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Indices of complement activation and coagulation changes in trauma patients

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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 (PT) 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.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Complement and coagulation cascades are crucial mediators in trauma-induced complementopathy and coagulopathy. Cross-talk between these two pathways remains unknown.

WHAT THIS STUDY ADDS

⇒ We revealed early correlations among plasma cascades after trauma, and the elevated factor VIII in the patients may be associated with an increased thrombotic risk after injury.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study provides a clue to a possible modulation of plasma cascades, a purposeful therapeutic approach for attenuating trauma-related morbidity and mortality.

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 tier 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.[5–8] 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).[9] 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 thromboinflammation (TI), 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 plasmin can cleave the key complement proteins leading to ComC activation.[12 13] Wu *et al* found that tranexamic acid can reduce pulmonary C5a levels in a rat model of polytrauma and hemorrhage.[14] Barthel and colleagues demonstrated that plasmin(ogen) is a ComC inhibitor, and the inhibitory effects of plasmin(ogen) on ComC are primarily mediated through the destruction of C3b.[15] Furthermore, the ComC in turn also regulates the FibC through upregulating plasminogen activator inhibitor-1 (PAI-1) expression,[16 17] binding to fibrin,[18] and interacting with plasminogen.[19] Another new interaction between FibC 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.[9 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 anticoagulation (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 chemoattractant 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 (7 vs 4 and 0.3 days),

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 (%)				
RBC	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 %.
ICU, 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).

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 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
HCt (%)	39.97±0.54	40.87±0.61	39.5±1.23	37.46±1.54
Platelets ($\times10^3$ /µL)	230±5.95	230±8.1	222±9.6	239±14.3
INR	1.07±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; HCt, hematocrit; INR, international normalized ratio; PTT, partial thromboplastin time; UTHSCSA, University of Texas Health Science Center-San Antonio; WBCs, white blood cells.

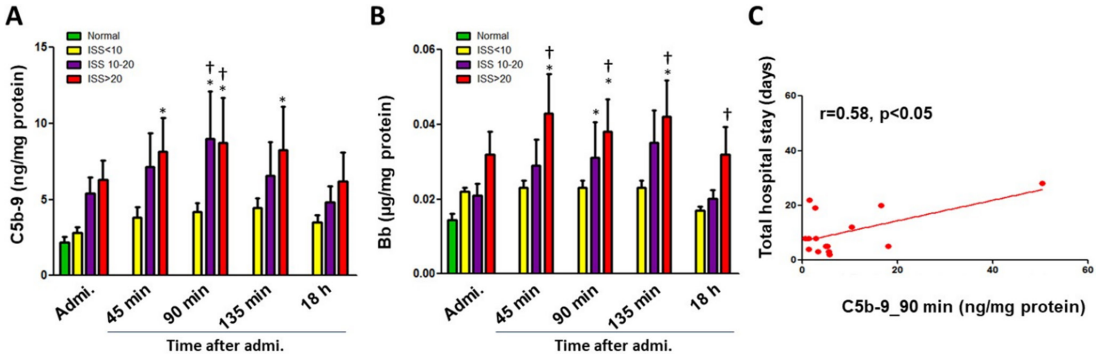

Figure 1 Correlation between activation of complement terminal/alternate pathways and severity of injury/total hospital stay of trauma patients. Plasma concentrations of C5b-9 (A) and Bb (B) in trauma patients (n=100) at admission (admi), 45, 90, 135 minutes, and 18 hours postadmission were presented. Panel C represents a positive correlation between plasma levels of C5b-9 at 90 minutes postadmission and hospital stay. Statistical analyses were performed by two-way ANOVA; * and † indicate p<0.05 vs healthy volunteers (normal, n=20) and ISS<10 patients (n=63), respectively. For correlation analysis, the Pearson correlation coefficient was calculated, including the 95% CI. P<0.05 values were considered significant. ISS=10–20 patients (n=19) and ISS>20 patients (n=18). ANOVA, analysis of variance; ISS, injury severity score.

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

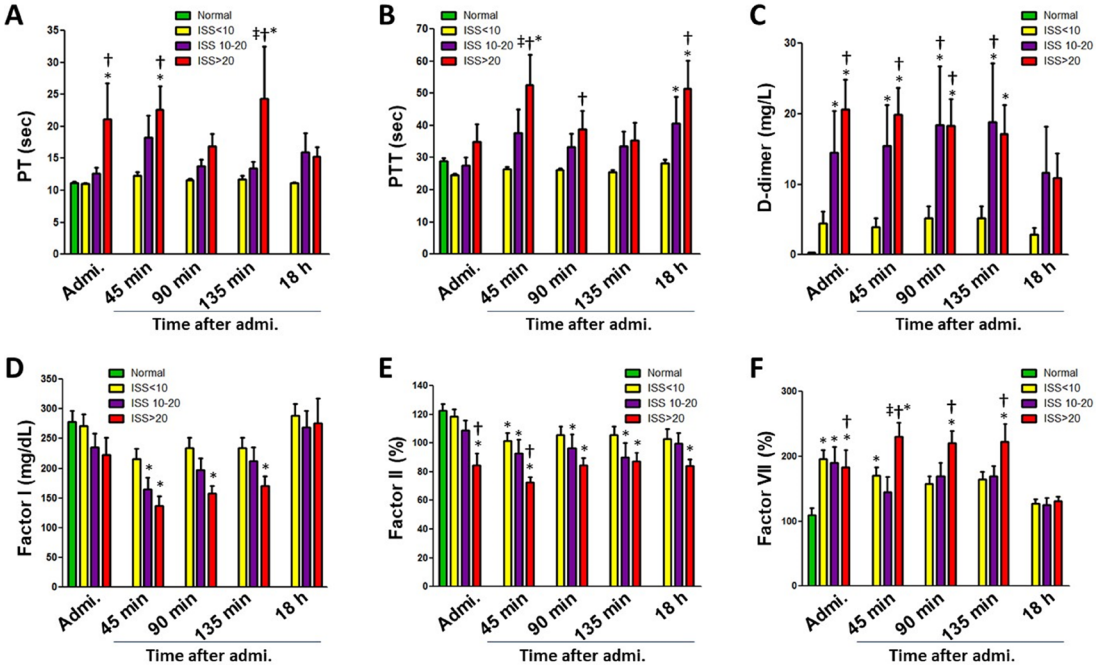

Figure 2 Alteration of coagulation parameters correlates with the severity of injury score in trauma patients (n=100). The blood samples were collected from healthy volunteers (normal, n=20) or trauma patients at admission (admi.), 45, 90, 135 minutes, and 18 hours postadmission, and coagulation parameters such as PT (A), PTT (B), D-dimer (C), factor I (D), factor II (E), and factor XII (F) in the plasma fractions were measured. 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; PT, partial thromboplastin; PTT, partial thromboplastin time.

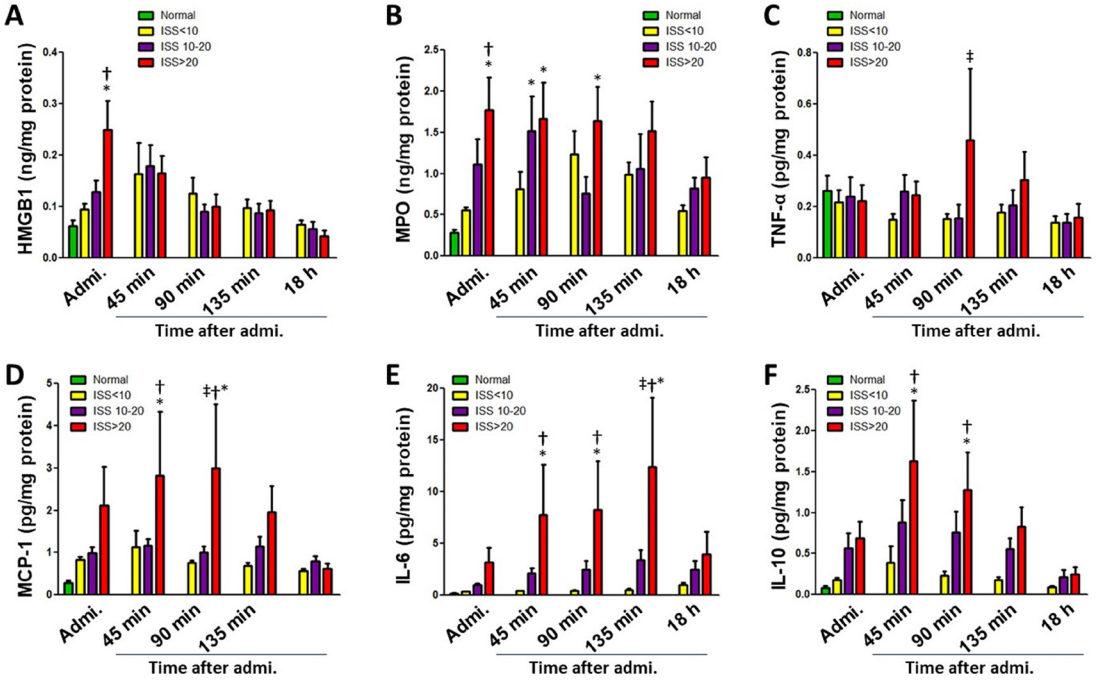

Figure 3 Systemic release of inflammatory cytokines in trauma patients. The plasma fractions isolated from blood samples collected from healthy volunteers (normal, n=20) or trauma patients (n=100) at admission (admi.), 45, 90, 135 minutes, and 18 hours postadmission were subjected to the analysis of 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 luminex 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 crosstalk 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.[27 28] The increased levels of C5b-9 and C5a correlated with injury severity, MOF and mortality have been reported before.[27 28] Different complement pathways contributed to complement activation in different trauma patient populations.[29] Previous studies, including our own, have addressed the detrimental facets of complement activation in trauma patients.[29 30] It is noteworthy that we also tested the efficacy of complement inhibitors in preclinical animal

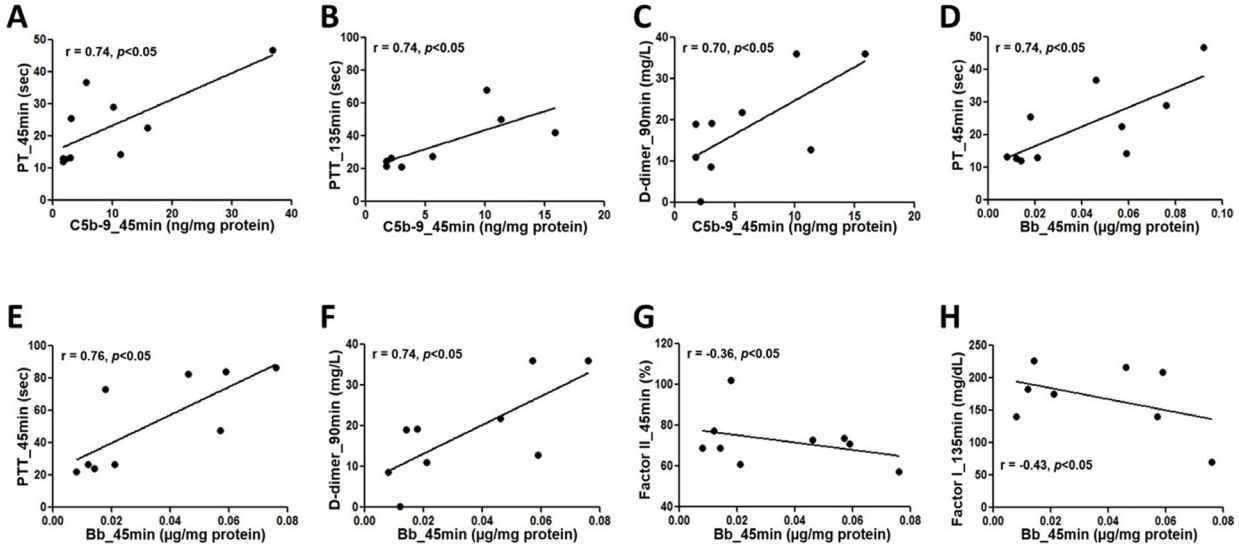

Figure 4 Correlation between complement activation and coagulation alteration. Plasma levels of C5b-9 at 45 minutes after admission positively correlated with PT at 45 minutes after admission (A), PTT at 135 minutes after admission (B) and D-dimer at 90 minutes after admission (C). Plasma concentrations of Bb at 45 minutes after admission positively correlated with PT at 45 minutes after admission (D), PTT at 45 minutes after admission (E) and D-dimer at 90 minutes after admission (F). Inverse correlation of plasma levels of Bb at 45 minutes after admission with plasma concentrations of factor II at 45 minutes after admission (G) and fibrinogen at 135 minutes after admission (H). The Pearson correlation coefficient was calculated, including 95% CI. P<0.05 values were considered to be significant for all analyses. PTT, partial thromboplastin time.

models and found that early complement inhibition after injury increased survival, improved hemodynamics and metabolism, and mitigated tissue damage.[31–36] However, the underlying mechanism of these phenomena and their clinical significance remains obscure. Therefore, further studies to analyze the timeframe 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.[30 31] 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.[37–39] 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.[40]

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.[5 10 11 41] 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.[12] 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, EP, and SIRS, CARS, or PICS, leading to trauma-induced MOF and mortality.[37 42] In the present study, plasma concentrations of C5b-9 and Bb at 45 minutes after admission positively correlated with plasma levels of PT, PTT, and D-dimer in severely injured patients. In contrast, we observe a negative correlation between Bb generation and plasma factor II concentration. These data hint us the potential crosstalk involving ComC, CoaC, and FibC after severe trauma. This study demonstrates trauma-related early activation of plasma cascades, including ComC, CoaC, and fibrinolytic cascades, and their mutual correlations in severe trauma patients. If there are controlled clinical associations with the activation of plasma cascades, the modulation of these cascades might benefit clinical outcomes for trauma patients. However, the other measured complement fragments (C3a and C4d) did not correlate with the aforementioned coagulation and fibrinolytic parameters (data not shown), suggesting that CoaC-induced and FibC-induced ComC activation may be mediated by direct targeting of complement terminal pathway (C5).

We are aware that this study has several limitations. First, since this is a single-center and small observational cohort study of major trauma patients, a much larger patient cohort should be enrolled to analyze the correlations of individual complications of trauma. Second, the effect of coagulation factor depletion may not be as functional biomarkers for coagulopathy. Third, only D-dimer measurement after trauma is not sufficient to analyze fibrinolytic status. Therefore, the employed approach in this study is limited to measure coagulation proteins and a fibrin degradation product that neither necessarily represents the activity nor sufficiently predicts the fibrinolytic status, respectively. In the future study, we further need to apply functional assays of CoaC (coagulation serine-protease activity) and standard thromboelastography (LY30) to analyze coagulation and fibrinolytic status, respectively.

In conclusion, we demonstrate that early activation of plasma cascades along with their intricate interactions in the severely injured patients may act as a linchpin leading to the development of TI, EP, and SIRS, CARS, or PICS. Nevertheless, this study also highlights that modulation of plasma cascades might be a therapeutic strategy for attenuating trauma-induced morbidity and mortality (online supplemental figure 3).

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