Indices of complement activation and coagulation changes in trauma patients

Zhangsheng Yang,1 Milomir O Simovic,1 Bin Liu,1 Matthew B Burgess,1 Andrew P Cap,1 Jurandir J DalleLucca,2 Yansong Li1,3,4

ABSTRACT
Objectives Early complementopathy and coagulopathy are shown often after trauma. However, the prevalence of any interplay between complement cascade (ComC) and coagulation cascade (CoaC) after trauma remains unclear. This study intended to explore whether complement-coagulation crosstalk exists, which may provide a reliable guide to clinical implications in trauma patients.

Methods This single-center cohort study of trauma patients enrolled 100 patients along with 20 healthy volunteers. Blood samples from patients were collected at admission, 45, 90, 135 minutes, and 18 hours after admission. Demographic characteristics were recorded, blood levels of ComC and CoaC factors, and inflammatory cytokines were measured by ELISA, clot-based assays, or luminex multiplex assay, and partial thromboplastin time (PTT) were assessed using a Behring blood coagulation system.

Results Compared with the healthy controls, plasma levels of complement factors (C5b-9 and Bb) and 11 tested inflammatory cytokines increased in moderately and severely injured patients as early as 45 minutes after admission and sustained higher levels up to 18 hours after admission. C5b-9 correlated positively to patients’ hospital stay. In parallel, the consumption of coagulation factors I, II, X, and XIII was shown throughout the first 18 hours after admission in moderately and severely injured patients, whereas PT, PTT, D-dimer, factor VII, and factor VIII values significantly increased from the admission to 135 minutes in moderately and severely injured patients. Along with an inverse correlation between plasma Bb, factors I and II, a positive correlation between C5b-9, Bb, D-dimer, PT, and PTT was evident.

Conclusions This study demonstrates trauma-induced early activation of plasma cascades including ComC, CoaC, and fibrinolytic cascade, and their correlation between plasma cascades in severe trauma patients. Our study suggests that the simultaneous modulation of plasma cascades might benefit clinical outcomes for trauma patients.

Level of evidence Prospective study, level III.

BACKGROUND
Trauma, a significant healthcare burden among civilians and military service members, is responsible for 5 million deaths per year worldwide,1 and 60% of total trauma-related deaths occur between the age of 1 and 44 in the USA.2,3 Trauma-related healthcare is also an overwhelming healthcare costs such as 671 billion dollars in the year of 2013 alone.3 Since 2003, military operations in Iraq and Afghanistan have resulted in many injuries.4 Although the mortality from trauma has diminished throughout the Iraq and Afghanistan conflicts due to advance the protection and rapid transportation to higher facilities, death from the trauma still represents a substantial problem. Two-third of major trauma patients die now as a result of causes other than exsanguination.5,6 Although these patients may withstand the initial wounding event by hemorrhage control and restoration of blood pressure and have potentially survivable injuries, they still can die due to early multiple-organ failure (MOF) caused by dysregulation of immune and coagulation systems during episode of prolonged field care.6,4 By and large, post-traumatic direct tissue damage and ischemia/reperfusion injury (IRI) trigger a multifaceted plasma cascade activation, including complement cascade (ComC), coagulation cascade (CoaC), kinin cascade (Kinc), and fibrinolytic cascade (FibC).6 Consequently, such multifaceted plasma cascade activation has a decisive role in IRI-triggered systemic inflammatory responses. Moreover, emerging evidence indicates that post-traumatic dysregulation of plasma cascades appears to act as a linchpin in the events of thrombininflammation (TT), endotheliopathy (EP), systemic inflammatory response syndrome (SIRS), compensatory anti-inflammatory response syndrome (CARS), or persistent inflammation, immunosuppression, and catabolism syndrome (PICS).5,10,11 These disorders further contribute to the incidence of...
trauma-induced MOF, thus resulting in poor clinical outcomes or death.5 10 11

The relationship between ComC and other plasma cascades is complex. Amara et al and Foley et al reported that plasminogen can cleave the key complement proteins leading to ComC activation.12 13 Wu et al found that tranexamic acid can reduce pulmonary CS levels in a rat model of polytrauma and hemorrhage.14 Barthel and colleagues demonstrated that plasminogen(ogen) is a ComC inhibitor, and the inhibitory effects of plasminogen(ogen) on ComC are primarily mediated through the destruction of C3b.15 Furthermore, the ComC in turn also regulates the FbC through upregulating plasminogen activator inhibitor-1 (PAI-1) expression,16 17 binding to fibrin,18 and interacting with plasminogen.19 Another new interaction between FbC and KinC can lead to hyperfibrinolysis-induced blood-brain barrier leakage by plasmin-dependent and bradykinin B2 receptor-dependent manner.20 Additionally, the CoaC and KinC specific serine proteases can activate the ComC.9 21 22 Previous studies also showed that tissue factor pathway inhibitor can inhibit the lectin pathway of ComC.23 In turn, complement components can also activate CoaC.7 24 25

Fundamentally, the multifaceted activation and interactions of plasma cascades highlight the complexity of the inflammatory response after trauma. However, the interplay among these cascades remains unknown and requires further investigation. Based on these premises, we hypothesized that there is a positive correlation between plasma cascades. Here we investigated the activation of some plasma cascades and their mutual interactions, which may provide a reliable guide to clinical implications in trauma patients.

MATERIALS AND METHODS

This prospective single-center cohort study evaluated severe trauma patients admitted directly to level 1 trauma center at the University of Texas Health Science Center-San Antonio (UTHSCSA). This study was conducted under a protocol reviewed and approved by the UTHSCSA and the US Army Medical Research and Development Command Institutional Review Board (HSC2012-0291-H). The study was granted a waiver of consent for blood sampling as a minimal risk intervention.

Patients and healthy subjects

For this prospective study, one hundred (100) patients suffering from blunt and penetrating trauma or these injuries alone were consented to and enrolled at admissions to the UTHSCSA Hospital’s emergency department (ED). The mechanisms of injury included motor vehicle collision (47%), auto vs pedestrian (16%), fall (14%), gunshot/stab wound (13%), and assault (10%). Enrollment of these patients was based on an age≥18 years with the following exclusion criteria: (i) admission to the hospital ward; (ii) prisoners; (iii) pre-existing therapeutic anti-coagulation (except for aspirin and/or ibuprofen); and (iv) coagulopathy or other coagulation disorders present prior to any trauma. Each patient was indexed and categorized based on an injury Severity Score (ISS)<10, 10 to 20, or >20.

In addition to the 100 pooled trauma patients, 20 healthy volunteers were recruited to serve as healthy controls. The inclusion criteria for these healthy volunteers were: (i) free of any acute illness during the two previous weeks; (ii) minimal body mass of 110 lbs (50 kg); and (iii) not pregnant. Healthy volunteers donated a one-time blood sample after signing a consent form per regulatory policy.

Clinical data

As described previously, ISS was used by certified coders to evaluate patient’s injuries as described previously.26 Other clinical data including mortality, total hospital stay days, total intensive care unit (ICU) stay length, and other basic demographic data were collected during the patient’s ICU and hospital stay and entered into an Oracle database.

Blood collection

Blood samples were collected from the trauma cohort at admission, 45, 90, 135 minutes, and 18 hours postadmission. Next, the blood samples were centrifuged and plasma from each blood sample was collected utilizing the standard procedure. Subsequently, aliquots of plasma were stored in cryoprecipitate polypropylene tubes at −80°C for further analysis. Blood samples collected from healthy volunteers were similarly processed.

Measurement of complement and coagulation factors

Activation of the complement alternative and terminal pathways was determined by measuring plasma levels of Bb fragments of complement factor B and soluble terminal complement complex C5b-9 using MicroVue Bb Plus EIA and C5b-9 Plus EIA (Quidel Corp., San Diego, CA), respectively. Blood levels of CoaC proteins (factor I and D-dimer) were measured using the ELISA kit (Abcam, Cambridge, MA). The plasma concentrations of other CoaC factors were determined by clot-based assays. All measurements were performed following the manufacturers’ instructions.

Measurement of inflammatory cytokines in plasma

The levels of high mobility group box 1 (HMGB1) and myeloperoxidase (MPO) in the plasma were measured by ELISA according to the manufacturer’s instructions by using HMGB1 kit (IBL International GmbH, Hamburg, Germany) and MPO kit (Hycult Biotech, Uden, Netherlands), respectively. The respective levels of proinflammatory cytokine (interleukin 6 (IL-6), IL-8, tumor necrosis factor α (TNF-α), monocyte chemotaxatant protein-1 (MCP-1), macrophage inflammatory proteins-1β (MIP-1β), and granulocyte-colony stimulating factor (G-CSF) and anti-inflammatory cytokine (IL-5, L-10, and IL-13) present in the plasma samples were analyzed using a Luminex assay kit (Bio-Rad Laboratories, Hercules, CA).

Statistical analysis

Group data were expressed as means±SEM. Serum complement and cytokine levels were compared using one-way or two-way analysis of variance followed by Tukey’s multiple comparison test or unpaired t-test with Welch’s correction. For correlation analyses, the Pearson correlation coefficient was calculated, including the 95% CI. Throughout this study, p<0.05 values were considered to be significant for all analyses.

RESULTS

Demographics, clinical characteristics, and outcomes

The clinical characteristics and demographics of the trauma patients analyzed (n=100) are presented in table 1. The total of 100 patients was divided into three subcategories based on their ISS as: mild, ISS<10 (n=63), moderate, ISS=10 to 20 (n=19); and severe, ISS>20 (n=18). As expected, severely injured group had the highest level of mortality (17% vs 5% and 0%) compared with moderately and mildly injured groups, respectively. Similarly, severely injured patients had a longer hospital stay (10 vs 8 and 4 days), a longer ICU stay (10 vs 8 and 4 days), a longer ICU stay (7 vs 4 and 0.3 days), a longer ICU stay (10 vs 8 and 4 days), a longer ICU stay (7 vs 4 and 0.3 days),
Injury (table 2). In particular, significantly elevated levels of plasma D-dimer from 45 to 135 minutes after admission were observed in moderately and severely injured patients when compared with healthy volunteers or less injured patients (p<0.05, figure 2B). A positive correlation was observed between plasma C5b-9 concentrations at 90 minutes after injury and total hospital stay days in severely injured patients (p<0.05, figure 1C). However, there were no significant changes in plasma levels of C4d and MBL which are the known markers of the activation of classical and lectin pathway, respectively (data not shown).

**Post-traumatic coagulopathy**

Severely injured patients had significantly prolonged PT from admission to 135 minutes after admission versus healthy volunteers (p<0.05) and less injured patients (p<0.05, figure 2A). Significantly prolonged PTT values were observed in severely injured patients at 45, 90 minutes, and 18 hours after admission when compared with healthy volunteers or less injured patients (p<0.05, figure 2B). As depicted in figure 2C, significantly elevated levels of plasma D-dimer from admission to 135 minutes after injury were observed in moderately and severely injured patients when compared with healthy volunteers (p<0.05) or mildly injured patients (p<0.05). In comparison to healthy volunteers, severely injured patients demonstrated significantly lower plasma levels of factor I from 45 to 135 minutes after admission (figure 2D), factor II throughout the

### Table 1 Demographics of UTHSCSA trauma patients

|                      | Total (n=100) | ISS<10 (n=63) | ISS=10–20 (n=19) | ISS>20 (n=18) |
|----------------------|--------------|--------------|-----------------|--------------|
| Age (years)          | 39 (18–84)   | 39 (18–77)   | 43 (19–74)      | 34 (19–84)   |
| Male gender (%)      | 79           | 75           | 84              | 89           |
| Race (%)             |              |              |                 |              |
| White race           | 45           | 43           | 57              | 39           |
| Hispanic race        | 49           | 52           | 32              | 55           |
| Black race           | 6            | 5            | 11              | 6            |
| Type of injury (%)   |              |              |                 |              |
| Blunt mechanism      | 87           | 87           | 79              | 94           |
| MVC                  | 47           | 46           | 47              | 61           |
| Pedestrian struck    | 16           | 17           | 0               | 22           |
| Fall                 | 14           | 14           | 21              | 5            |
| Assault              | 10           | 10           | 11              | 6            |
| Penetrating          | 13           | 13           | 21              | 6            |
| Pre-hospital intubation (%) | 14      | 5           | 32              | 33           |
| Blood transfusion (%) | 15           | 6            | 26              | 33           |
| Plasma               | 8            | 2            | 16              | 22           |
| Platelet             | 1            | 0            | 5               | 0            |
| Mortality (%)        | 4            | 0            | 5               | 17           |
| Total hospital stay (days) | 6 (1–46) | 4 (1–46) | 8 (1–34) | 10 (1–34) |
| Total ICU stay (days) | 2 (0–26) | 0.3 (0–10) | 4 (0–19) | 7 (0–26)  |

Data of age, total hospital stay and total ICU stay are presented as mean (range), others are shown as %. ICG, intensive care unit; ISS, injury severity score; MVC, motor vehicle crash; RBC, red blood cell; UTHSCSA, University of Texas Health Science Center-San Antonio.

and a more requirement in blood transfusions as compared with mildly injured patients, respectively.

Blood chemistry analyses revealed the prevalence of higher levels of white blood cells (WBCs), sodium, potassium, blood urea nitrogen, creatinine, and lactate in moderately and severely injured patients (ISS>10) as compared with patients with mild injury (table 2). In particular, significantly elevated levels of WBCs were observed at admission. International normalized ratio (INR) had significantly higher values in severely injured patients than in less (moderately and mildly) injured patients. Partial thromboplastin time (PTT) had prolonged values in severely injured patients but without a significant difference compared with less injured patients (table 2). Of note, the values of other clinical parameters including hemoglobin (Hb), hematocrit (Hct), calcium, and magnesium were lower in severely injured patients than the patients with less injury (table 2).

**Formation of C5b-9 and generation of Bb after trauma**

As shown in figure 1A and a robust increase in plasma C5b-9 levels was observed at admission, and plasma C5b-9 levels reached a significant difference from 45 to 135 minutes after admission in all injury groups of patients when compared with respective levels in healthy volunteers. Moderately and severely injured patients had significantly higher plasma C5b-9 levels than mild injured patients (p<0.05, figure 1A). Concentrations of plasma Bb in severely injured patients (ISS>20) began to increase at admission and reached statistical significance throughout the entire observation period compared with healthy volunteers (p<0.05, figure 1B). Severely injured patients had significantly higher levels of plasma Bb than those mildly injured patients (p<0.05, figure 1B). A positive correlation was observed between plasma C5b-9 concentrations at 90 minutes after injury and total hospital stay days in severely injured patients (p<0.05, figure 1C). However, there were no significant changes in plasma levels of C4d and MBL which are the known markers of the activation of classical and lectin pathway, respectively (data not shown).

### Table 2 Clinical parameters of UTHSCSA trauma patients

|                      | Total (n=100) | ISS<10 (n=63) | ISS=10–20 (n=19) | ISS>20 (n=18) |
|----------------------|--------------|--------------|-----------------|--------------|
| WBC (10^9 /L)        | 12.35±0.56   | 10.71±0.6    | 14.38±1.41 **   | 15.87±1.33 **|
| Hb (g/dL)            | 13.79±0.21   | 14.13±0.25   | 13.52±0.5      | 12.89±0.56   |
| HCl (%)              | 39.97±0.54   | 40.87±0.61   | 39.5±1.23      | 37.46±1.54   |
| Platelets (×10^11 /μL) | 230±5.95    | 230±8.1      | 222±9.6        | 239±4.3      |
| INR                  | 1.0±0.02     | 1.04±0.02    | 1.06±0.03      | 1.18±0.07 **|
| PTT (seconds)        | 27.6±0.77    | 27.8±0.67    | 25.05±0.98     | 29.6±3.43    |
| Sodium (mEq/L)       | 140.11±0.33  | 139.65±0.45  | 141.16±0.6     | 140.6±0.64   |
| Potassium (mEq/L)    | 3.74±0.05    | 3.71±0.06    | 3.77±0.09      | 3.83±0.11    |
| BUN (mg/dL)          | 13.53±0.58   | 13.33±0.83   | 13.58±0.86     | 14.18±1.19   |
| Creatinine (mg/dL)   | 0.88±0.04    | 0.85±0.04    | 0.89±0.07      | 1.0±0.01     |
| Calcium (mg/dL)      | 8.47±0.07    | 8.62±0.08    | 8.27±0.18      | 8.15±0.19    |
| Magnesium (mEq/L)    | 1.87±0.02    | 1.89±0.03    | 1.86±0.04      | 1.83±0.06    |
| Lactate (mmol/L)     | 2.68±0.16    | 2.42±0.18    | 2.84±0.43      | 3.46±0.44    |

Values are expressed as mean±SEM Statistical analyses were performed by unpaired t-test with Welch’s correction to compare groups of ISS=10–20 and ISS>20 to the group of ISS<10.

*P<0.05, **p<0.01.

BUN, blood urea nitrogen; Hb, hemoglobin; HCl, hematocrit; INR, international normalized ratio; PTT, partial thromboplastin time; UTHSCSA, University of Texas Health Science Center-San Antonio; WBCs, white blood cells.

Yang Z, et al. Trauma Surg Acute Care Open 2022;7:e000927. doi:10.1136/tsaco-2022-000927
entire observation period (figure 2E), factors V, factor X, and protein C at 45 minutes (online supplemental figure 1A,C, E), and factor XIII at admission and 45 minutes after admission (p<0.05) (online supplemental figure 1D). Our analysis also indicates that moderately injured patients had decreased concentrations of factors I (figure 2D) and X (online supplemental figure 1C) at 45 minutes, factor II (figure 2E) from 45 minutes to 135 minutes, and factor XIII (online supplemental figure 1D) at admission, 45 minutes, and 18 hours after injury (p<0.05). There was significantly decreased in plasma factor II (figure 2E) at admission and 45 minutes and factor X (online supplemental figure 1C) at admission in severely injured patients compared with mildly injured patients (p<0.05). Conversely, plasma factor VIII concentrations were significantly higher in severely injured patients from admission to 135 minutes and in less injured patients only at the first 45 minutes after admission (p<0.05). Plasma levels of factor VIII returned to normal levels by 18 hours after admission. Severely injured patients had significantly higher levels of factor VIII from admission to 135 minutes after injury when compared with less injured patients (p<0.05) (online supplemental figure 1B).

**Inflammatory cytokines response after trauma**

We found that the levels of both HMGB1 (figure 3A) and MPO (figure 3B) were significantly elevated in severely injured patients at their time of admission when compared with the healthy and moderately injured subjects. The MPO levels in severe trauma patients were significantly higher in severely injured patients when compared with the healthy and moderately injured subjects. The MPO levels in severe trauma patients were significantly higher in severely injured patients when compared with the healthy and moderately injured subjects.
Figure 3  Systemic release of inflammatory cytokines in trauma patients. The plasma fractions isolated from blood samples collected from healthy volunteers (normal, n=20) or trauma patients (n=100) at admission (admi.), 45, 90, 135 minutes, and 18 hours postadmission were subjected to the analysis of systemic response of inflammatory cytokines such as HMGB1 (A), MPO (B), TNF-α (C), MCP-1 (D), IL-6 (E), and IL-10 (F) by ELISA or lumines multiplex assay. Statistical analyses were performed by two-way ANOVA, *, †, and ‡p<0.05 vs healthy volunteers, ISS<10 patients (n=63), and ISS=10–20 patients (n=19), respectively. ANOVA, analysis of variance; ISS, injury severity score; MCP, monocyte chemoattractant protein; MPO, myeloperoxidase.

remained elevated until 90 minutes after admission. The MPO levels increased early and reached a statistical significance at 45 minutes after admission in moderately injured patients (figure 3B). TNF-α (figure 3C) and IL-8 (online supplemental figure 2B) had little change between the healthy and injured patient populations with a single spike in concentration observed at the 90 minutes time point for severely injured group compared with both less injured groups (figure 3C and online supplemental figure 2B). MCP-1 levels increased in all injured groups at each time point. A statistically significant change of MCP-1 levels was found at the 45-minute and 90-minute time points for severely injured group compared with other groups (figure 3D). MIP-1β levels showed significant increase in severely and moderately injured groups from admission through 135 minutes postadmission compared with healthy volunteers (online supplemental figure 2E). A similar trend showed IL-6 (figure 3E) and G-CSF (online supplemental figure 2D) with significantly elevated levels at 45-minute and 90-minute time points, respectively, peaking at 135 minutes before diminishing at 18 hours. As shown in online supplemental figure 2A), IL-5 expression showed a consistent difference between severely injured and mildly injured patients from admission until 135 minutes after admission. The IL-13 showed a similar trend (online supplemental figure 2C). Another cytokine, IL-10, showed significant elevations peaking at 45 minutes postadmission in severely injured patients (figure 3F).

Correlation between complement activation and coagulation parameters
Cumulatively, our data indicate a positive correlation between plasma C5b-9 concentrations and PT levels at 45 minutes (p<0.05; figure 4A), PTT levels at 135 minutes (p<0.05; figure 4B) and D-dimer at 90 minutes (p<0.05; figure 4C) after admission. Moreover, a positive correlation was observed between plasma Bb levels at 45 minutes and PT at 45 minutes (p<0.05; figure 4D), PTT at 45 minutes (p<0.05; figure 4E), and D-dimer at 90 minutes (p<0.05; figure 4F) after admission. However, there was a negative correlation of plasma Bb at 45 minutes with factor II at 45 minutes (p<0.05; figure 4G) and factor I at 135 minutes (p<0.05; figure 4H) after admission.

DISCUSSION
In the current study, we investigated the dysregulation and cross-talk of plasma cascades and inflammatory responses in blood samples of trauma patients during 18 hour-period since ED admission. Primarily, the data from this study in trauma patients revealed a correlation between early alternative/terminal ComC activation, coagulopathy and cytokine storm with clinical outcome in severely injured patients. Besides, early coagulopathy consisting of increasing D-dimer, consumption of CoaC factors, and prolonged PT and PTT were evident in the population of severely injured patients. Furthermore, we observed an early correlation among plasma cascades after trauma and the elevated factor VIII in the patients may be associated with an increased thrombotic risk after injury.

Indeed, the excessive complement activation might lead to the development of early MOF via triggering inflammation, vascular leak, cell lysis, thrombosis, and acidosis. The increased levels of C5b-9 and C5a correlated with injury severity, MOF and mortality have been reported before. Different complement pathways contributed to complement activation in different trauma patient populations. Previous studies, including our own, have addressed the detrimental facets of complement activation in preclinical animal models.
models and found that early complement inhibition after injury increased survival, improved hemodynamics and metabolism, and mitigated tissue damage. However, the underlying mechanism of these phenomena and their clinical significance remains obscure. Therefore, further studies to analyze the time-frame and activation of complement pathway after trauma, and the relation between complement activation and ISS may assist in diagnosing and treating complementopathy based on the specific status of the individual patient. Such gained knowledge will provide reliable guidance for possible complement modulation therapy. In the current study, we found early elevation of plasma levels of C5b-9 and Bb, but not C4d (data not shown), after trauma at 45 minutes after admission, the degree of activation being proportional to ISS and alternative pathway as the predominant activated complement pathway, which is consistent with a previous report in major trauma patients. Our present investigation further reinstates that the activation of both complement terminal and alternative pathways might serve as early diagnostic biomarkers and therapeutic targets for severely injured trauma patients. Early complement activation (at 90 minutes after admission) positively correlated with the length of hospital stay in severely injured patients, thus suggesting that early complement terminal activation might be as useful prognostic biomarkers for trauma patients.

Trauma-induced coagulopathy (TIC) is a serious complication in both civilians and military trauma patients. TIC is a complicated process consisting of early hypercoagulopathy (thrombosis) stemming from tissue factor exposure and tissue PAI-1 release, and subsequent hypocoagulopathy and hyperfibrinolysis (bleeding) via protein C activation and tissue plasminogen activator release. Both thrombotic and bleeding phenotypes are associated with increased MOF and mortality. In this study, we observed that severely injured patients had increased PT, PTT, and INR from admission to 18 hours after admission. Depletion of coagulation factors in the plasma after trauma might induce prolonged PT, PTT, and INR. Indeed, in present study, early decreased plasma levels of specific coagulation proteins: factors I (fibrinogen), II, X, and XIII showed in moderately and severely injured patient, and factor V and protein C in severely injured patients from admission to 135 minutes after admission, indicate that severe trauma triggers more CoaC protein consumption or CoaC activation after injury.

The decreased coagulation proteins returned to healthy levels at 18 hours after admission may be caused by appropriate interventions with plasma transfusion and/or hepatic neosynthesis of coagulation proteins. The reported data from this study demonstrating the simultaneous increase in the plasma levels of D-dimer with the prolonged PT, PTT, and INR in the early phase (from admission to 135 minutes) of severely injured patients might be associated with hypercoagulation and hyperfibrinolysis after trauma.

In the present study, we also detected an increase in plasma levels of factor VIII within 135 minutes after admission, suggesting factor VIII might be a risk factor for thrombosis in trauma patients. Factor VIII is a plasma sialoglycoprotein that plays a pivotal role in normal hemostasis by acting as a critical cofactor for the activated factor IX. It has been recognized for many years that factor VIII deficiency in hemophilia patients results in a significant bleeding diathesis. In contrast, during recent years, an increasing body of work indicates that high plasma levels of factor VIII may serve to increase thrombosis.

In terms of inflammatory responses, the results presented here revealed that the patients with a higher ISS demonstrated significantly higher levels of inflammatory cytokine expression. Moreover, in these trauma patients, the expression of proinflammatory cytokines and anti-inflammatory cytokines was concomitant rather than sequential. Besides, our results also showed that severely injured patients had a longer ICU and total hospital stay as well as a higher mortality rate compared with less injured patients. Overall, our observations suggest that this early inflammatory response may be related to the morbidity and mortality observed in trauma patients, as reported before. Therefore, the modulation of inflammatory responses in severe trauma patients should be implemented.
Severe blunt trauma can activate a multifaceted network of plasma cascades. Traditionally, the plasma cascades such as ComC, CoaC, FibC, and KinC are considered separate distinguishable cascades. Nevertheless, these systems belong to complex inflammatory networks and exhibit similar characteristics regarding the specialized functions of their activators and inhibitors. The mounting evidence implicates that the interplay between these cascades escalates rapid amplification of these cascades which essentially contributes to the development of immunothrombosis, and eventually, progression to TI, MOF, and mortality. In the present study, plasma concentrations of EP, and SIRS, CARS, or PICS, leading to trauma-associated immunothrombosis, and eventually, progression to TI, et al. 2022; Yang Z et al. 2022; Yang Z, et al. 2022. Funding this study involves human participants and was approved by the US Army Medical Research and Development Command. This study was conducted under a protocol reviewed and approved by the UTHSCSA Institutional Review Board (HSC.2012-0291-H). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available on reasonable request.

Supplemental material This content has been supplied by the author(s), it has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

Acknowledgements The authors gratefully thank Mr. Charles Guymon for assessing the coagulation factors and Dr. Subrata Haldar for critical review and editing of the article.

References

1. WHO. Injuries and violence: the facts. 2014. https://www.who.int/violence_injury_prevention/media/news/2015/Injury_violence_facts_2014/en/ (23 Dec 2019).
2. Florence C, Haegierch T, Simon T, Zhou C, Luo F, Medical EL. Estimated Lifetime Medical and Work-Loss Costs of Emergency Department-Treated Nonfatal Injuries—United States, 2013. MMWR Mortal Wkly Rep 2015;64:1078–82.
3. Florence C, Simon T, Haegerich T, Luo F, Zhou C. Estimated Lifetime Medical and Work-Loss Costs of Fatal Injuries—United States, 2013. MMWR Mortal Wkly Rep 2015;64:1074–7.
4. Le TD, Gueym JM, Nnamani NS, Gross KR, Chuung KK, Stockinger ZT, Nessen SC, Pusateri AE, Akers KS. A 12-year analysis of Nonbattle injury among US service members deployed to Iraq and Afghanistan. JAMA Surg 2018;153:800–7.
5. Lord JM, Midwinter MI, Chen I-F, Belli A, Brohi K, Kovacs EJ, Koenderman L, Kubes P, Lifflord RJ. The systemic immune response to trauma: an overview of pathophysiology and treatment. Lancet 2014;384:1455–65.
6. Cannon JW. Hemorrhagic shock. N Engl J Med 2018;378:370–9.
7. Dutton RP, Stansbury LG, Leone S, Kramer E, Hess JR, Scalea TM. Trauma mortality in mature trauma systems: are we doing better? an analysis of trauma mortality patterns, 1997-2008. J Trauma 2010;69:620–6.
8. Mannucci PM, Levi M. Prevention and treatment of major blood loss. N Engl J Med 2007;356:2301–11.
9. Dueckhoff C, Rieben R. Ischaemia/Reperfusion injury: effect of simultaneous inhibition of plasma cascade systems versus specific complement inhibition. Biochem Pharmacol 2014;88:12–22.
10. Sausaia A, Moore FA, Moore EE. Postinjury inflammation and organ dysfunction. Crit Care Clin 2017;33:167–91.
11. Huber-Lang M, Lambris JD, Ward PA. Innate immune responses to trauma. Nat Immunol 2018;19:327–41.
12. Amara U, Flierl MA, Rittirsch D, Klos A, Chen H, Acker B, Brückner UB, Nilsson B, Gebhardt F, Lambris JD, et al. Molecular intercommunication between the complement and coagulation systems. J Immunol 2010;185:5628–36.
13. Foley JH, Walton BL, Aleman MM, O’Byrne AM, Lei V, Hamasser M, Foley KA, Wolberg AS, Conway EM. Complement activation in arterial and venous thrombosis is mediated by plasmin. EbiolMedicine 2016;5:175–82.
14. Wu X, Dubick MA, Schwacha MG, Cap AP, Darlington DN. Tranexamic acid attenuates the loss of lung barrier function in a rat model of Polytrauma and hemorrhage with resuscitation. Shock 2017;47:500–5.
15. Barbelt D, Schinder S, Zipfel PF. Plasminogen is a complement inhibitor. J Biol Chem 2012;287:18831–42.
16. Speidel WS, Kastl SP, Hutter R, Katsaros KM, Kaun C, Bauriedel G, Maurer H, Guer K, Badimon JJ, Wolff J. The complement component C5a is present in human corneal lesions in vivo and induces the expression of MMP-1 and MMP-9 in human macrophages in vitro. Faseb J 2011;25:35–44.
17. An G, Ren G, An F, Zhang C. Role of C5a-C5aR axis in the development of atherosclerosis. Sci China Life Sci 2014;57:790–4.
18. Howes J-M, Richardson VR, Smith KA, Schroeder V, Sonani R, Shore A, Hess K, Aijan R, Rease JR, Keen JN, et al. Complement C3 is a novel plasma clot component with anti-fibrinolytic properties. Diab Vasc Dis Res 2012;9:216–25.

Patient consent for publication Not applicable.

Ethics approval This study involves human participants and was approved by the US Army Medical Research and Development Command. This study was conducted under a protocol reviewed and approved by the UTHSCSA Institutional Review Board (HSC2012-0291-H). Participants gave informed consent to participate in the study before taking part.

Acknowledgements The authors gratefully thank Mr. Charles Guymon for assessing the coagulation factors and Dr. Subrata Haldar for critical review and editing of the article.

Contributors YL and JJD supervised the project. ZY, MOS, and MBB drafted the manuscript. YL and BL conducted the luminex and ELISA measurements. ZY, MOS, and BL performed the data statistical analysis. APC, JJD, MOS, and YL participated in the data interpretation and edit the manuscript.

Funding This study was supported by a grant from the US Army Medical Research and Development Command (grant C_086_2014_USAISR).

Disclaimer The views expressed in this article are those of the authors and do not reflect the official policy or position of the U.S. Army Medical Department, Department of the Army, DoD, or the U.S. Government.

Competing interests None declared.

ORCID iD

Yang Z http://orcid.org/0000-0002-8341-1539
Cavenaghe ME, Teixeira AF, Filho AS, Souza GO, Vasconcellos SA, Heinemann MB, Nascimento ALTO. Characterization of a novel protein of Leptospira interrogans exhibiting plasmogenase, vitronectin and complement binding properties. Int J Med Microbiol 2019;309:116–29.

Marcos-Contreras OA, Martínez de Lizarrondo S, Bardou I, Orset C, Pruvost M, Anfray A, Frigout Y, Hamet Y, Labrousse L, Montaner J, et al. Hyperfibrinolysis increases blood-brain barrier permeability by a plasmin- and bradykinin-dependent mechanism. Blood 2016;128:2423–34.

Hoht JJ, Wells JD, Jones SE, Yoza BK, McCall CE. Complement mediates a primed inflammatory response after traumatic lung injury. J Trauma Acute Care Surg 2014;76:601–9.

Bosilj F, Peerschke EL, Ghrebietski B, Tedesco F. Cross-Talk between the complement and the kinin system in vascular permeability. Immunol Lett 2011;140:7–13.

Keiser MP, Pouv RB, Kamp AM, Patiwaal S, Marsman G, Hart MH, Zeeleider S, Kuipers TW, Wouters D. Tfpi inhibits lectin pathway of complement activation by direct interaction with MASP-2. Eur J Immunol 2015;45:544–50.

Mizuno T, Yoshioka K, Mizuno M, Shimizu M, Nagano F, Okuda T, Tsuibo N, Maruyama S, Nagamatsu T, Imai M. Complement component 5 promotes lethal thrombosis. Sci Rep 2017;7:42714.

Subramanian S, Jurk K, Hobohm L, Jäckel S, Saffarzadeh M, Schwierczek K, Wenzel F, Langer F, Reinhardt C, Ruf W. Distinct contributions of complement factors to platelet activation and fibrin formation in venous thrombus development. Blood 2017;129:2291–302.

Baker SP, O’Neill B, Haddon W, Long WB. The injury severity score: a method for describing patients with multiple injuries and evaluating emergency care. J Trauma 1974;14:187–96.

Karaseu E, Nilsson B, Köhl J, Lambiris JD, Huber-Lang M. Targeting complement pathways in Polytrauma- and sepsis-induced multiple-organ dysfunction. Front Immunol 2019;10:5343.

Rittirsch D, Redl H, Huber-Lang M. Role of complement in multiorgan failure. Clin Dev Immunol 2012;2012:1–10.

Li Y, Zhao Q, Liu B, Dixon A, Cancio L, Dubick M, Dalle Lucca J. Early complementopathy predicts the outcomes of patients with trauma. Trauma Surg Acute Care Open 2019;4:e000217.

Burk A-M, Martin M, Flierl MA, Rittirsch D, Helm M, Lampl L, Bruckner U, Stahl GL, Blom AM, Perl M, et al. Early complementopathy after multiple injuries in humans. Shock 2012;37:348–54.

Li Y, Yang Z, Chavko M, Liu B, Ademeri OA, Simovic MO, Dubick MA, Cancio LC. Complement inhibition ameliorates blast-induced acute lung injury in rats: potential role of complement in intracellular HMGB1-mediated inflammation. PLoS ONE 2016;13:e0205394.

Campbell JC, Li Y, van Amersfoort E, Relan A, Dubick M, Sheppard F, Pusateri A, Niemeyer D, Tsokos GC, Dalle Lucca J. C1 inhibitor limits organ injury and prolongs survival in swine subjected to battlefield simulated injury. Shock 2016;46:177–88.

Li Y, Chavko M, Slack JL, Liu B, McCarron RM, Ross JD, Dalle Lucca J. Protective effects of decay-accelerating factor on blast-induced neurotrauma in rats. Acta Neuropathol Commun 2013;1:52.

Dalle Lucca JJ, Li Y, Simovic MO, Slack JL, Cap A, Falabella MJ, Dubick M, Lebeda F, Tsokos GC. Decay-Accelerating factor limits hemorrhage-instigated tissue injury and improves resuscitation clinical parameters. J Surg Res 2013;179:159–67.

Dalle Lucca JJ, Li Y, Simovic M, Pusateri AE, Falabella M, Dubick MA, Tsokos GC. Effects of C1 inhibitor on tissue damage in a porcine model of controlled hemorrhage. Shock 2012;38:82–91.

Dalle Lucca JJ, Simovic M, Li Y, Moratz C, Falabella M, Tsokos GC. Decay-Accelerating factor mitigates controlled hemorrhage-instigated intestinal and lung tissue damage and hyperkalemia in swine. J Trauma 2011;71:5151–60.

Satyam A, Graef ER, Lapshak PH, Tsokos MG, Dalle Lucca JJ, Tsokos GC. Complement and coagulation cascades in trauma. Acute Med Surg 2019;6:329–35.

Schmitz FCF, Manolov V, Morgenstern J, Fleming T, Hettmeier S, Uhle F, Al-Saeedi M, Hackert T, Bruckner T, Schöchl H, et al. Acute fibrinolysis shutdown occurs early in septic shock and is associated with increased morbidity and mortality: results of an observational pilot study. Ann Intensive Care 2019;9:19.

Leeper CM, Neal MD, Billiar TR, Spery JL, Gaines BA. Overresuscitation with plasma is associated with sustained fibrinolysis shutdown and death in pediatric traumatic brain injury. J Trauma Acute Care Surg 2018;85:12–17.

Jenkins PV, Rawley G, Smith OP, O’Donnell JS. Elevated factor VIII levels and risk of venous thrombosis. Br J Haematol 2012;157:653–63.

Reikerås O. Immune depression in musculoskeletal trauma. Inflamm Res 2010;59:409–14.

Engelmann B, Massberg S. Thrombosis as an intravascular effector of innate immunity. Nat Rev Immunol 2013;13:34–45.