A prospective observational study of acute traumatic coagulopathy in traumatic bleeding from the battlefield

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BACKGROUND: Acute trauma coagulopathy (ATC) after military trauma has not been comprehensively studied. ATC is defined as a prolonged prothrombin time ratio (PTr) or reduced clot amplitude (A5) in viscoelastic testing. Compared to civilian trauma, military trauma has more injuries from explosions and gunshot wounds (GSWs), potentially leading to a different pathophysiology for traumatic coagulopathy. This study aimed to characterize military ATC on admission to a military hospital in Afghanistan and to explore any differences due to the mechanism of injury.

METHODS: Severely injured military casualties were enrolled in the study. Blood samples were taken on admission and after routine testing, waste plasma was prepared, frozen, and transported to the United Kingdom for in-depth hemostatic analysis.

RESULTS: Seventy-seven percent of casualties had ATC defined by a PTr greater than 1.2 and 19% when defined by rotational thromboelastometry (ROTEM) A5 less than 36 mm. Coagulation factor depletion correlated with degree of shock, particularly factor V ($p < 0.01$), factor X ($p < 0.01$), and fibrinogen levels ($p < 0.01$). Thrombin generation was well preserved. Fibrinolytic biomarkers were raised correlating with the degree of shock ($p < 0.01$), and 8% of casualties had hyperfibrinolysis on ROTEM analysis. Plasmin-antiplasmin complexes ($p < 0.01$) and D-dimer levels ($p = 0.01$) were higher and clot firmness lower ($p = 0.02$) in those injured by explosion compared to GSW's.

CONCLUSIONS: ATC was present and correlated with shock, similar to civilian trauma. Thrombin generation remained adequate. Fibrinogen and factor V levels were disproportionately low but still sufficient to allow clot formation. Fibrinolysis is a key feature, probably due to a tissue plasminogen activator surge at the time of injury. Blast injuries are associated with a greater activation of fibrinolysis than GSWs.

Trauma causes approximately 4 million deaths annually, accounting for approximately 10% of all global deaths, and is the leading cause of death in men aged under 40.[1] Most potentially preventable trauma deaths result from bleeding; this is especially true in the military setting.[2] *Acute traumatic coagulopathy* (ATC) is the term used for the impairment in hemostasis seen on admission to a hospital after trauma.[3] There are many views in the literature as to the correct definition for coagulopathy after trauma. In many studies, ATC has been characterized by a prolonged prothrombin time ratio (PTr > 1.2) compared with

ABBREVIATIONS: A5 = clot amplitude; APTT = activated partial thromboplastin time; ATC = acute trauma coagulopathy; GSWs = gunshot wounds; INR = international normalized ratio; ISS = injury severity score; MCF = maximum clot firmness; MERT = Medical Emergency Response Team; PAI-1 = plasminogen activator inhibitor type 1; PAP = plasmin-antiplasmin complexes; PTr = prothrombin time ratio; RCC = red blood cell concentrate; ROTEM = rotational thromboelastometry; TF = tissue factor; TM = thrombomodulin; tPA = tissue plasminogen activator; TXA = tranexamic acid; vWF = von Willebrand factor.

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normal and/or rotational thromboelastometry (ROTEM) EXTEM clot amplitude (A5) less than 36 mm.[4] ATC is thought to be driven by shock and systemic tissue hypoperfusion and is independently associated with the extent of bleeding, the need for blood components, late multiorgan failure and a four-fold increase in mortality.[3,5,6]

Increased fibrinolytic activity plays an important role in ATC. Patients with viscoelastic evidence of hyperfibrinolysis on admission have an increased mortality compared with those who do not,[7] and the early use of an antifibrinolytic (tranexamic acid) reduces mortality in civilian (Clinical Randomization of an Antifibrinolytic in Significant Haemorrhage 2 trial [CRASH-2])[8] and military patients (Military Application of Tranexamic Acid in Trauma Emergency Resscitation Study [MATTERS]).[9] There is a growing body of research on the pathobiology of civilian ATC. Brohi et al.[2] demonstrated that there was anticoagulation despite no critical loss in factor activity or levels and both Brohi's study and Raza et al.[10] described the fibrinolytic activity of civilian trauma patients. These features of military ATC have not been described despite interest in the pathobiology of massive military bleeding from the time of the US-Vietnam war in the late 1960s. These early studies showed prolongation of PTr and activated partial thromboplastin time (APTT) in cohorts of military trauma patients.[11,12] Cohen et al.[5] recently published an analysis of INR and ROTEM in assessing the presence of ATC and likelihood of requiring massive transfusion. Studies have also looked at ATC in animal models of military trauma, for example, Watts et al.[13] To our knowledge there have been no published papers describing hemostatic assays as well as conventional testing in military trauma. We felt it was important to study military trauma separately from civilian trauma due to the differences between military injuries and trauma health care systems compared to their civilian counterparts. In addition, the causes of injury are different in military trauma: In civilian trauma, explosions are rare, but in recent military conflicts, battlefield casualties were principally injured in explosions, which involve primary, secondary, and tertiary blast injuries. Gunshot wounds (GSWs) are common in military trauma; this includes high-velocity bullets that cause significant local soft tissue disruption without the whole-body injury seen in explosions. The use of sophisticated military personal protective equipment and first aid protocols have allowed severely injured causalities to reach medical care alive, when in previous conflicts they would have died on the battlefield. In addition, military personnel are trained in trauma first aid to a greater degree than civilians; thus, tourniquet use is started as soon as a casualty is injured, and evacuation is rapid and direct to a trauma hospital. Military trauma differs from civilian trauma in the health and age range of casualties, for those with military trauma tend to be fitter and younger than the ages affected by civilian trauma; the latter includes the growing group of elderly trauma victims.[14]

The aim of this prospective observational study was to characterize military ATC by exploring the hemostatic profile of those with traumatic hemorrhage brought directly from the battlefield and to assess the impact of shock on the development of ATC. Our secondary aim was to explore the effect of mechanism of injuries (blast or gunshot wounds) on the hemostatic manifestations of ATC.

METHODS

Study design

A prospective observational study. Casualties requiring full trauma team activation (Table 1) that presented to the Joint Force Role 3 Field Hospital in Camp Bastion, Afghanistan, between November 2011 and August 2013 were eligible. This field hospital approximated to a civilian trauma center.

Ethics

Because these casualties were all severely injured, bleeding, and shocked trauma patients, informed consent or seeking alternative legal representation before enrollment was not possible. Once patients were evacuated to their home country from Afghanistan or to another facility in Afghanistan, it

TABLE 1. Trauma team activation criteria according to Clinical Guidelines for Operations, Joint Service Publication 999

Penetrating trauma	Gunshot or shrapnel wound Blast injury (mine/improvised explosive device/grenade) Stab wound
Blunt trauma	Motor vehicle crash with ejection Motorcyclist or pedestrian hit by vehicle >30 km/h Fall >5 meters Fatality in the same vehicle Entrapment and/or crush injury Interhospital trauma transfer meeting activation criteria
And	
Anatomy	Injury to two or more body regions Fracture to two or more long bones Spinal cord injuryAmputation of a limb Penetrating injury to head, neck, torso, or proximal limb Burns >15% BSA in adults **or** >10% in children **or** airway burns Airway obstruction
Or	
Physiology	Systolic blood pressure <90 mm Hg or pulse >120 bpm (adults) Respiratory rate < 10 or > 30 per minute (adults); SpO_2 <90% Depressed level of consciousness or fitting Deterioration in the emergency department Age >70 years Pregnancy >24 weeks with torso injury

was not possible to follow up and therefore gain retrospective consent if the subjects regained capacity.

We used residual plasma (excluding the buffy coat) that would normally be discarded after routine coagulation testing on admission. This sample was viewed as clinical waste. There was an additional 4.5-mL sample taken; however, this volume was a negligible amount in relation to the patients' blood loss and the volume of blood taken for clinical assessment.

Advice was sought regarding the requirement for a formal ethics submission, and in view of the negligible blood volume and clinical waste, a formal submission was not required by the UK Ministry of Defense Research Ethics Committee although the US Army granted ethical approval (log number M-10242) for their study participants.

Both the UK and US advice was that informed consent was not required, as this was a "minimal risk study" to the subjects, thereby meeting the regulatory requirements for waiver of consent.

Study setting

Military trauma evacuation systems evolved during the Afghanistan conflict. During this study, casualties were evacuated from the battlefield either by the UK Medical Emergency Response Team (MERT), the US Air Force "Pedro" rescue helicopters, or US army "Dustoff" helicopters. Ground ambulance was available, but the majority of the casualties arrived via helicopter. Each evacuation platform had different medical and military capabilities. MERT had consultant anesthetic or emergency physicians on board, who could perform advanced medical interventions and administer prehospital blood products to multiple casualties. Pedro had pararescue staff with a more limited interventional capacity but were quicker to reach nearby casualties, and by the end of this study, these platforms were able to administer blood products. Dustoff had less advanced medical interventions but were more frequently in the air and able to pick up casualties soon after injury. Where possible, the helicopter sent to retrieve the casualty was matched to the casualty's triage status as described by the ground troops, with consideration given to the on-board medical capability, the distance to the incident, casualty numbers and tactical situation on the ground.

Patient selection

All adults (>18 years), who met the criteria for full trauma team activation (Table 1) were considered and assessed against inclusion and exclusion criteria (Table 2). The logistical constraints of performing research in a military environment and the lack of 24-hour dedicated researchers prevented enrollment of consecutive patients. This study was performed by clinical staff, whose primary role was to provide clinical care, not to do research. Therefore, casualties were enrolled only if the investigators were in the hospital and not required for the clinical care of the casualties. In addition, casualties were enrolled only during the principal investigators deployments, a total of around 5 months during the study period.

TABLE 2. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Full trauma team activation	Civilian casualties
Coalition forces	Age <18 y
Explosive injury	Died before start of surgery
Gunshot wound or penetrating fragment wound not associated with explosion	Isolated brain injury
Evidence of shock:	Pregnancy
Clinical suspicion associated with any of the following criteria:	
• Base deficit >6	
• Systolic blood pressure <90 mm Hg	
• Pulse >120 bpm	
• Depressed Glasgow Coma Score	
Clinical suspicion of requirement for blood transfusion	Enemy forces

Data collection and analysis

Clinical parameters and local laboratory results were collected. Some information, such as the injury severity score (ISS), was produced retrospectively, while other data, due to the nature of military trauma, was verified after the event using UK or US trauma registry searches.

Blood sampling technique

As part of routine care, blood was taken immediately on admission from a freshly inserted 14G cannula or central venous line. Emergency intraosseous access samples were excluded.

Sample analysis

Full blood count samples were collected into ethylenediaminetetraacetic acid tubes (Vacutainer, Beckton Dickinson) and analyzed locally (Coulter Ac·T diff, Beckman Coulter).

Blood gas analysis samples were collected into preheparinized syringes from arterial or central venous lines after discarding dead space blood. Analysis was performed immediately using a point-of-care blood gas device (i-STAT, Abbott) using an EG7+ cartridge.

Whole blood (4.5 mL) was collected into sodium citrate collection tubes (Vacutainer, Beckton Dickinson), with a final ratio of blood to sodium citrate of 9:1. Thromboelastometry analysis was performed using ROTEM (TEM International GmbH) within 5 minutes of sample collection, according to the manufacturer instructions, for EXTEM, FIBTEM, and APTEM and compared with our normal ranges. Unfortunately, there were no dedicated research ROTEM analyzers, so the clinical machines were used. Hospital protocols were that ROTEM's should be run until Li30 was reached (after approximately 30 minutes) Therefore, Li60 was often not reached, and consequently Li30 was used as the assessment of fibrinolysis.

The remaining blood in the citrate tubes was centrifuged at 3000 × *g* for 20 minutes. Residual plasma was divided into 500-μL aliquots and immediately frozen at –30°C. The samples were returned to St Thomas' Hospital, London, in closed boxes in "dry ice" and stored at –80°C until analysis.

TABLE 3. Results for all 53 casualties

	Median (range)	Normal range
Background data		
Injury to admission time (min)*	68 (30–177)	
Injury severity score*	20 (1–75)	
Pulse (bpm)	110 (36–183)	
Systolic pressure (mm Hg)	122 (45–173)	
Base deficit (mmol/L)*	6 (4–26)	
Temperature (°C)	36 (28.7–38.6)	
Prehospital Transfusion		
Red cell concentrates (units)†	1 (0–6)	
Fresh frozen plasma (units)†	0 (0–6)	
Full blood count		
Hemoglobin (g/L)	126 (92–177)	
Platelet count (10^9/L)	248 (60–403)	
Coagulation assays		
Prothrombin ratio*	1.3 (1–2.2)	<1.2
Prothrombin time (sec)*	18.6 (13.8–30.9)	11.7-15.9
Activated partial thromboplastin time (sec)*	32.4 (14.4–114)	26.1-38.1
Clauss fibrinogen (g/L)	2 (0.6–3.2)	1.52-4.12
Factor II (IU/dL)	78 (39–116)	50-150
Factor V (IU/dL)	53 (13–106)	50-150
Factor VII (IU/dL)	67 (36–143)	50-150
Factor X (IU/dL)	78 (33–118)	50-150
Prothrombin fragment 1 + 2 (pmol/L)†	1556 (24–2566)	200-1200
Fibrinolysis assays		
D-Dimer (ng/mL)	3016 (257–8648)	0-145
Plasmin antiplasmin (μg/mL)*	5082 (931–37000)	150-800
Tissue plasminogen activator (ng/mL)*	15 (3–108)	1-15
Plasminogen activator inhibitor-1 (ng/mL)*	32 (11–119)	0-33
Other hemostatic assays		
von Willebrand factor (%)†	248 (40–1633)	50-138
Soluble thrombomodulin (ng/mL)*	4 (3–17)	12-145
Soluble tissue factor (pg/mL)*	199 (39–580)	40-300
ROTEM parameters		
EXTEM clotting time (sec)*	70 (41–206)	25-90
EXTEM amplitude 5 min (mm)*	42 (8–53)	32-71
EXTEM amplitude at 10 min (mm)*	52 (13–62)	40-72
EXTEM clot formation time (sec)*	94 (61–1718)	43-173
EXTEM maximum clot firmness (mm)*	60 (23–69)	44-74
FIBTEM maximum clot firmness (mm)	11 (4–18)	9-25

* Transformed data.
† Data resistant to transformation.
Data presented as median (range). Injury to admission time is defined as the time from injury to arrival in the emergency department at the Role 3 hospital in Bastion.

Hemostatic analysis

Levels of D-dimers, plasminogen activator inhibitor type 1 antigen (PAI-1), and soluble thrombomodulin (TM; Diagnostica Stago), D-dimer intra-assay variable 3%, interassay variable 4%, PAI-1 (intra, 3%; inter, 3%) TM (intra, 4.8%; inter, 4%), plasmin-antiplasmin complexes (PAP; intra, 4.2%, inter, 7.3%) (Immuno Diagnostic Systems Ltd), tissue plasminogen activator (tPA) antigen (intra, 5%; inter, 4%) (TCoag UK Sales), soluble tissue factor (TF; intra, 6%; inter, 5%) (Invitech), prothrombin fragment 1 + 2 (intra, 6%; inter, 9%) (Sysmex UK Ltd), and von Willebrand factor (vWF) antigen (intra, 4.8%; inter, 6%; in-house assay) were measured using sandwich enzyme-linked immunosorbent assays following the manufacturer's instructions. Prothrombin time, APTT, Clauss fibrinogen, factor II, factor V, factor VII, and factor X levels were measured using reagents (HaemosIL, Werfen UK) using an analyzer (ACL 300R, Werfen UK). Manufacturers' instructions were followed for all assays and normal ranges were established using healthy controls.

Statistical analysis

Statistical analysis was performed with statistical software (NCSS, version 8). Data were assessed for normality using the Anderson-Darling test. Nonparametric data was transformed to a normal distribution with a suitable transformation as

TABLE 4. Results for correlations with admission base deficit

	p value ± R^2
Full blood count	
Platelet count (10^9/L)	0.10 + 0.062
Coagulation assays	
Prothrombin ratio	<0.01 − 0.38
Prothrombin time (sec)	<0.01 + 0.39
Activated partial thromboplastin time (sec)	<0.01 + 0.41
Clauss fibrinogen (g/L)	<0.01 + 0.19
Factor II (IU/dL)	<0.01 + 0.30
Factor V (IU/dL)	<0.01 + 0.41
Factor VII (IU/dL)	0.19 + 0.04
Factor X (IU/dL)	<0.01 + 0.23
Prothrombin fragment 1 + 2 (pmol/L)	0.37 − 0.033
Fibrinolysis assays	
D-Dimer (ng/mL)	0.11 − 0.06
Plasmin-antiplasmin (μg/mL)	<0.01 − 0.31
Tissue plasminogen activator (ng/mL)	<0.01 − 0.49
Plasminogen activator inhibitor-1 (ng/mL)	0.93 − <0.001
Other hemostatic assays	
von Willebrand factor (%)	0.94 − 0.026
Soluble thrombomodulin (ng/mL)	0.24 − 0.035
Soluble tissue factor (pg/mL)	0.31 + 0.033
ROTEM parameters	
EXTEM clotting time (sec)	<0.01 + 0.23
EXTEM amplitude 5 min (mm)	0.73 + 0.004
EXTEM amplitude at 10 min (mm)	0.57 + 0.009
EXTEM clot formation time (sec)	0.67 + 0.0002
EXTEM maximum clot firmness (mm)	0.62 + 0.018

Data were nonparametric so Spearman's rank correlations were used.

indicated in the Results section. Comparisons between group means used a t test for independent samples. Correlations were made between hemostatic variables and base deficit with Spearman's rank correlations. An alpha level of rejection of p less than 0.05 was used to assign statistical significance.

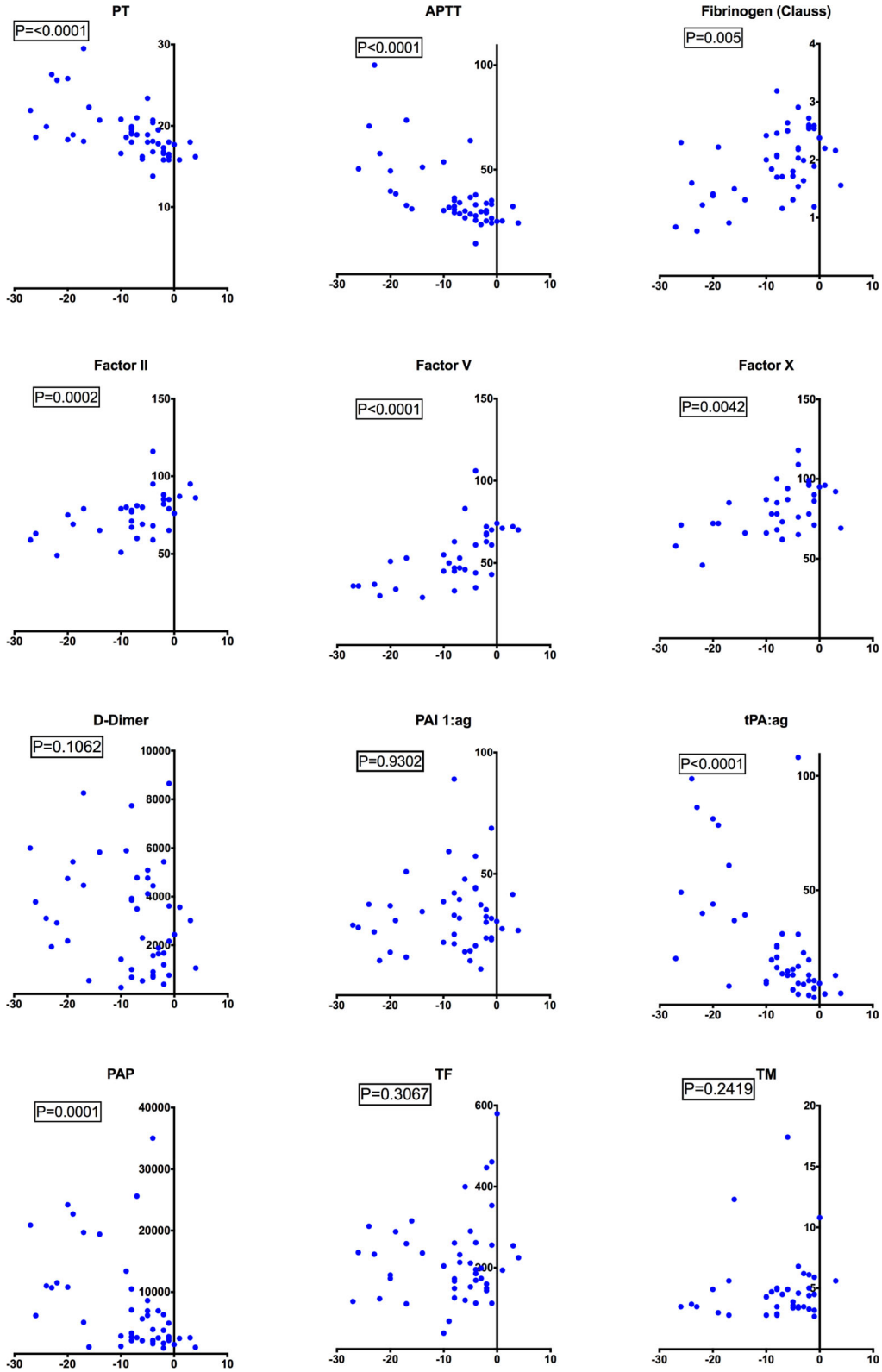

Fig. 1. Correlations with base deficit values using Spearman's rank correlation.

RESULTS

Results are expressed as median (range) unless stated otherwise. During the study period, 69 eligible patients were assessed. Five patients were excluded: a child; a local national contractor; one found to be a member of enemy forces; one with head injury alone; and one who had been transferred in from another hospital. Another 11 patients were excluded: 7 because of blood collection errors, and 4 had incomplete plasma collection. Thus, 53 patients were included in the analysis.

Clinical details and blood product use prior to admission

Admission clinical and hemostatic data is shown in Table 3. Time from injury to admission was 68 (30-177) minutes. All 53 patients were male, aged 25 (19-60), with only one older than 35. None were receiving antithrombotic medication. Patients were severely injured and tachycardic. Four were receiving active cardiopulmonary resuscitation on arrival, and one had a carotid pulse but no recordable blood pressure. The remainder had a recordable blood pressure, 12 (23%) had a systolic blood pressure of 100 or less on admission, and 48% had a base deficit greater than 6 mmol/L.

Twenty-eight (54%) patients received blood products in transit. Five patients received 1 unit of red blood cell concentrate (RCC) alone; all others received both RCC and fresh frozen plasma. Forty-two percent (22/53) received 1 g of tranexamic acid (TXA) before admission.

Characterization of hemostatic changes

On admission, 41 (77%) patients fulfilled the definition of ATC (PTr > 1.2), and 25% had fibrinogen levels less than

TABLE 5. Median (range) values stratified by mechanism of injury

	BLAST (n = 36)	GSW (n = 17)	BLAST vs. GSW p value	Normal range
Background data				
Injury to admission time (min)	56 (30–130)	75 (45–177)	0.19	
Injury severity score	29 (1–75)	16 (2–75)	0.29	
Pulse (bpm)	115 (36–183)	89 (39–120)	0.28	
Systolic pressure (mm Hg)	118 (45–160)	128 (58–173)	0.2	
Base deficit (mmol/L)	7 (3–25)	4 (4–26)	0.22	
Temperature (°C)	35 (31.6–38.6)	36.7 (28.7–37.6)	0.45	
Prehospital transfusion				
Red cell concentrates (units)	1 (0–6)	0 (0–2)	0.002	
Fresh frozen plasma (units)	1 (0–6)	0 (0–1)	0.004	
Full blood count				
Hemoglobin (g/L)	119 (92–177)	136 (98–149)	0.39	
Platelet count (10^9/L)	243 (60–403)	264 (85–378)	0.39	
Coagulation assays				
Prothrombin ratio	1.4 (1–2.2)	1.2 (1.1–1.9)	0.08	<1.2
Prothrombin time (sec)	18.9 (13.8–30.9)	16.6 (15.6–26.3)	0.18	11.7-15.9
Activated partial thromboplastin time (sec)	33 (14.7–114)	30.2 (14.4–100)	0.25	26.1-38.1
Clauss fibrinogen (g/L)	1.7 (0.6–3.2)	2.4 (0.8–2.8)	0.02	1.52–4.12
Factor II (IU/dL)	77 (39–116)	80 (51–109)	0.66	50-150
Factor V (IU/dL)	47 (13–106)	67 (36–83)	0.09	50-150
Factor VII (IU/dL)	66 (36–143)	72 (45–94)	0.84	50-150
Factor X (IU/dL)	78 (33–118)	87 (49–117)	0.34	50-150
Prothrombin fragment 1 + 2 (pmol/L)	1777 (24–2566)	1121 (157–2299)	0.55	200-1200
Fibrinolysis assays				
D-Dimer (ng/mL)	3976 (676–8648)	1418 (257–3781)	<0.001	0-145
Plasmin-antiplasmin (μg/mL)	6982 (1639–37000)	2177 (931–17100)	<0.001	150-800
Tissue plasminogen activator (ng/mL)	20 (3–108)	10 (4–86)	0.06	1-15
Plasminogen activator inhibitor-1 (ng/mL)	34 (11–89)	28 (17–119)	0.99	0-33
Other hemostatic assays				
von Willebrand factor (%)	253 (40–1633)	233 (148–1435)	0.47	50-138
Soluble thrombomodulin (ng/mL)	4 (3–6)	5 (3–17)	0.04	12-145
Soluble tissue factor (pg/mL)	205 (69–461)	194 (39–580)	0.45	40-300
ROTEM parameters				
EXTEM clotting time (sec)	71 (41–206)	68 (48–133)	0.90	25-90
EXTEM amplitude 5 min (mm)	42 (8–53)	42 (35–51)	0.35	32-71
EXTEM amplitude at 10 min (mm)	52 (13–62)	52 (45–61)	0.28	40-72
EXTEM clot formation time (sec)	94 (61–1718)	94 (62–130)	0.25	43-173
EXTEM maximum clot firmness (mm)	61 (23–69)	60 (51–68)	0.36	44-74
EXTEM LY30	100 (0-100)	100 (1-100)	0.61	
FIBTEM maximum clot firmness (mm)	10 (4–18)	12 (8–18)	0.13	9-25

BLAST injury is due to an explosion regardless of the type of explosion, or whether the explosion was in a confined space, such as a vehicle, or in the open, such as a foot patrol. It includes primary blast injury from the blast wave, as well as secondary and tertiary injuries from fragments and additional injuries.
GSW (gunshot wound) is defined as those with an isolated gunshot wound and includes both high-velocity and low-velocity weapons.
Injury to admission time is the time from injury to arrival in the emergency department at the Bastion Role 3 hospital.

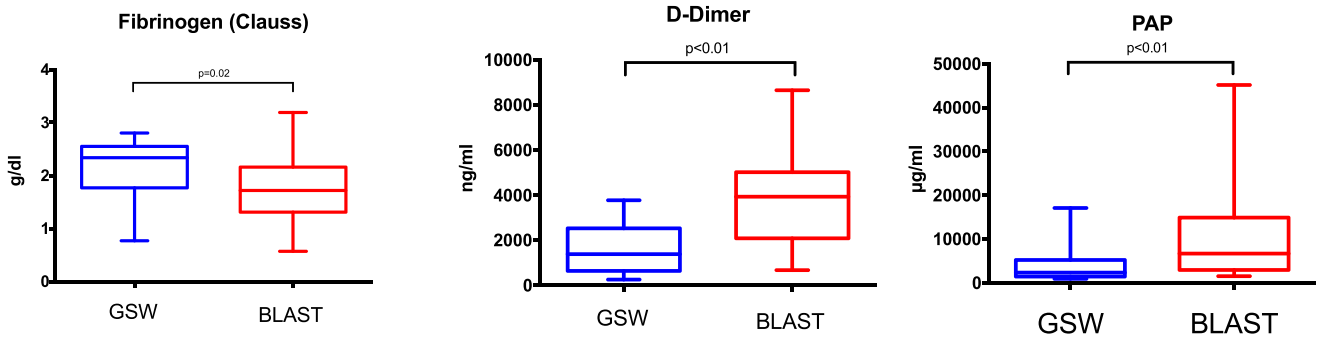

Fig. 2. Fibrinolysis and fibrinogen levels in BLAST and GSW groups.

1.5 g/L. In contrast, only four (8%) had platelet counts less than 100×10^9/L, and none had platelets less than 50×10^9/l. vWF levels were increased, at 248% (40–1633%), except for one patient who had levels suggesting undiagnosed von Willebrand disease (40%).

ROTEM showed that 10% had a prolonged EXTEM clotting time and 19% had A5 less than 35 mm, but only 8% had an EXTEM maximum clot firmness (MCF) below the normal range. There were normal or increased prothrombin fragment 1 + 2 levels in the majority of patients showing uncompromised thrombin generation. Soluble TM and soluble TF levels appeared unaffected.

Profound fibrinolytic activation was observed as reflected in high levels of D-dimer (3016 [257-8648] ng/mL) and plasmin-antiplasmin (5082 [931-37,000] µg/mL) were seen and 51% had increased tPA levels. However, only 8% had ROTEM hyperfibrinolysis, and approximately half of these received TXA before blood sampling and half did not.

Impact of shock

Table 4 and Fig. 1 show the correlations between hemostatic variables, ROTEM parameters, and base deficit. PTr, aPTT, PAP, and tPA showed positive correlations with shock levels ($p < 0.01$). Clauss fibrinogen levels ($p < 0.01$), factor V ($p < 0.01$), and factor X ($p < 0.01$) levels had an inverse relationship with base deficit. EXTEM clotting time correlated with base deficit ($p < 0.01$), and was the only ROTEM parameter that showed significant differences as shock increased.

Impact of the mechanism of injury (Table 5)

Thirty-six of 53 (68%) had explosion/blast injury (BLAST) and 17 of 53 (32%) were injured by GSWs. The BLAST group received more prehospital RCC ($p = 0.002$) and fresh frozen plasma ($p = 0.004$). There was no difference in prehospital transport times, ISS, base deficit, systolic blood pressure, or pulse rate between BLAST and GSW groups.

PTr, aPTT, and coagulation factor levels were similar between BLAST and GSW. Clauss fibrinogen levels were also significantly lower in the BLAST group (1.7 [0.6–3.2]) versus GSW group (2.4 [0.8–2.8]; $p = 0.02$). However, D-dimer levels were significantly higher in the BLAST injury (3976 [676–8648] versus GSW (1418 [257–3781] ng/mL, $p < 0.001$) as well as PAP levels (BLAST, 6982 [1639–37,000] vs GSW, 2177 [931–17,100] ng/mL; $p < 0.001$) (Fig. 2). Despite these changes showing massively increased fibrinolytic turnover, however, there were no differences in ROTEM fibrinolytic parameters between the groups.

DISCUSSION

This is a unique study showing, for the first time, detailed hemostatic changes in patients with military trauma at hospital admission. As expected, the casualties were mainly young men with major injury and were shocked. ATC was common in those who were severely injured. There was relative preservation of coagulation factor levels except for factor V and fibrinogen. Markers of coagulation and fibrinolytic activation/turnover were significantly elevated and the most marked hemostatic changes were present in those who were most shocked.

In addition, subgroup analysis investigating the effect of mechanism of injury, blast versus gunshot, revealed greater fibrinolysis and lower fibrinogen levels in the BLAST group, despite no significant difference in the extent of shock or injury severity.

There was profound fibrinolytic activation as measured by D-dimers and PAP, with levels 50 times the upper limit of the normal range tPA is the main activator of plasminogen, but our study showed disproportionately low tPA levels when compared to the PAP and D-dimer levels. There may be a number of explanations for this. First, tPA has a short half-life of approximately 2.5 minutes. tPA is released from preformed stores in endothelial cells, peaking within the first 30 minutes following injury and hemorrhage in animal studies.[15] Therefore, a tPA surge soon after injury would be missed since most patients arrived at the hospital about 60 minutes after injury. Second, there may be other mechanisms of activation of plasminogen during traumatic injury. For example, Gall et al.[16] suggests that a plasminogen receptor S100A10 is released from the endothelium during

trauma and may be independently activating fibrinolysis. Whatever the mechanism, our data show that fibrinolysis is prevalent in severely injured military casualties and that there was greater fibrinolytic activation with BLAST and GSWs, which most likely accounts for the effectiveness of TXA in reducing trauma and hemorrhage mortality in this population.[9]

There was evidence of consumption of all the coagulation factors, with the lowest factor levels and most prolonged PTr and APTT in those who were most severely shocked. It is notable that median levels of fibrinogen fell disproportionately more than the other factors as described in other bleeding patients.[17] Previous in vitro studies[18] suggest that the falls in fibrinogen and factor V relate to fibrinolytic breakdown of these molecules. Plasmin, as well as causing fibrin breakdown, will cause fibrinogenolysis and a transient activation of factor V before subsequent lysis.[19] This may explain the disproportionately low fibrinogen levels in the BLAST group, with greater activation of plasmin-induced fibrinogenolysis. Factor V can also be cleaved at Arg 506 by activated protein C to generate an anticoagulant molecule, and this may be another mechanism responsible for the fall in factor V seen in ATC.[20]

Despite reduced coagulation factor levels, thrombin generation was preserved, as shown by the normal or increased levels of prothrombin fragment 1 + 2, a molecule shed when prothrombin is activated to thrombin. Plasma vWF levels can be incremented quickly by releasing their stores from Weibel Palade bodies within the endothelial cells. vWF is the main ligand for platelet adhesion and the carrier molecule for factor VIII. vWF levels were reassuringly increased in all groups, similar to civilian trauma[21] facilitating platelet adhesion to damaged tissues.

TM is a surface glycoprotein expressed on endothelial cells of arteries, veins, and microvasculature necessary for the activation of protein C. During endothelial cell activation, TM is endocytosed. Thus, the presence of soluble TM is thought to represent cleavage of TM when the endothelium is injured. Previous studies of soluble TM levels after civilian trauma have shown varied results, either unaltered or raised levels.[20–22] Our study showed soluble TM levels were within normal limits in military trauma.

Soluble tissue factor levels were also within normal limits. Assuming that our assay is reliable, it suggests that even if there is release of TF into the circulation at the time of injury, it is not sustained.

We found that the ROTEM showed less sensitivity to both coagulation and fibrinolytic changes of ATC than conventional assays. Of note, conventional coagulation testing with PTr was more sensitive than ROTEM in detecting ATC, for 77% fulfilled the criteria for ATC by PTr >1.2 (77%) but only 19% fulfilled the ROTEM criteria of an EXTEM A5 less than 35 mm. It is also interesting that ROTEM analysis only revealed abnormal lysis parameters in 8% of our casualties and yet 51% had a tPA level greater than normal and all had increased D-dimer and PAP levels.

ROTEM analysis in military trauma has been investigated before. In 2013, Woolley et al described abnormal admission ROTEM's in 39% of casualties, although at that time the definition of an MCF less than 40 mm was used rather than and A5 less than 35 mm. This study proposed that ROTEM was more sensitive when used as a hybrid score, rather than a single parameter. These findings are similar to Cohen et al.,[23] who described 40 combat casualties in Afghanistan. They considered an "integrated score" where patients had either an international normalized ratio (INR) greater than 1.2, or an A5 less than 35 mm or a Li30 less than 97%. Twenty-eight of 40 casualties met at least one of these definitions, although 22 of 40 (55%) had an abnormal INR, and 15 of 40 (37%) had an A5 less than 35 mm. Only 3 of 40 (8%) had a LY30 less than 97% (a more sensitive definition than our definition of <94%). Cohen's paper concludes that the addition of ROTEM to the INR with an abnormality in at least one of these parameters is a more sensitive method of detecting massive transfusion.

We acknowledge that Li60 would be useful in addition to Li30 in the assessment of fibrinolysis; however, the throughput of casualties in the hospital meant that often ROTEM machines were required for further clinical analysis, and so, as per hospital protocols, analysis was stopped after Li30 was reached. Poor sensitivity to fibrinolysis has been demonstrated before in civilian trauma,[21] and Gall et al.[16] suggested that soluble S100A10 maybe interfering with ROTEM analysis of fibrinolysis. Our results suggest further studies of thromboelastography in military trauma are required. The lack of sensitivity of ROTEM to diagnose hyperfibrinolysis is important because some centers withhold TXA treatment if viscoelastic testing does not demonstrate hyperfibrinolysis. The results from this study would suggest that TXA would still be of benefit in these patients despite a normal ROTEM.

While we are unable to formally compare our study population with a civilian control group, we have assessed our results against the published civilian literature. It is often suggested that military patients will be younger, fitter, and take less medication than those patients reported in civilian studies, and therefore comparison is not possible. The median age in this study was 25, in comparison with median ages between 35 and 40 in civilian studies. This age difference is unlikely to be significant since most people under the age of 40 will not be on medication and the physiological differences between 25-year-olds and 40-year-olds will have less impact than suggested. It is interesting to note that the factor levels reported in this study were similar to those seen in civilian trauma.[24]

There are a number of limitations to our study: First, we did not have dedicated, full-time researchers in Afghanistan to conduct the study. All of the investigators who collected samples had other primary roles that would always take priority over enrolling patients. Second, the clinical workload was such that often investigators were not available as they were recuperating from busy clinical shifts. Finally, patients were enrolled only when the investigators were deployed to

Afghanistan, which was for about 5 months in total during the entire study period. This made enrollment extremely challenging; hence, only 53 casualties had full data sets. We acknowledge that this is a small sample size, but it nonetheless remains unique. Our study is also complicated by the heterogeneity of military trauma and the different types of casualty evacuation as well as the evolution of prehospital treatments during the study. Each of the three types of helicopter used had their own treatment protocols, meaning that the treatment available to the casualty was dictated by the mechanism of retrieval. On occasion, multiple casualties arrived simultaneously, necessitating an immediate decision as to which casualty was most likely to meet the inclusion criteria for our study. This means that the casualties recruited are representative of military trauma but are not a comprehensive cross section.

Furthermore, treatment protocols changed over the course of the study. The British military prehospital use of blood components was established in 2009, and, after CRASH-2[7] (describing the benefits of TXA) was published in 2010-2011; a strategic decision was made to give TXA prehospital if possible.

Thus, the preadministration of TXA and blood components was determined by two factors: the clinical state of the patient and whether the prehospital team had the capability to administer TXA or blood products.

It is possible that the difference in prehospital treatment between the BLAST and GSW groups limits the ability to detect a significant difference in hemostatic parameters between these groups. Sicker patients tended to get the UK MERT with a different medical capability, earlier adoption of TXA and blood transfusion during the study period and different prehospital times. Our study was not designed to explore these differences, so this analysis was not included.

CONCLUSION

The main conclusion of our study is that ATC in military trauma is strikingly similar to ATC due to civilian trauma, as evidenced by the profound activation in coagulation and fibrinolysis at hospital presentation and their correlation with degree of shock. Despite the prolonged prothrombin time, ATC is characterized by a combination of adequate thrombin generation, fibrinogen consumption, and fibrinolysis. These findings are reassuring, as they suggest that the developments in the hemostatic management of civilian trauma can inform military trauma and vice versa. Certainly, the finding of fibrinolytic activation justifies the use of TXA at a biologic level. It is interesting to note the lack of sensitivity of Li30 at detecting hyperfibrinolysis, a consideration for clinicians who require viscoelastic evidence of fibrinolysis before administering TXA.

A novel finding is that blast injury is associated with a more extensive activation of fibrinolysis than gunshot wounds. This raises the question as to whether the currently used dose of TXA is adequate or whether larger doses or a more powerful antifibrinolytic might further improve survival.

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CONFLICT OF INTERESTS

The authors have disclosed no conflicts of interest.

AUTHORS CONTRIBUTION

The idea was conceived and developed by BJH, TW, EK, SW, JD and MM. TW, EK, and others took and locally prepared the samples. KP analyzed all samples in the United Kingdom. BG performed statistical analysis. BJH and TW wrote the paper. EK, SW, BG, KP, JD, and MM read, commented on, and agreed on the final paper.

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