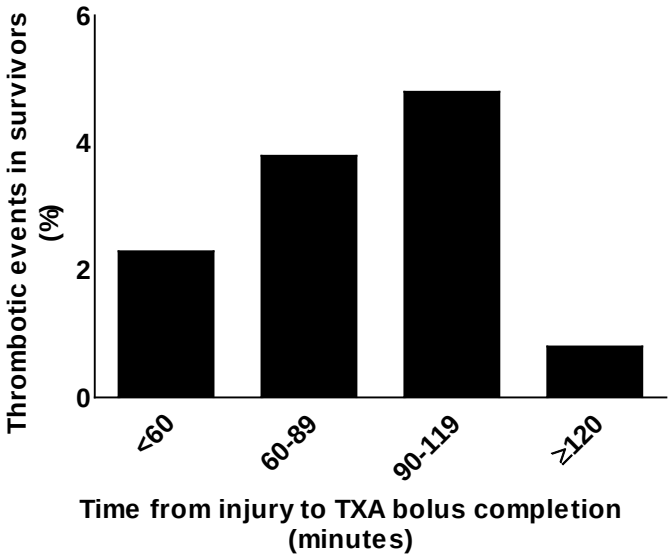


Figure 53 Thrombotic events in survivors from single bolus group only, stratified by time TXA bolus ends

Chi-square test for trend p=0.62

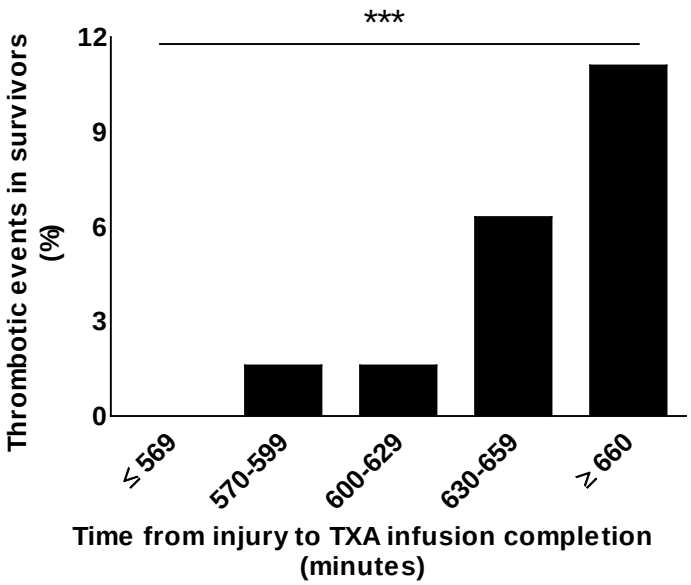


Figure 54 Thrombotic events in survivors from bolus + infusion group only, stratified by time TXA infusion ends

Chi-square test for trend ***p=0.0007

5.4.4 Thrombotic Events

Patients experiencing a thrombotic event within 28 days of injury were compared to those who did not (Table 17). Although not statistically significant, a pattern towards more women developing a thrombotic event was noted (p=0.05). There was no significant difference in age, mechanism or severity of injury and degree of shock on admission. Admission ROTEM parameters were similar with no evidence of hypercoagulability on admission in those developing thromboses. Although not reaching statistically significance, 28-day mortality differed between the two subgroups, with 96% of those who developed a thrombotic event surviving beyond 28-days from injury. Patients with a thrombotic event had a tendency towards greater blood transfusion requirements during the first 24 hours from injury and higher rates of MODS although neither of these differences reached statistical significance. Both critical care and hospital lengths of stay were greater in those survivors who developed a thrombotic complication.

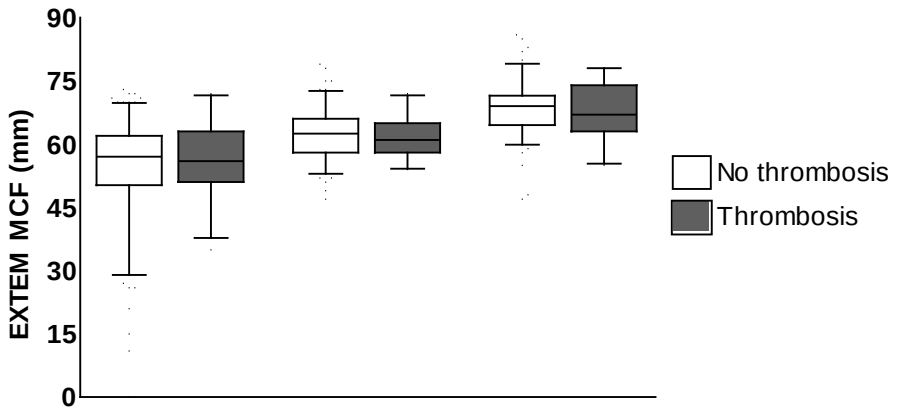
All patients received at least a bolus dose of TXA, but patients with thrombotic events tended towards later administration of the bolus (78 vs 70 minutes, p=0.13), with fewer receiving the bolus either pre-hospital or within the first hour from injury, although these differences were not statistically significant (Table 17). However, the time from injury to the TXA infusion beginning was significantly longer in patients who proceeded to develop a thrombotic complication (239 vs 154 minutes, p=0.048).

Table 17 Characteristics of patients surviving beyond 24 hours from injury who did and did not develop a thrombotic event

	No Thrombotic Event	Positive Thrombotic Event	*p*
Number of patients	156	27	
Number of thrombotic events	0	29	
Number of venous events	-	14	
Number of arterial events	-	15	
Age, years	32 (24 – 50)	38 (28 – 58)	0.12
Male, n (%)	121 (78%)	16 (59%)	0.05
Blunt, n (%)	104 (67%)	21 (78%)	0.37
Injury Severity Score	26 (17 – 38)	29 (20 – 43)	0.42
Glasgow Coma Scale	12 (6 – 15)	14 (9 – 15)	0.21
Base deficit, mmol/L	7.5 (4.1 – 12.3)	7.6 (4.6 – 11.9)	0.91
INR > 1.2, n (%)	44 (28%)	8 (30%)	1.00
Admission EXTEM MCF, mm	59 (54 - 63)	56 (51 – 63)	0.50
Admission FIBTEM MCF, mm	11 (7 – 15)	11 (8 – 13)	0.91
Admission EXTEM ML, %	4 (2 – 6)	4 (2 – 6)	0.78
Pre-hospital TXA, n (%)	114 (73%)	18 (67%)	0.49
Received TXA bolus within first hour, n (%)	52 (34%)	6 (22%)	0.37
Time from injury to 1st bolus, minutes	70 (47 – 91)	78 (60 – 99)	0.13
Received TXA Infusion, n (%)	58 (37%)	12 (44%)	0.52
Time from injury to infusion, minutes	154 (112 – 209)	239 (150 – 360)	0.048
Major Haemorrhage, n (%)	99 (63%)	22 (81%)	0.08
Massive Haemorrhage, n (%)	27 (17%)	9 (33%)	0.07
28-day mortality, n (%)	28 (17.9%)	1 (3.7%)	0.08
	n=137	n=26	
MODS, n (%)	113 (82%)	25 (96%)	0.13
Critical care LOS, days	7 (3 – 17)	14 (8 – 23)	0.006
Hospital LOS, days	24 (10 – 47)	35 (24 – 56)	0.04

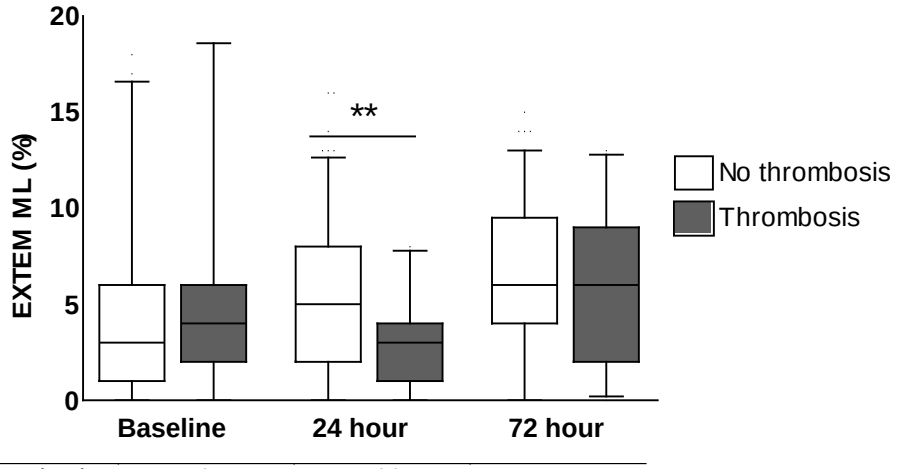
LOS, length of stay for survivors only. MODS, multiple organ dysfunction syndrome for those patients admitted to critical care.

Functional coagulation on admission and at 24- and 72-hours was compared between those who did and did not develop a later thrombotic complication. There was no evidence of hypercoagulability in terms of ROTEM clot strength at any sampling point during the first 72-hours from injury in the thrombosis group (Figure 55). Twenty-four hours after injury, the thrombosis group did, however, have lower levels of functional fibrinolysis (ML: 3 vs 5 %, p=0.002; Figure 56) and higher D-dimer levels (23.9 vs 13.3 mg/L FEU, p=0.02; Figure 57) compared to the no thrombosis group. By 72-hours there was no apparent difference between the groups.



	Baseline	24 hour	72 hour
# No Thrombosis	184	126	117
# Thrombosis	27	23	23

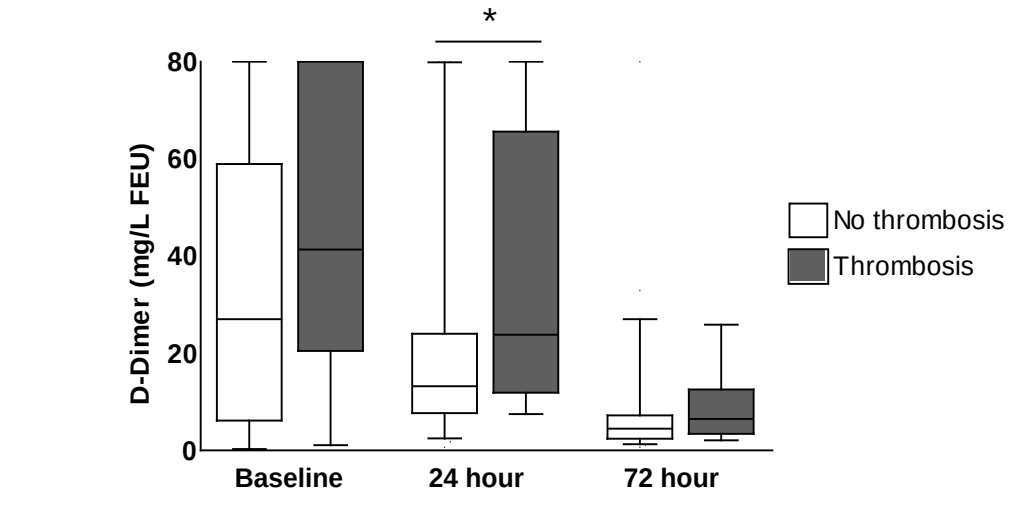
Figure 55 EXTEM Maximum clot firmness (MCF) over the first 72-hours from injury



# No Thrombosis	184	126	117
# Thrombosis	27	23	23

Figure 56 EXTEM Maximum lysis (ML) over the first 72-hours from injury

****p=0.002**



# No Thrombosis	65	63	56
# Thrombosis	16	16	12

Figure 57 D-dimer levels over the first 72-hours from injury

***p=0.02**

The no thrombosis and thrombosis groups were further subdivided by dosing regimen of TXA received. A similar pattern at 24-hours of lower ML (Figure 58) and higher D-dimer (Figure 59) levels in patients who developed thrombosis was seen, with no statistically significant difference in levels identified between the single bolus and bolus + infusion treatment regimens.

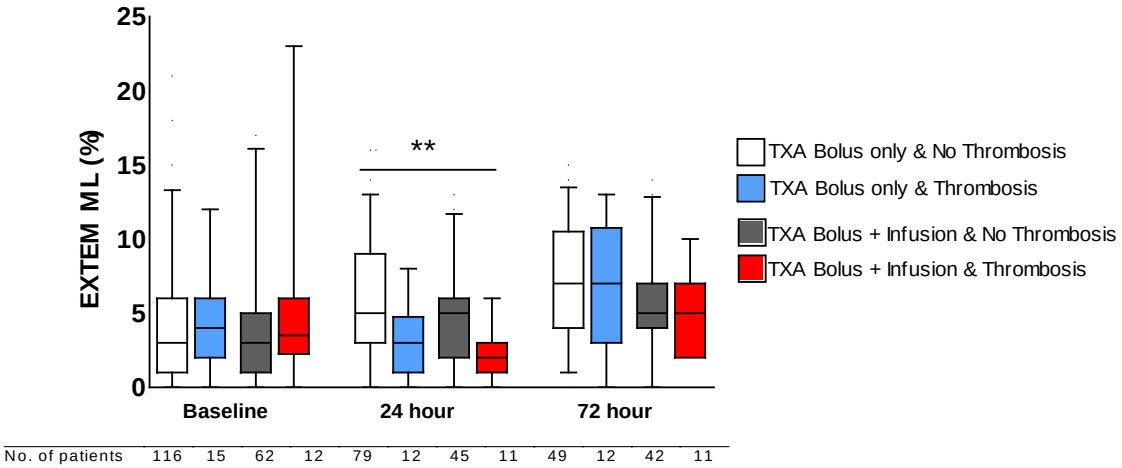


Figure 58 EXTEM Maximum lysis (ML) over the first 72-hours from injury stratified by TXA dosing regimen and thrombotic events

****p=0.006 comparing the four groups at 24-hours (Kruskal-Wallis) with ML in TXA bolus + infusion & thrombosis group significantly lower than in the TXA bolus only & no thrombosis group on Dunn's multiple comparison test (2 vs 5 %, p<0.05).**

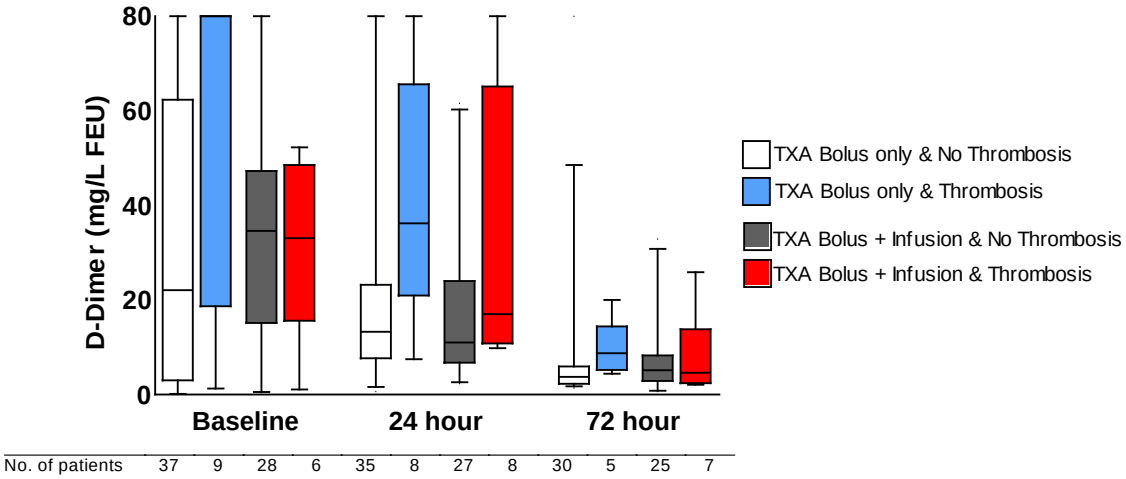


Figure 59 D-dimer levels over the first 72-hours from injury stratified by TXA dosing regimen and thrombotic events

D-dimer levels at 24-hours were higher in patients with thrombosis in both the single bolus and bolus + infusion TXA groups, but did not reach statistical significance (Kruskal-Wallis test at 24 hours, p=0.08).

5.4.5 Risk-adjusted effect of TXA infusion and time to bolus on late outcomes

The previous univariate analysis suggested that TXA therapy may be associated with the development of later thrombotic events and MODS. Late TXA therapy is known to be associated with an increased risk of mortality. Therefore, to investigate the independence of the effect the TXA infusion versus a single bolus of TXA and the effect time from injury to first TXA bolus has on the outcomes of 28-day mortality, MODS and thrombotic events, several multi-variate models were created. Patients surviving beyond 24 hours were included in the analysis.

5.4.5.1 28-day mortality

Univariate analysis of all admission variables on 28-day mortality was performed (Table 18) and factors achieving a univariate significance of p<0.2 were entered into a multivariate logistic analysis.

Table 18 Univariate analysis of admission variables on 28-day mortality

	Survived	**Died**	***p***
Number of patients	154	29	
Age, years	33 (24 – 50)	34 (26 – 54)	0.51
Male, n (%)	118 (77%)	19 (66%)	0.24
Blunt, n (%)	103 (67%)	22 (76%)	0.39
Injury Severity Score	25 (17 – 38)	38 (26 – 52)	**0.0007**
ED Admission SBP, mmHg	100 (82 – 122)	91 (69 – 126)	0.39
ED Glasgow Coma Scale	14 (8 – 15)	3 (3 – 9)	**<0.0001**
Base deficit, mmol/L	7.3 (3.8 – 11.3)	11.3 (6.6 – 22.0)	**0.0009**
INR	1.2 (1.1 – 1.3)	1.3 (1.2 – 1.5)	**0.009**
24hr PRBC total, units	5 (2 – 8)	9 (5 – 16)	**0.002**
Time: injury - TXA bolus, minutes	71 (52 – 92)	70 (46 – 96)	0.93
Received TXA Infusion, n (%)	56 (36%)	14 (48%)	0.23

Receiving the TXA infusion was not associated with 28-day mortality following multivariate analysis (Table 19). Factors associated with mortality were admission GCS and base deficit.

Table 19 Multivariate logistic regression: the effect of TXA infusion on 28-day mortality

Variable	Odds Ratio (95% CI)	*p*
ISS	1.03 (0.99 – 1.08)	0.15
GCS	0.80 (0.70 – 0.91)	**0.001**
Base deficit	1.09 (1.01 – 1.18)	**0.03**
INR	1.55 (0.06 – 44.09)	0.80
24hr PRBC total	1.03 (0.95 – 1.12)	0.49
TXA Infusion	0.49 (0.14 – 1.80)	0.29

There was a positive signal towards a longer time from injury to administration of the TXA bolus being associated with 28-day mortality following multivariate analysis although the significance did not reach the $p<0.05$ threshold (Table 20). Admission GCS and base deficit were again associated with mortality in this model.

Table 20 Multivariate logistic regression: the effect of time from injury to TXA bolus on 28-day mortality

Variable	Odds Ratio (95% CI)	*p*
ISS	1.02 (0.98 – 1.07)	0.31
GCS	0.80 (0.70 – 0.91)	**0.001**
Base deficit	1.09 (1.01 – 1.18)	**0.02**
INR	2.58 (0.10 – 64.33)	0.56
24hr PRBC total	1.01 (0.93 – 1.11)	0.76
Minutes from injury – TXA bolus	1.02 (1.00 – 1.04)	0.06

5.4.5.2 Thrombotic events

Univariate analysis of all admission variables on occurrence of thrombotic events within 28-days of injury was performed (Table 21) and factors achieving a univariate significance of $p<0.2$ were entered into a multivariate logistic analysis.

Table 21 Univariate analysis of admission variables on occurrence of thrombotic events

	No thrombotic event	**Thrombotic event**	***p***
Number of patients	153	27	
Age, years	32 (24 – 50)	38 (28 – 58)	**0.12**
Male, n (%)	118 (77%)	16 (59%)	**0.05**
Blunt, n (%)	103 (67%)	21 (78%)	0.37
Injury Severity Score	26 (17 – 38)	29 (20 – 43)	0.42
ED Admission SBP, mmHg	98 (81 – 122)	102 (75 – 127)	0.83
ED Glasgow Coma Scale	12 (6 – 15)	14 (9 – 15)	0.21
Base deficit, mmol/L	7.5 (4.1 – 12.3)	7.6 (4.6 – 11.9)	0.91
INR	1.2 (1.1 – 1.3)	1.2 (1.1 – 1.3)	0.24
24hr PRBC total	5 (2 – 8)	7 (4 – 10)	**0.03**
Time: injury - TXA bolus, minutes	70 (47 – 91)	78 (60 – 99)	0.11
Received TXA Infusion, n (%)	58 (38%)	12 (44%)	0.52

The TXA infusion was found not to be associated with thrombotic events after risk-adjustment with multivariate analysis (Table 22). The time from injury to delivery of the TXA bolus was also not associated with thrombotic events on multivariate analysis (Table 23). There was a signal towards female gender being associated with thrombotic event occurrence, but this factor did not reach statistical significance.

Table 22 Multivariate logistic regression: the effect of TXA infusion on thrombotic events

Variable	Odds Ratio (95% CI)	*p*
Age	1.01 (0.99 – 1.04)	0.34
Male Gender	0.48 (0.20 – 1.18)	0.11
24hr PRBC total	1.04 (0.98 – 1.09)	0.22
TXA Infusion	1.23 (0.52 – 2.93)	0.64

Table 23 Multivariate logistic regression: the effect of time from injury to TXA bolus on thrombotic events

Variable	Odds Ratio (95% CI)	*p*
Age	1.01 (0.98 – 1.03)	0.53
Male Gender	0.44 (0.19 – 1.09)	0.08
24hr PRBC total	1.03 (0.97 – 1.09)	0.30
Minutes from injury – TXA bolus	1.01 (0.99 – 1.02)	0.28

5.4.5.3 MODS

Univariate analysis of all admission variables on occurrence of MODS within 7-days of injury was performed (Table 24) and factors achieving a univariate significance of p<0.2 were entered into a multivariate logistic analysis.

Table 24 Univariate analysis of admission variables on occurrence of MODS

	No MODS	**MODS**	***p***
Number of patients	25	135	
Age, years	29 (23 – 48)	36 (25 – 52)	0.22
Male, n (%)	21 (84%)	95 (70%)	0.22
Blunt, n (%)	13 (52%)	107 (79%)	**0.004**
Injury Severity Score	19 (11 – 31)	30 (22 – 43)	**0.0007**
ED Glasgow Coma Scale	15 (14 – 15)	10 (3 – 14)	**<0.0001**
Base deficit, mmol/L	5.4 (3.4 – 10.3)	7.9 (4.9 – 13.8)	**0.08**
INR	1.1 (1.1 – 1.2)	1.2 (1.1 – 1.3)	**0.01**
24hr PRBC total	3 (2 – 6)	6 (3 – 9)	**0.0001**
Time: injury - TXA bolus, minutes	73 (46 – 82)	70 (52 – 94)	0.88
Received TXA Infusion, n (%)	7 (28%)	58 (43%)	0.19

Receiving the TXA infusion was not found to be associated with development of MODS after multivariate analysis (Table 25). A higher GCS was protective against MODS whilst higher blood transfusion requirements were independently associated with MODS development. There was a signal towards blunt rather than penetrating injury being associated with MODS development.

Table 25 Multivariate logistic regression: the effect of TXA infusion on MODS

Variable	Odds Ratio (95% CI)	*p*
Penetrating injury	0.20 (0.04 – 1.09)	0.06
ISS	1.04 (0.98 – 1.10)	0.26
GCS	0.71 (0.57 – 0.88)	**0.002**
Base deficit	0.99 (0.88 – 1.12)	0.89
INR	0.39 (0.00 – 70.63)	0.73
24hr PRBC total	1.64 (1.21 – 2.21)	**0.001**
TXA Infusion	2.43 (0.56 – 10.52)	0.23

The time from injury to treatment with the TXA bolus was not independently associated with MODS after multivariate analysis (Table 26).

Table 26 Multivariate logistic regression: the effect of time from injury to TXA bolus on MODS

Variable	Odds Ratio (95% CI)	*p*
Penetrating injury	0.19 (0.03 – 1.12)	0.07
ISS	1.05 (0.99 – 1.11)	0.14
GCS	0.68 (0.54 – 0.86)	**0.001**
Base deficit	1.02 (0.90 – 1.15)	0.80
INR	0.20 (0.00 – 43.92)	0.56
24hr PRBC total	1.68 (1.24 – 2.27)	**0.001**
Minutes from injury – TXA bolus	1.01 (0.99 – 1.04)	0.42

5.4.5.4 Summary of the effect of TXA on late clinical outcomes after multivariate analysis

The effect of the TXA infusion on late outcomes and the effect of time from injury to receiving the TXA bolus on late outcomes after risk-adjustment by multivariate analysis are summarised in Figure 60 and Figure 61 respectively.

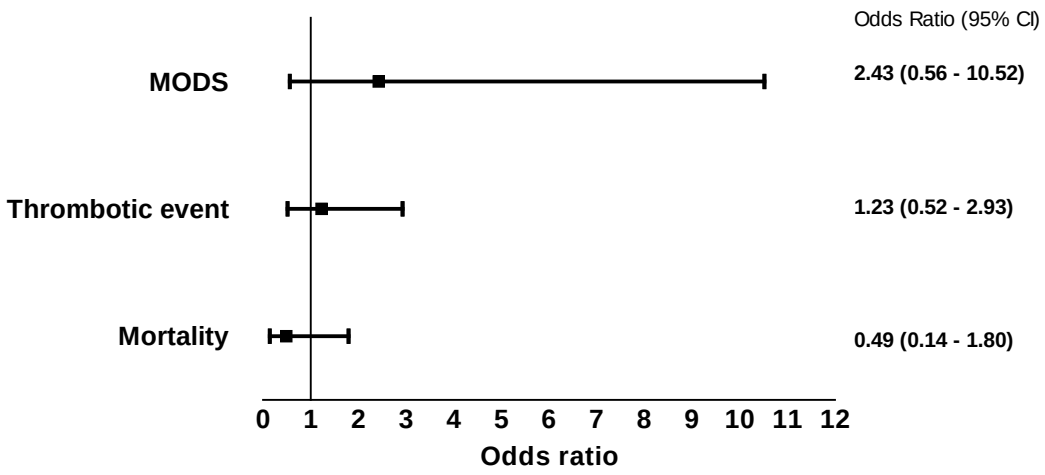


Figure 60 The effect of the TXA infusion on late clinical outcomes

Forest plot shows odds ratio and 95% confidence intervals.

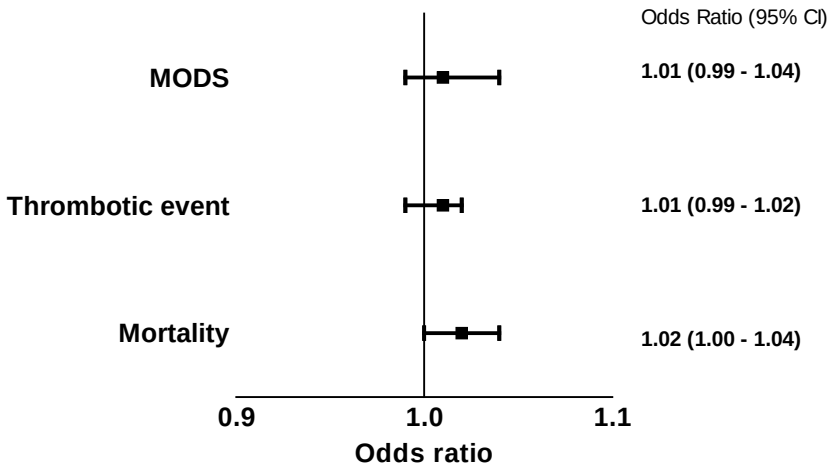


Figure 61 The effect of time from injury to receiving the TXA bolus on late clinical outcomes

Forest plot shows odds ratio and 95% confidence intervals.

5.5 DISCUSSION

In this study of 215 patients treated with TXA for traumatic haemorrhage, no beneficial effect in functional coagulation or fibrinolysis on admission, during bleeding or at 24-hours was measured from the TXA bolus + infusion over a single bolus. A non-statistically significant higher rate of thrombotic events was identified on univariate analysis in those receiving bolus + infusion, in the context of increased clot strength and reduced fibrinolysis on ROTEM at 72-hours in this group compared to patients receiving a single TXA bolus. TXA dosed as a bolus + infusion has previously been shown to reduce the risk of death from haemorrhage and in this study we found no significant difference in haemorrhage-related deaths between the two dosing regimens studied. A linear trend between later completion of the TXA infusion and increased thrombotic events was identified in this cohort of patients on univariate analysis. However, after adjusting for all variables with multivariate analysis, receiving the TXA infusion was not associated with thrombotic events, MODS or mortality. The odds ratio of the TXA infusion was 0.5 for mortality suggesting a possible underpowered effect and highlighting the need for clinical trial data to explore this effect further. Time from injury to treatment with the TXA bolus was similarly not associated with development of MODS or thrombosis but there was a non-statistically significant signal towards this variable being associated with mortality. Patients who developed a thrombotic event had lower ROTEM ML and yet higher D-dimers at 24-hours.

In this observational study, timing of administration of the TXA infusion was seen to vary widely. Whilst this likely reflects the difficulty of implementing a specific IV treatment for a bleeding trauma patient for clinical teams who find themselves within a stressful situation requiring prioritisation of many competing tasks, the reality of the treatment actually

delivered is that it was not as described in the CRASH-2 trial. The median time from bolus to infusion starting was 84 minutes. One factor likely contributing to this delay is that the bolus was given pre-hospital in the majority of cases whilst the infusion would not be expected to start until arrival in the hospital. It is not known whether a bolus followed by immediate infusion within the three hour window post-injury has the same beneficial effect as a bolus followed by a delayed infusion. However, the finding of increased subsequent thrombotic events in patients who had TXA infusions running beyond 10.5 hours after injury on univariate analysis suggests that any delay in therapy may be important with regards to development of thrombotic complications.

Earlier treatment with TXA following injury is known to provide the most beneficial effects in terms of reducing the risk of bleeding due to haemorrhage.[139] A recently published meta-analysis of the CRASH-2 trial data combined with data from the WOMAN trial, which assessed the effects of TXA in post-partum haemorrhage, examined the effect of delay in treatment on the effectiveness of TXA.[140] In a combined analysis of data from over 40,000 patients, the study reported that whilst immediate treatment resulted in the greatest improvement in survival (by at least 70%), for every 15 minute delay in treatment, the survival benefit reduced by 10%. After three hours, there was no improvement in survival with TXA. Treatment delay was not found to alter the risk of vascular occlusive events. The current study has also shown no association between time to receiving the TXA bolus and development of thrombotic complications. Only one patient in this study received the TXA bolus beyond three hours from injury. In spite of this our analysis suggests that increased time from injury to administration of the TXA bolus could be associated with mortality, even when the TXA bolus is administered within three hours from injury. Increasing the sample size could help to elucidate whether this factor becomes more significant.

Accurate diagnosis of fibrinolytic status in a clinically useful timeframe is challenging. The methods currently available to the clinician include laboratory measurement of D-dimer or point-of-care diagnostics such as TEG and ROTEM. However, as we have demonstrated in this body of work (Chapter 3), current ROTEM assays are capable of detecting only the highest extremes of tPA-mediated hyperfibrinolysis and do not detect all increased fibrinolytic activity. Trauma resuscitation guidelines recommend empiric treatment with TXA in suspected trauma haemorrhage for this reason. The challenge, however, is knowing if a patient is still lytic or not after treatment with the first 1g bolus of TXA. A patient with persistently high fibrinolytic activity may derive benefit from an additional dosage of TXA whilst in another patient whose fibrinolytic system has already been effectively 'switched-off' any further TXA treatment could potentially cause harm. Mapping the timescale for the temporal changes in the fibrinolytic system in response to initial injury, blood product resuscitation, therapeutics such as TXA and surgery through use of existing biomarker assays e.g. tPA, PAP is a priority. This may lead to improved understanding with regards the optimal timing for TXA therapy or other haemostatic agents post injury. In addition, development of improved point-of-care diagnostics capable of rapid, accurate assessment of fibrinolytic activity is required.

However, despite a recent change in practice at the RLH with omission of the TXA infusion based on expert opinion and increased use of VHA in the clinical setting, the current best evidence-based practice in order to improve trauma haemorrhage outcomes is to deliver TXA in-line with the CRASH-2 trial of a bolus followed by immediate infusion. The Author acknowledges that deviation from this evidence-based practice has the potential to result in

poorer outcomes for patients. This further strengthens the argument for another trial to urgently investigate the optimal dose of TXA in trauma haemorrhage.

This study has a number of limitations. Firstly, it was an observational study that recorded the incidence of symptomatic thrombotic events, with no routine screening for asymptomatic VTE and therefore the incidence of thrombotic events reported may be an underestimate. A standardised mechanical and pharmacological thromboprophylaxis protocol is in place within our institution, although methods of thromboprophylaxis and adherence with the guideline were not recorded in this study and therefore the influence of prophylaxis in the reported results is unknown. The number of patients studied and the number of thrombotic events identified were relatively small, which may have limited our ability to detect differences in outcomes associated with the different TXA dosing regimens. In particular, we are able to make no assessment of the effect later TXA treatment had in protecting against death due to bleeding due to the small sample size and small number of deaths secondary to haemorrhage. The difference identified in deaths secondary to haemorrhage in the unadjusted analysis did not reach statistical significance, however, a lack of power in this study limits our ability to know if there is a true difference or not in this outcome between dosing regimens.

5.6 CONCLUSION

The principle findings of this study are that a single bolus of TXA appears to exert the same effect as measured by functional coagulation and D-dimer as a bolus + infusion. In patients receiving the TXA infusion, thrombotic complications increased in a linear fashion the longer the delay to starting the infusion and therefore the later patients were exposed to a TXA

infusion after injury. However, after adjusting for all other variables, neither the TXA infusion nor the time from injury to administration of the TXA bolus were found to be associated with development of thrombotic events. A single bolus of TXA may, however, provide a lower effective therapeutic dose in trauma haemorrhage with reduced potential for thrombotic complications. This theory would require further testing in a randomised control trial. Future research should aim to improve point of care diagnostics of fibrinolytic activity status and characterise the temporal changes in the coagulation and fibrinolytic systems that occur in response to injury and resuscitation which could enable individualised therapies such as early high dose thromboprophylaxis in an individual at high-risk of thrombotic complications.

5.7 Specific Contributions of the Author

Having attended several trauma conferences where concerns had been raised surrounding the potential thrombotic complications of TXA use, the Author conceived this study to investigate the effect of the TXA infusion and timing of TXA treatment on outcomes in our patients recruited to RLH. The Author created the dataset used within this study. The study design was planned with KB and RD. The analysis was performed by the Author. Data interpretation was carried out by the Author, KB and RD. This chapter was written and edited by the Author after receiving comments from RD and KB. The Author created all data tables and figures within the chapter.

Chapter 6

CONCLUSIONS, LIMITATIONS & FUTURE RESEARCH DIRECTIONS

6.1 SUMMARY OF FINDINGS

The focus of this thesis has been to explore the different fibrinolytic phenotypes which contribute towards early trauma-induced coagulopathy (TIC) and to examine the effects of the antifibrinolytic Tranexamic acid (TXA) in trauma haemorrhage.

The role for TXA in patients presenting to hospital with low levels of fibrinolysis as detected by VHA was uncertain. In Chapter 3, patients presenting with low levels of ROTEM detected fibrinolysis were characterised and discovered in fact to be a heterogeneous group. D-dimer level in combination with ML is able to discriminate those patients with poor clinical outcomes. Patients with the ML_{LOW}+DD_{LOW} phenotype have low injury severity and good outcomes. However, those with the ML_{LOW}+DD_{HIGH} phenotype are severely injured, coagulopathic, with a hyperfibrinolytic biomarker profile and associated poor clinical outcomes. The mechanism of occult hyperfibrinolysis in this phenotype does not appear to be driven by tPA and instead a potential alternative mechanism of plasminogen activation, mediated by S100A10, was identified which also artificially lowers ML *ex-vivo*. The finding of an occult hyperfibrinolytic phenotype supports the continued empiric use of TXA in trauma haemorrhage.

In order to address the knowledge gap around the effect TXA has on TIC, Chapter 4 examined the effect of TXA on functional coagulation and fibrinolysis during trauma haemorrhage. Early empiric TXA completely avoids functional hyperfibrinolysis both on admission and during haemorrhage (up to transfusion of the 12 unit PRBC). D-dimer levels in those treated with TXA were correspondingly lower at the end of massive transfusion. TXA additionally provided a degree of protection against TIC with greater clot strength after massive haemorrhage in those receiving TXA compared to patients who did not.

Finally, Chapter 5 investigated the effect of the TXA infusion and timing of treatment on clinical outcomes including thrombotic events. In a separate study of bleeding trauma patients, those receiving a TXA bolus + infusion were compared to those receiving a single TXA bolus. No difference in functional measures of coagulation were identified between the dosing regimens during initial haemorrhage or at 24-hours. However, at 72-hours, those receiving TXA bolus + infusion demonstrated increased clot strength and a correspondingly lower ML. In those treated with a TXA bolus + infusion, thrombotic events were discovered on univariate analysis to increase in a linear fashion the longer the time to administering the TXA infusion after injury. The incidence of thrombotic events increased if exposure to the TXA infusion continued beyond 10.5 hours from injury. However on multivariate analysis, the TXA infusion and time from injury to TXA bolus administration were not associated with thrombotic events. In theory, a single TXA bolus may provide a lower effective therapeutic dose in trauma haemorrhage; countering hyperfibrinolysis yet with a reduced potential for thrombotic complications. This theory requires further evaluation in a randomised control trial to investigate the effect of only administering a TXA bolus on patient outcomes.

6.2 Strengths & Limitations of This Work

The collaboration of researchers from six European trauma centres working together as part of the INTRN partnership is an important feature of the work contained within the thesis. The study described in Chapter 3 involved over 900 patients recruited to the ACIT II study from five out of these six partner sites. This greatly enhanced the study sample size and dataset available which in turn strengthened the results and enabled robust conclusions to be made. No previous study has assayed plasma samples from as many trauma patients for biomarkers of coagulation and fibrinolysis to investigate VHA-hypofibrinolysis. On a personal

level, collaboration with international partner sites provided me the opportunity to meet and engage with fellow researchers abroad and develop connections for future work and research.

The Royal London Hospital is the busiest major trauma centre in the United Kingdom and treats a large volume of severely injured and bleeding trauma patients. This permitted the recruitment of a large volume of bleeding 'code-red' patients over a relatively short time period and enabled the effect of TXA in this cohort of patients to be studied. In the final study described, patients were enrolled 24 hours a day, seven days a week, further increasing study recruitment. A strength of the study is that Nominated Consultee agreement allows rapid patient enrolment and permits baseline blood samples to be taken as soon as the patient arrives in the ED and within 2 hours of injury. This early sampling time point permits investigation of the early fibrinolytic component of Acute Traumatic Coagulopathy. One limitation of attempting to obtain serial samples from severely injured bleeding patients for an observational study of this nature is that occasionally clinical need takes priority and for example a PRBC unit sample may not be possible to obtain if the patient is transferred between resus and the operating theatre during the sampling window. Whilst correct that clinical need takes priority over the observational study, this results in missing data and fewer data points available for the PRBC unit samples in particular within the datasets analysed.

A limitation of the results presented within this thesis is that they are obtained from observational data and can therefore suggest associations but not determine causation. This should be borne in mind when considering patient outcome data in particular.

Despite the documented limitations of both D-dimer levels and ROTEM-detected fibrinolysis, these were the only measures of fibrinolysis by which the two TXA dosages in chapter 5 could be compared. Due to limitations on time it was not possible to measure biomarkers of fibrinolysis e.g. PAP, for this study cohort. It is planned that these stored samples will be analysed by our group in the future in order to expand on the work presented here.

6.3 Future Research Directions

This body of work represents our research groups initial steps towards understanding the changes in the fibrinolytic system following injury and in doing so has raised further important questions and opened many potential avenues for future research to investigate.

Accurate assessment of fibrinolytic activity is limited by the current lack of a sufficiently rapid yet sensitive diagnostic tool. The work presented within this thesis has highlighted the limitations of current ROTEM assays to detect fibrinolytic activity. As understanding of the drivers and control mechanisms for fibrinolytic activation are better understood, collaboration between trauma researchers and the medical device industries should be encouraged with an aim of developing novel assays and point-of-care platforms which permit serial assessment of fibrinolytic status to aid clinical acumen and guide treatment.

In the meantime, future studies aimed at better understanding the mechanisms of fibrinolytic activation should utilise measurement of specific individual protein biomarkers e.g. PAP, tPA, PAI-1. The novel finding of a potential role for S100A10 in mediating increased fibrinolytic activity in those patients who present with elevated D-dimers yet low ML requires further evaluation. Whilst we have identified a potential explanation for “fibrinolytic

shutdown", this work represents merely the start of our journey towards fully understanding the fibrinolytic changes following injury. A later fibrinolytic shutdown may exist and experimental models to measure fibrinolytic protein mediators including S100A10 during the first 24 – 48 hours are required. The effect of TXA in this specific phenotype is unclear and will require further clinical trial investigation. The mechanisms of exposure and shedding of membrane-bound S100A10 and its function when free in the plasma requires exploration. Both clinical and experimental studies will be required to understand the role of S100A10 and assess its potential as a therapeutic target in trauma-induced coagulopathy.

Given the advances in pharmaceutical engineering since TXA was first described over 50 years ago, it seems unlikely that such an old drug will remain the optimal agent to treat trauma patients. Whilst it remains the only evidence-based therapy available for treatment of hyperfibrinolysis in trauma, a randomised trial to determine optimal therapeutic dose should be considered. As understanding of the pathways that drive excessive fibrinolytic activation advances, including the role of uPA and S100A10, this may lead to the development of novel and more efficacious therapeutics. The fibrinolytic system is highly dynamic, evolving over time following injury and yet studies have on the whole been limited to measurement of fibrinolysis at the point of ED arrival. Future studies should focus on serial sampling to fully characterise the temporal changes that occur in the coagulation and fibrinolytic systems in response not just to the initial trauma, but over subsequent hours and days following resuscitation, surgery and antifibrinolytic therapy.

6.4 Final Conclusions

Trauma haemorrhage remains a leading cause of morbidity and mortality worldwide. Advances in our understanding of the pathophysiology leading to trauma-induced coagulopathy are an important means by which targeted therapeutic interventions can be developed. Tranexamic acid in trauma haemorrhage is known to improve survival and in this body of work has been shown to reduce overt functional hyperfibrinolysis and if dosed empirically has the potential to also be of benefit to patients with an occult hyperfibrinolytic phenotype. The findings of this work represent an initial step towards understanding the fibrinolytic response to traumatic injury and understanding the effect of TXA in trauma haemorrhage. Future research is required to define the mechanisms driving hyperfibrinolysis and trauma-induced coagulopathy, with the aim of enabling superior diagnostics and novel therapeutics to be developed in order to improve overall outcomes for trauma patients.

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APPENDICES

APPENDIX 1

Mechanism of occult hyperfibrinolysis in trauma patients

Clinical characteristics and coagulation profiles were similar to the main cohort (Table A27 and Table A28). Urokinase-type plasminogen activator (uPA) levels were similar across all ML and DD groups (Figure A62). Plasma S100A10 levels were low in the DD_{LOW} cohorts but significantly elevated in both the ML_{LOW}+DD_{HIGH} and ML_{HIGH}+DD_{HIGH} patients (Figure A63A). Plasma S100A10 levels were strongly correlated with both PAP ($r=0.58$, $p<0.001$) and D-dimer ($r=0.56$, $p<0.001$) levels (Figure A63B & C). S100A10 levels were strongly associated with injury severity (Figure A63D). In particular, patients with TBI (AIS Head 3+) had much higher plasma levels of S100A10 than those without (S100A10: 437 vs 55 pg/ml, $p<0.001$). TBI patients with higher plasma S100A10 (above the median 120pg/ml) had a 58% mortality, compared to only 6% for those with low S100A10 levels ($p<0.001$), as well as a higher incidence of coagulopathy (31% vs 0%, $p=0.009$) and PRBC transfusion (38% vs 6%, $p=0.01$).

As S100A10 is known to bind tPA and plasmin, we further hypothesized that S100A10 would bind these in the ROTEM cup, thus reducing *ex-vivo* the availability of plasmin and therefore the VHA-measured fibrinolysis. We added 10ng/ml S100A10 to the whole blood of 6 healthy volunteers and saw a significant reduction in %ML (Figure A63E). In the ML_{LOW} and ML_{NORMAL} combined cohort, plasma S100A10 levels negatively correlated with %ML ($r=-0.26$, $p<0.001$, Figure A63F) but were still positively correlated with PAP ($r=0.56$, $p<0.001$) and D-dimer levels ($r=0.28$, $p<0.001$). There was no relationship between plasma levels of S100A10 and plasma tPA levels.

Table A27 Characteristics and outcomes of the extended biomarker subgroup

Number of Patients	ML_{NORMAL} 68	ML_{LOW} + DD_{LOW} 51	ML_{LOW} + DD_{HIGH} 45	ML_{HIGH} + DD_{LOW} 8	ML_{HIGH} + DD_{HIGH} 7
Admission characteristics					
Age, years	28 (23-45)	38 (27- 50)^	50 (34-62)*	27 (23-45)	58 (24-59)
Male, n (%)	58 (85%)	44 (86%)	34 (76%)	7 (88%)	6 (86%)
GCS	15 (14-15)	15 (14-15)	11 (4-15)**	15 (13-15)	8 (4-12)
SBP, mmHg	138 (116-154)	138 (118-151)	128 (99-164)	131 (120-175)	120 (78-162)
BD, mmol/L	0 (-1-1)	1.5 (-1-4)^	2.5 (-1-5)*	1 (-2-16)	17 (9-23)++
INR > 1.2, n (%)	3 (1%)	0 (0%)	10 (24%)**	1 (13%)	3 (43%)
EXTEM ML, %	7 (6-10)	3 (2-4)^^	3 (2-4)	17 (16-96)	100 (23-100)
D-Dimer, ng/ml	4124 (664 – 11356)	4224 (1041 – 11260)	91700 (59388 – 140173)**	2824 (689 – 1658)	109193 (46600 – 336490)
Injury to sample time, minutes	83 (62-99)	88 (67-103)	98 (82-110)*	85 (73-115)	104 (85-106)
Injury characteristics					
Blunt, n (%)	47 (69%)	40 (78%)	44 (98%)*	6 (75%)	7 (100%)
ISS	10 (5-21)	10 (4-17)	29 (20-38)**	9 (5-29)	27 (25-41)
AIS Head & Neck ≥ 3, n (%)	0 (0-1)	0 (0-2)	4 (0-5)**	0 (0-0)	0 (0-5)
AIS Thorax ≥ 3, n (%)	0 (0-3)	0 (0-3)	3 (1-4)**	0 (0-3)	3 (0-4)
AIS Abdo/pelvis ≥ 3, n (%)	0 (0-2)	0 (0-0)	0 (0-2)	0 (0-2)	0 (0-1)
AIS Extremity ≥ 3, n (%)	1 (0-2)	1 (0-2)	2 (0-3)	2 (0-3)	0 (0-4)
Fluid & Blood product totals					
Pre-baseline crystalloid, ml	0 (0-0)	0 (0-250)^^	225 (0-500)*	0 (0-450)	500 (250-1500)
Pre-baseline PRBC, units	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
12hr Crystalloid, ml	100 (0-2075)	250 (0-1500)	1500 (400-2900)*	0 (0-2000)	1250 (500-4900)
12hr PRBC, units	0 (0-0)	0 (0-0)	0 (0-4)**	0 (0-20)	11 (4-20)++

Outcomes					
Massive Transfusion, n (%)	0 (0%)	0 (0%)	2 (4%)	2 (29%)	4 (50%)$^{+}$
VTE, n (%)	0 (0%)	0 (0%)	1 (2%)	0 (0%)	0 (0%)
Critical Care LOS (survivors)	0 (0-0)	0 (0-0)	5 (1-12)**	0 (0-0)	10 (8-11)
Hospital LOS (survivors)	7 (2-15)	7 (2-13)	24 (14-45)**	6 (1-12)	39 (38-39)
24hr mortality, n (%)	1 (2%)	1 (2%)	6 (13%)*	1 (13%)	5 (71%)$^{+}$
28-day mortality, n (%)	2 (3%)	2 (4%)	15 (33%)**	2 (25%)	5 (71%)
Time to death, days	2 (1-2)	3 (1-11)	1 (0-6)	2 (0-3)	0 (0-0)$^{+}$

All values are median with interquartile range unless specified. Continuous data compared with Mann-Whitney U-test. Categorical data compared with Fisher's exact test. ^p<0.05, ^^p<0.001, ML_{NORMAL} vs ML_{LOW} + DD_{LOW}. *p<0.05, **p<0.001, ML_{LOW} + DD_{HIGH} vs ML_{LOW} + DD_{LOW}. $^{+}$p<0.05, $^{++}$p<0.001, ML_{HIGH} + DD_{HIGH} vs ML_{LOW} + DD_{HIGH}. GCS, Glasgow coma score. SBP, systolic blood pressure. BD, base deficit. INR, international normalized ratio. ML, maximum lysis. ISS, injury severity score. AIS, abbreviated injury score. PRBC, packed red blood cells. VTE, venous thromboembolism. LOS, length of stay in days

Table A28 Coagulation profiles in the extended biomarker subgroup

Number of Patients	ML_{NORMAL} 68	ML_{LOW} + DD_{LOW} 51	ML_{LOW} + DD_{HIGH} 45	ML_{HIGH} + DD_{LOW} 8	ML_{HIGH} + DD_{HIGH} 7
Fibrinogen, g/L	2.2 (1.7-2.7)	2.1 (1.7-2.5)	1.6 (1.1-2.0)**	1.8 (0.7-2.5)	0.7 (0.5-1.2)$^{+}$
Prothrombin Fragments 1+2, pmol/L	694 (335-1457)	818 (393-1420)	4924 (2966-8293)**	492 (432-1149)	4491 (1789-11738)$^{+}$
α2 antiplasmin, u/dL	103 (90-114)	93 (102-116)	71 (53-93)**	108 (31-140)	51 (10-59)$^{+}$
Plasminogen Activator Inhibitor-1, ng/ml	18.3 (10.5-26.1)	24.9 (14.3-58.0)^	30.2 (19.2-49.3)	18.3 (9.2-35.3)	25.35 (15.6-44.2)$^{+}$
Tissue plasminogen activator, ng/ml	11.1 (7.1-16.6)	17.5 (8.4-23.5)^	15.4 (10.5-26.5)	9.5 (5.5-27.8)	51.0 (23.6-84.8)$^{+}$
Plasmin-antiplasmin complex, ng/ml	2544 (1716-4690)	1536 (1012-3053)^	16094 (9539-20261)**	2231 (1309-6849)	33816 (14587-37768)$^{+}$
Urinary plasminogen activator, pg/ml	126 (0-233)	104 (0-249)	134 (33-212)	12 (0-221)	81 (0-215)
S100A10, pg/ml	50 (0-185)	9 (0-187)	1333 (128-2553)**	25 (0-322)	1281 (308-2869)$^{+}$

Mann-Whitney U-test for all comparisons. ^p<0.05, ^^p<0.001, ML_{NORMAL} vs ML_{LOW} + DD_{LOW}. *p<0.05, **p<0.001, ML_{LOW} + DD_{HIGH} vs ML_{LOW} + DD_{LOW}. $^{+}$p<0.05, $^{++}$p<0.001, ML_{HIGH} + DD_{HIGH} vs ML_{LOW} + DD_{HIGH}.

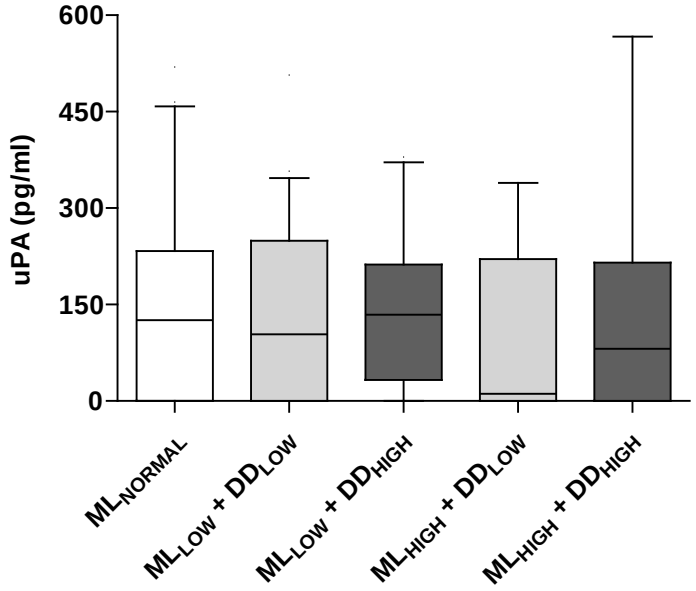

Figure A62 Urokinase-type plasminogen activator (uPA) levels in patients grouped by fibrinolytic profile

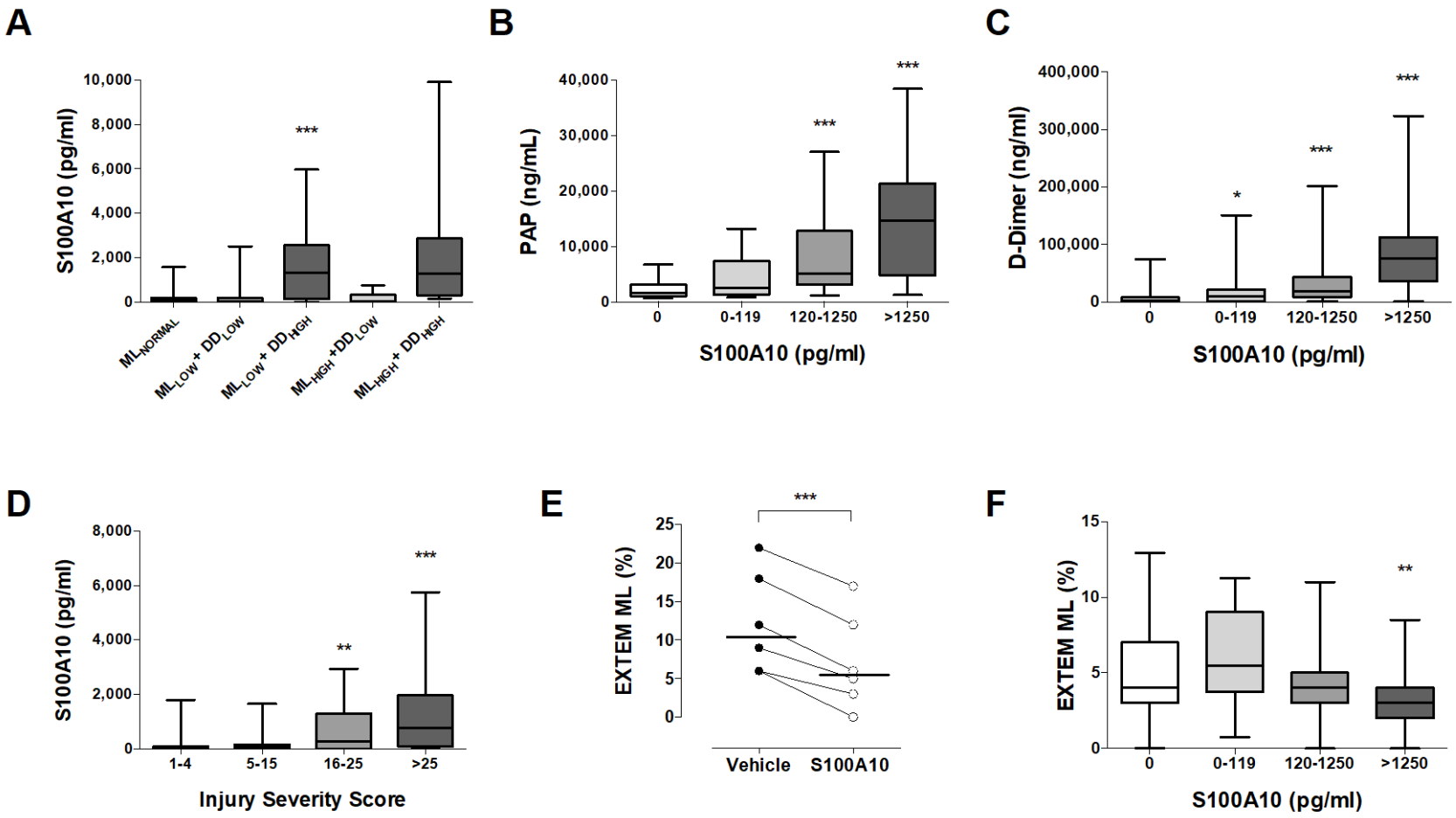

Figure A63 S100A10 is associated with occult hyperfibrinolysis but reduces clot breakdown on viscoelastic testing.

A: Levels of S100A10 in trauma patients grouped by fibrinolytic profile. ***p<0.001, $ML_{LOW}DD_{HIGH}$ vs ML_{NORMAL}, Mann-Whitney U-test. B and C: Relationship between S100A10 and plasmin-antiplasmin (PAP) complex levels (B) and D-Dimer levels (C). S100A10 in quartiles; *p<0.05, ***p<0.001 vs Q1, Kruskall-Wallis test with Dunn's multiple comparisons test D: S100A10 levels in patients with mild (ISS 1-4), moderate (ISS 5-15), severe (ISS 16-25) and critical (ISS>25) injuries. ** p<0.01, ***p<0.001 vs ISS 0-4, Kruskall-Wallis test with Dunn's multiple comparisons test. E: Effect of S100A10 on maximum clot lysis (ML) measured by viscoelastic testing. Rotational thromboelastometry (ROTEM) was performed in tissue factor-stimulated blood samples from healthy volunteers (n=6) in the presence of recombinant S100A10 (10ng/ml) or vehicle. Lines indicate mean. ***p<0.001, paired T-test. F: Clot lysis on ROTEM in trauma patients from ML_{LOW} and ML_{NORMAL} groups stratified by S100A10 quartiles. ** p<0.01 vs Q1, Kruskall-Wallis test with Dunn's multiple comparisons test. Box-whisker plots represent interquartile range with 10^{th}-90^{th} percentiles.

APPENDIX 2

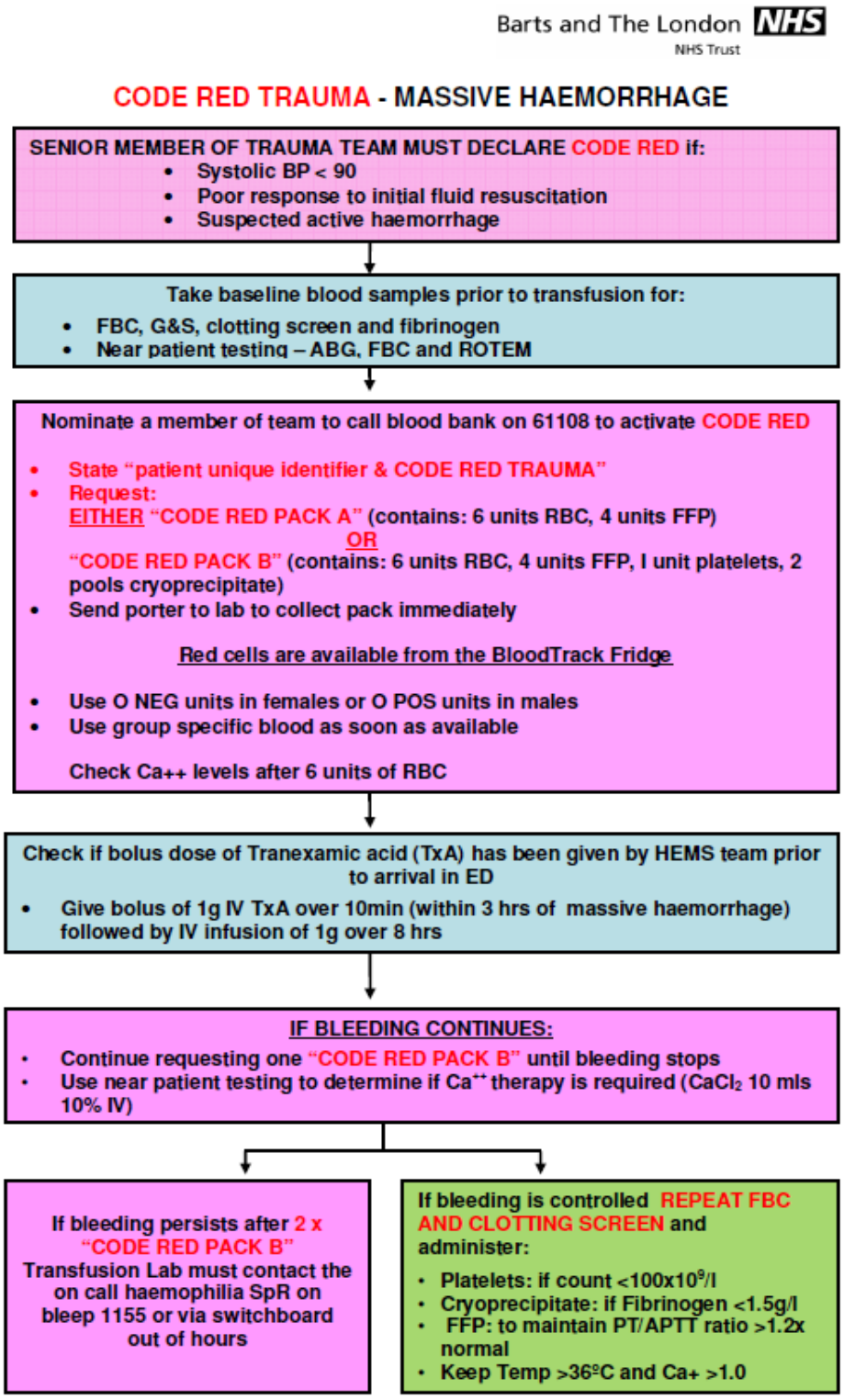

Figure A64 The Royal London Hospital Major Haemorrhage Protocol (revised July 2011)

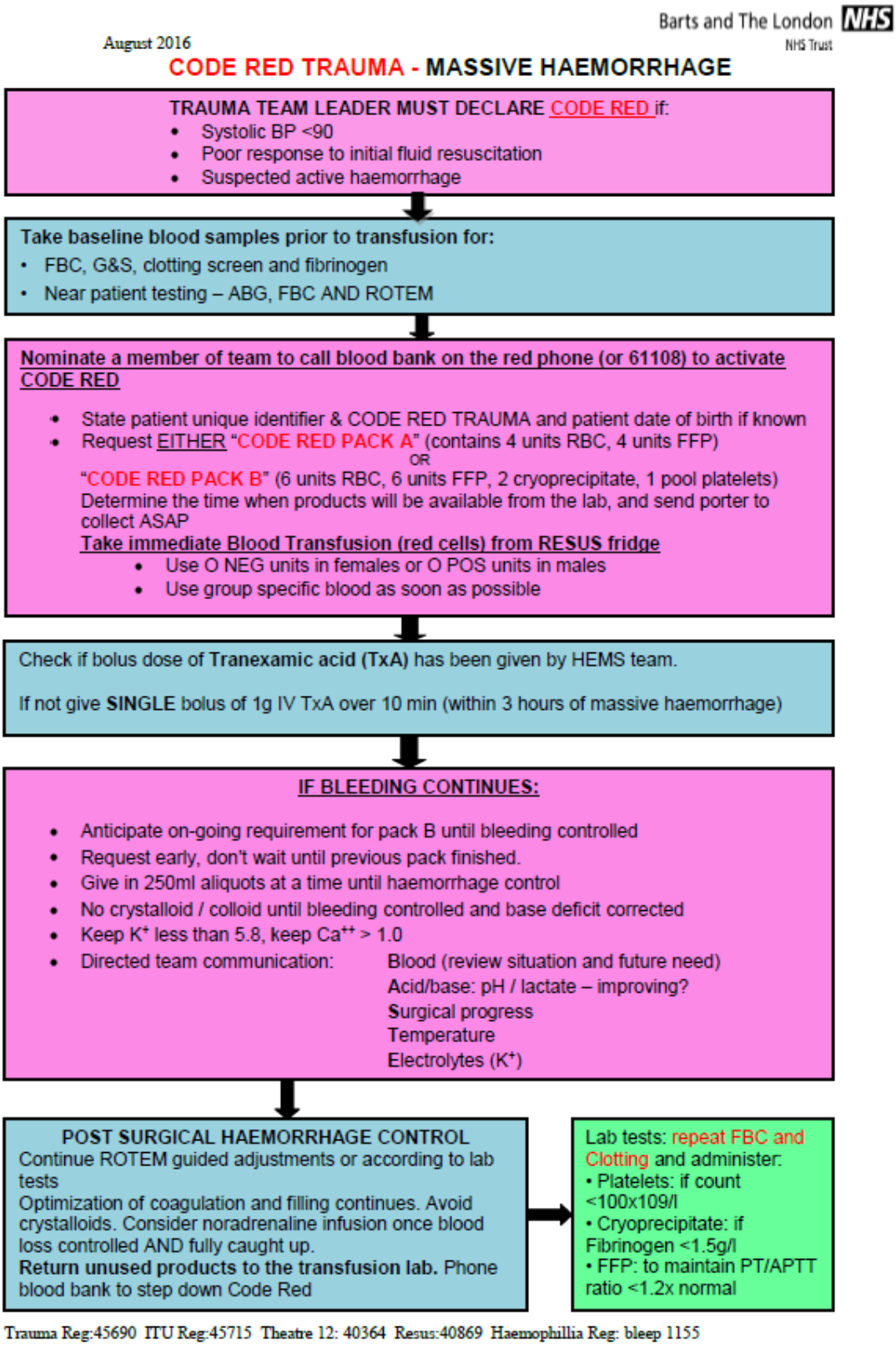

Figure A65 The Royal London Hospital Major Haemorrhage Protocol (Revised Aug 2016)

APPENDIX 3

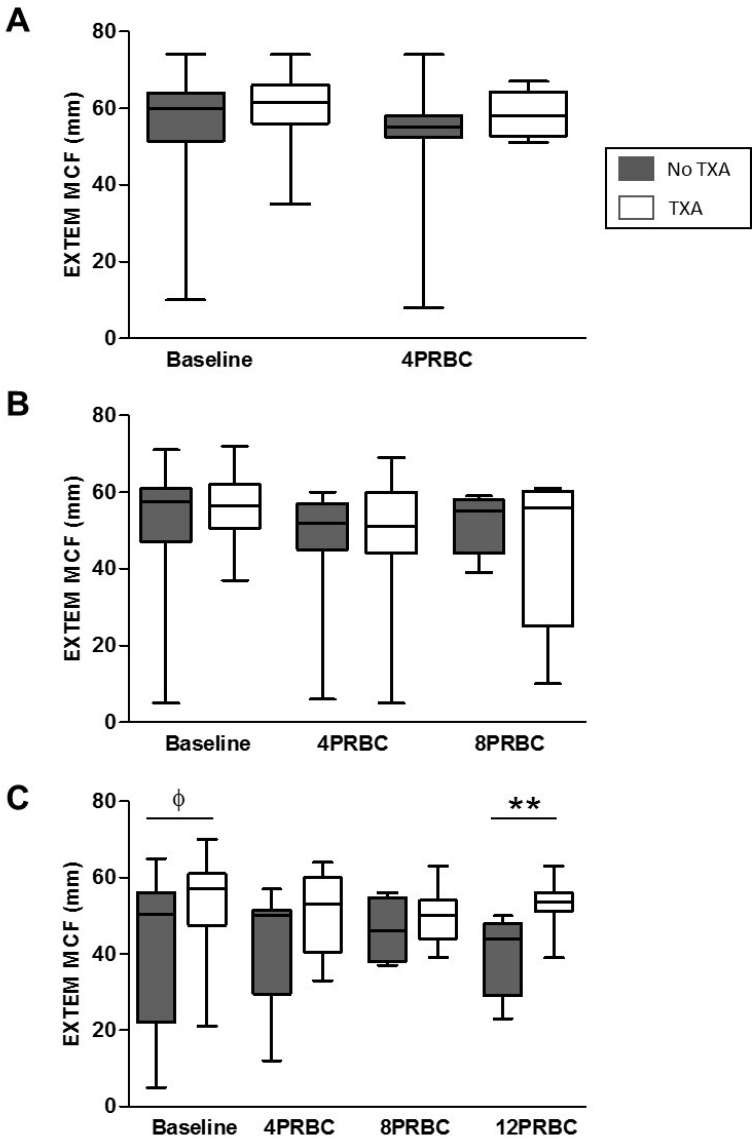

Figure A66 Change in EXTEM Maximum Clot Firmness (MCF) during bleeding episode stratified by PRBC transfusion requirements.

A. Patients receiving 4 – 7 units PRBC. B. Patients receiving 8 – 11 units PRBC. C. Patients receiving 12 units or more PRBC. EXTEM MCF at baseline: No TXA vs TXA 51 (22 – 56) vs 57 (48 – 61) mm, p=0.02. EXTEM MCF after 12 units PRBC: No TXA vs TXA 44 (29 – 48) vs 54 (51- 56), p=0.005. Box and whisker plots show median, interquartile range, maximum and minimum values. ϕp<0.05, **p<0.001. PRBC, packed red blood cells.

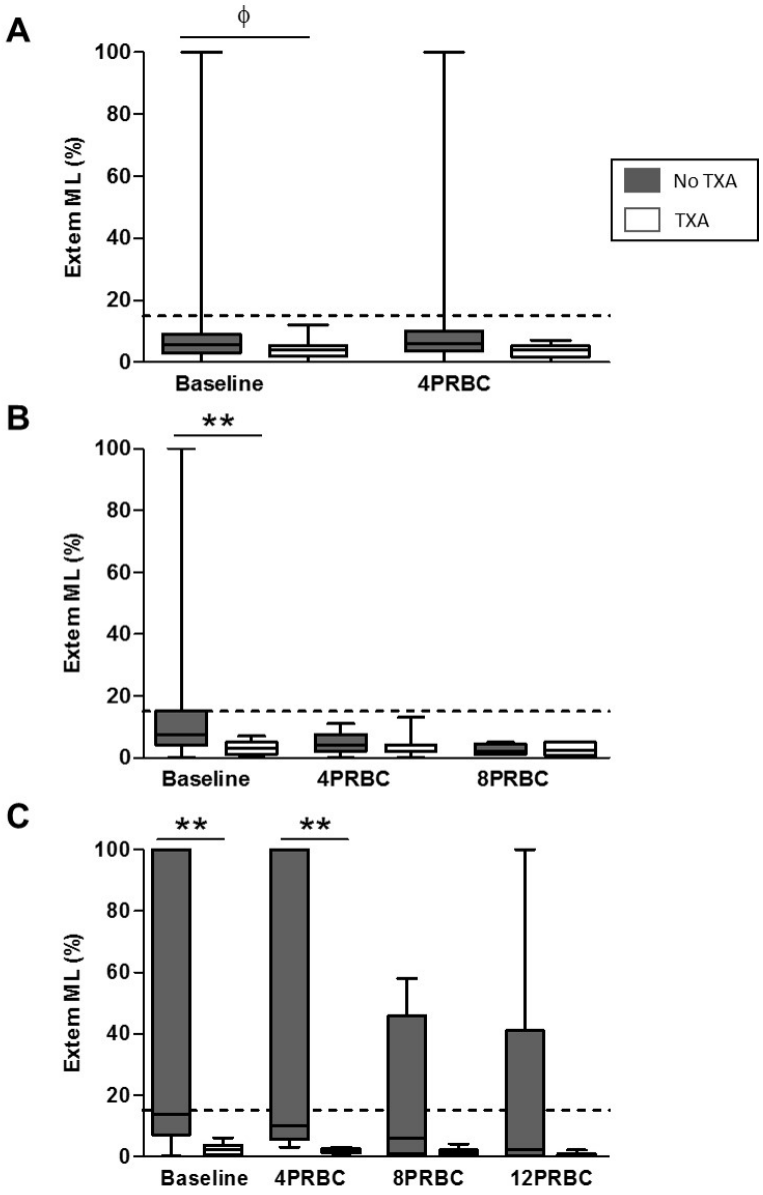

Figure A67 Change in EXTEM Maximum Lysis (ML) during bleeding episode stratified by PRBC transfusion requirements.

A. Patients receiving 4 – 7 units PRBC. EXTEM ML at baseline: No TXA vs TXA 6 (3 – 9) vs 4 (2 – 5) %, p=0.03. B. Patients receiving 8 – 11 units PRBC. EXTEM ML at baseline: No TXA vs TXA 8 (4 – 15) vs 3 (1 – 5) %, p=0.001. C. Patients receiving 12 units or more PRBC. EXTEM ML at baseline: No TXA vs TXA 14 (7 – 100) vs 2 (1 – 4) %, p<0.0001. EXTEM ML after 4 units PRBC: No TXA vs TXA 10 (6 – 100) vs 2 (1 – 3) %, p=0.0005. Box and whisker plots show median, interquartile range, maximum and minimum values. ϕp<0.05, **p<0.01. PRBC, packed red blood cells.

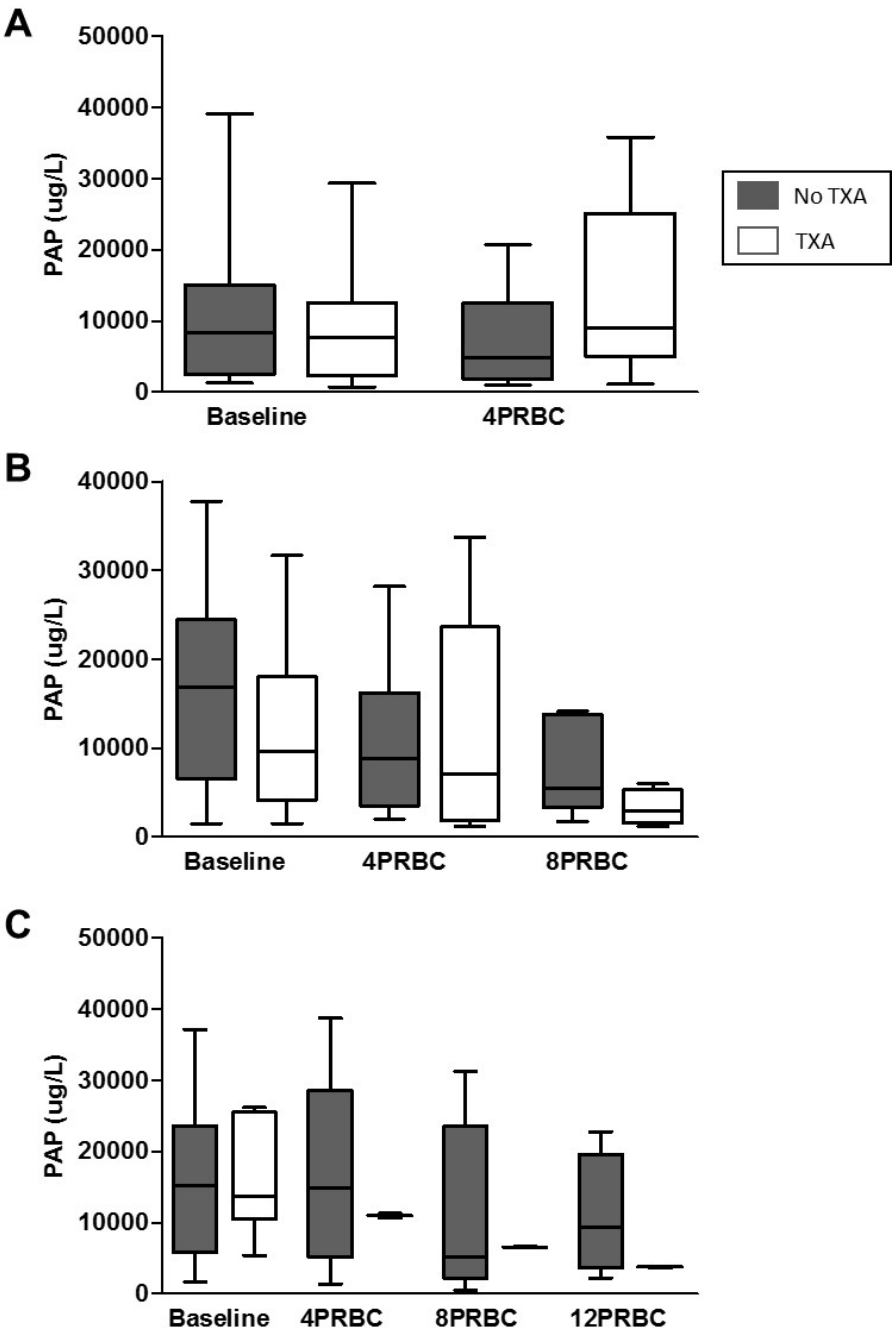

Figure A68 Change in plasmin-α2-antiplasmin complex (PAP) levels during bleeding episode stratified by PRBC transfusion requirements.

A. Patients receiving 4 – 7 units PRBC. B. Patients receiving 8 – 11 units PRBC. C. Patients receiving 12 units or more PRBC. Box and whisker plots show median, interquartile range, maximum and minimum values. PRBC, packed red blood cells.

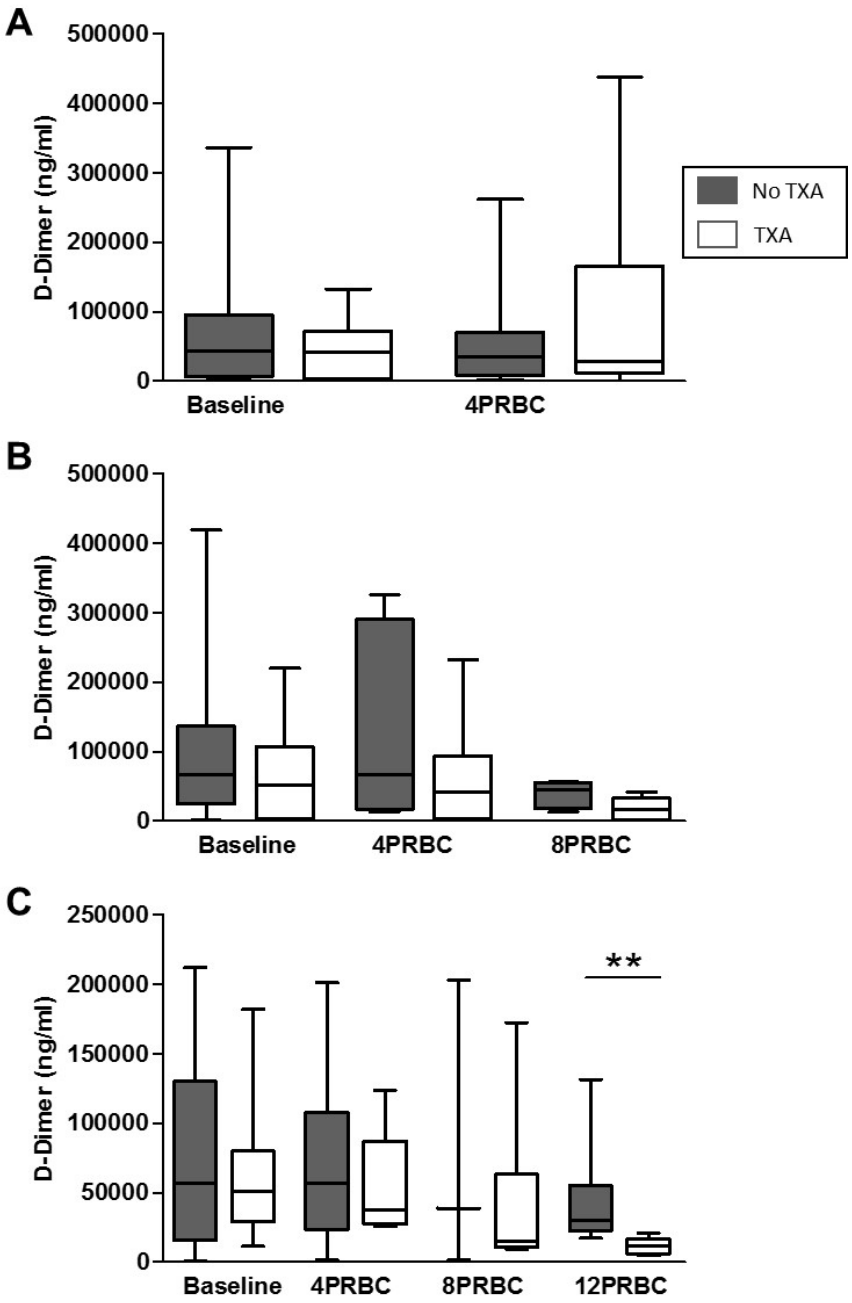

Figure A69 Change in D-dimer levels during bleeding episode stratified by PRBC transfusion requirements.

A. Patients receiving 4 – 7 units PRBC. B. Patients receiving 8 – 11 units PRBC. C. Patients receiving 12 units or more PRBC. D-dimer level after 12PRBC: No TXA vs TXA 30380 (22710 – 54960) vs 12050 (5843 – 16780) ng/ml, p=0.005. Box and whisker plots show median, interquartile range, maximum and minimum values. **p<0.01. PRBC, packed red blood cells.

APPENDIX 4

Related Publications (not directly resulting from work presented within this thesis)

Book chapter

Gall L, Brohi K. Trauma-Induced Coagulopathy. In: Thomas D, Thompson J, Ridler B, eds. All Blood Counts — A manual for blood conservation and patient blood management. tfm Publishing Limited, Shrewsbury, UK. 2016: 315-327

Published Articles

Baksaas-Aasen, **Gall L**, Eaglestone S, Rourke C, Juffermans NP, Goslings JC, Naess PA, van Dieren S, Ostrowski SR, Stensballe J, Maegele M, Stanworth SJ, Gaarder C, Brohi K, Johansson PI. iTACTIC – implementing treatment algorithms for the correction of trauma-induced coagulopathy: study protocol for a multicentre, randomised controlled trial. *Trials* 2017; 18:486

Vulliamy P, Gillespie S, **Gall LS**, Green L, Brohi K, Davenport RA. Platelet transfusions reduce fibrinolysis but do not restore platelet function during trauma hemorrhage. *J Trauma Acute Care Surg* 2017; 83:388-397

Published Abstract

Jones TF, Gillespie S, **Gall LS**, Davenport RA, Brohi K. T-12: Venous thromboembolic events after traumatic injury are associated with early changes to the fibrinolytic system. *Shock* 2015; 44 Suppl 2: 24.

Collaborative research as part of the TACTIC project

Balvers K, van Dieren S, Baksaas-Aasen K, Gaarder C, Brohi K, Eaglestone S, Stanworth S, Johansson PI, Ostrowski SR, Stensballe J, Maegele M, Goslings JC, Juffermans NP, on behalf of the Targeted Action for Curing Trauma-Induced Coagulopathy (TACTIC) Collaborators. Combined effect of therapeutic strategies for bleeding injury on early survival, transfusion needs and correction of coagulopathy. *Br J Surg* 2017; 104:222-229

Schafer N, Driessen A, Frohlich M, Sturmer EK, Maegele M and TACTIC partners. Diversity in clinical management and protocols for the treatment of major bleeding trauma patients across European level I Trauma Centres. *Scand J Trauma Resusc Emerg Med* 2015; 23:74