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ORIGINAL RESEARCH REPORT

Early posttraumatic changes in coagulation and fibrinolysis systems in isolated severe traumatic brain injury patients and its influence on immediate outcome

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KEYWORDS
Trauma induced coagulopathy;
Coagulation pathway analysis;
Isolated traumatic brain injury;
Outcome

Abstract
Objective/background: Early coagulopathy in isolated severe traumatic brain injury occurs despite the lack of severe bleeding, shock, and fluid administration. We aimed to correlate coagulation activation/inhibition, thrombin generation and fibrinolysis with the development of acute trauma induced coagulopathy (TIC) and its effects on early mortality in isolated severe traumatic brain injury (iSTBI) patients.
Methods: A prospective screening of iSTBI patients was done for two years. History of anticoagulants, liver disease, hypotension, extracranial injuries, transfusion, brain death were excluded. TIC was defined as international normalized ratio (INR) $\geq$ 1.27 and/or prothrombin time (PT) $\geq$ 16.7 seconds and/or activated partial thromboplastin Time (aPTT) $\geq$ 28.8 seconds on admission following iSTBI. Analysis of tissue factor (TF), tissue factor pathway inhibitor (TFPI), protein C (PC), protein S (PS), thrombin/antithrombin complex (TAT), soluble fibrin monomer (sFM), tissue plasminogen activator (tPA) and plasminogen activator inhibitor-1 (PAI-1) was done. Cases were categorized as presence or absence of TIC and 20 healthy controls participants were included.

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Results: A total of 120 cases met the inclusion criteria, aged 35.7 ± 12.12 years, 96% males. TIC was identified in 50 (41.6%). TIC occurred independently of age, sex, Glasgow coma scale (GCS) but was associated with acidosis (60%; $p = .01$). Following iSTBI significant decline was seen in coagulation activation. Thrombin generation and fibrinolysis were markedly increased. TF, TFPI, PC and PS were low in TIC compared with control. Significant depletion of PS was seen in TIC versus No-TIC. TBI patients with depleted PS had an odds ratio (OR) of 7.10 (1.61—31.2) for TIC. Receiver operating characteristic curve (ROC) analysis depicted area under the curve (AUC) of 0.73 (95% confidence interval [CI] 0.63—0.84) with a cut-off of $\geq$74 of PS (specificity 63.9%, sensitivity 72.7%). In-hospital mortality was higher in TIC group (44%) compared with no-TIC (20%) with OR of 4.73 (95% CI 1.68—13.3) and hazard ratio [HR] of 2.8 (95 % CI 1.2—6.4).

Conclusion: Incidence of TIC in iSTBI is 41.6%, with 4.7 times odds for mortality. Traumatic brain injury causes enhanced coagulation activation, inadequate inhibition, exacerbation of thrombin generation, and subsequent increased fibrinolysis. ROC curve analysis revealed a cut-off of PS $\leq$ 74 with specificity 63.8%, sensitivity 72.7% for development of TIC.

Introduction

Background

Traumatic brain injury (TBI) is the leading cause of early mortality after injury, with only hemorrhagic shock consistently more lethal [1]. By contrast to TBI, 30—40% of mortality rate has been reported for hemorrhage, and hemorrhagic shock [1]. Development of trauma-induced coagulopathy (TIC) results in dysfunction and severely compromised hemostatic performance and exacerbates bleeding. The term ''trauma-induced coagulopathy" was introduced to describe a process that impairs blood coagulation and increases blood loss by a possible deregulation of the intrinsic coagulation system in trauma patients [2]. Approximately 25% of TBI are complicated by hemorrhagic shock, and result in a two- to three-fold elevated risk of poor outcome [1] (i.e., an early hypocoagulable state and bleeding to death or a late hypercoagulable state and the development of multiple organ failure) [3]. Although TBI patients have limited blood loss and receive restricted fluid infusion, accompanying coagulopathy leads to progression of hemorrhagic lesions, with an overall prevalence of 32.7% [4—6]. Subsequent to severe injury and tissue hypoperfusion, TIC develops within minutes of injury, pathogenesis includes a substantial tissue factor release, altered protein C (PC) homeostasis, microparticle upregulation, and platelet hyperactivity [2]. Although the incidence and outcome of patients with TBI associated acute coagulopathy have been outlined thoroughly throughout the literature there is a paucity of published data on TIC pathophysiology subsequent to isolated severe TBI. We designed this study to answer three defined key clinical questions: (1) what are the pathophysiological relationships between coagulation, thrombin generation, and fibrinolysis in severe isolated TBI patients?; (2) whether acute coagulofibrinolytic derangements contribute towards unfavorable outcomes in severe isolated TBI patients?; (3) can the circulating levels of coagulation, thrombin generation, fibrinolysis severe in early prognostication and identification of acute trauma-induced coagulopathy following brain injury?

Objectives

We aimed to detect the possible marker which can predict the development of early coagulopathy after brain injury. Our primary objective was to correlate parameters of coagulation activation/inhibition, thrombin generation, and fibrinolysis with the development of TIC in isolated severe TBI patients. Our secondary objective was to study the effects of TIC on transfusion requirements, organ dysfunction, sepsis, and mortality.

Materials and methods

Study design and sample analysis

We conducted a prospective observational control study (The Strengthening the Reporting of Observational Studies in Epidemiology's (STROBE) criteria followed), in a level 1 trauma care center in north India, for the duration of 2.5 years (August 2014 to January 2016).

Setting and participants

In the present study, plasma biomarkers from a total of 561 patients with the highest level of trauma activation were investigated. Of these, only admitted patients suffering from isolated severe TBI were included.

This excluded patients < 20 years and > 50 years of age ($n = 43$), secondary admission ($n = 131$), polytrauma ($n = 121$), hypotension ($n = 14$) and history of antiplatelet therapy ($n = 2$). Retrospectively two patients were excluded due to brain death.

Management of TBI

Immediately on arrival at the emergency department (ED), treatment was given in accordance with guidelines of Advanced Trauma Life Support (ATLS). All patients underwent brain computer tomography (CT) after detailed neurological evaluation and initial resuscitation.

Defining isolated TBI

Head injuries [7] were defined as severe TBI if they met any of the following three criteria: (1) Glasgow Coma Scale (GCS) $\leq$ 8; (2) head abbreviated injury scale (AIS) $\geq$ 2; and (3) intracranial hematoma on CT scan of the head (cerebral contusion; subarachnoid, subdural, or epidural hemorrhages). Severe TBI without any extracranial injuries was defined as isolated severe traumatic brain injury.

Defining TBI associated coagulopathy

Patients were stratified by conventional coagulation tests as coagulopathic (international normalized ratio (INR) $\geq$ 1.27 and/or prothrombin time (PT) $\geq$ 16.7 and/or activated partial thromboplastin time (aPTT) $\geq$ 28.8) on hospital admission. This definition of TIC was chosen based on the study by Greuters et al. [8] and our institutional current clinical practice and experience.

Variables

Information on baseline demography, clinical, physiologic, and laboratory tests were prospectively compiled from patient records and the institutional trauma registry. Admission and follow-up CT scans were independently evaluated, and the type of head injury based on the radiologic findings was classified into acute subdural hematoma (ASDH), acute epidural hematoma (AEDH), intracerebral hematoma/contusion (ICH), and traumatic subarachnoid hemorrhage (TSAH). Acidosis was defined as arterial blood pH < 7.3 and arterial blood HCO3 < 20 mEq/L. Hypoperfusion was defined as the presence of an arterial BD (base deficit) > 6 mmol/L at admission. The data included admission vital signs, complete blood count, biochemical profile, pH, BE (base excess, conventional plasma-based coagulation tests (PT, aPTT and INR), 24-hour blood transfusions (packed RBCs, plasma, and platelets). The primary patient outcome was in-hospital mortality (up to 30 days). Secondary patient outcome was sepsis (blood culture positive), hepatic failure defined as serum total bilirubin > 3 mg/dL and aspartate aminotransferase > 80 IU/L [two times the upper limit of normal] and renal failure defined as serum creatinine > 2 and creatinine clearance < 100 mg/dL [9].

Sample size

The anticipated TIC rate being > 40%, to detect the difference in patients who do and do not develop coagulopathy with 90% power and 95% confidence limits, we need at least 35 patients with TIC. Therefore, the estimated sample size was at least 100 isolated severe TBI patients.

Blood sampling and investigations

To minimize the impact of confounding therapy, a one-time sample was collected in the ED (< 12 hours of injury). 10-mL sample of blood was obtained prior to transfusion of fluids and/or blood products. Blood was transferred into vacutainer tubes containing 3.2% citrate and inverted to ensure proper anticoagulation. After spinning plasma was aliquoted and frozen for later analysis. The enzyme-linked immunosorbent assay (ELISA) analyses were conducted using the following commercially available kits for Tissue Factor [Human Tissue Factor ELISA Kit (CD142); cat no: ab108903; Abcam plc., USA]; endothelial protein C receptor (EPCR) (Asserachrom sEPCR, Catalog No. 00264; Diagnostic Stago, Inc. USA); tissue factor pathway inhibitor (TFPI) (Asserachrom Total TFPI, Catalog No. 00261; Diagnostic Stago, Inc. USA); thrombin/antithrombin complex (TAT) (IMUBIND TAT ELISA, Catalog No. ADG833; Sekisui Diagnostics GmbH, Germany); tissue plasminogen activator (tPA) (Asserachrom tPA, Catalog No. 00948; Diagnostic Stago, Inc. USA); plasminogen activator inhibitor (PAI) (Asserachrom PAI-1, Catalog No. 00949; Diagnostic Stago, Inc. USA). PC was analyzed in citrate plasma, using STA-Stachrom protein C kit (Catalog No. 00671; Diagnostica Stago, S.A.S, France; normal range: 70—130%); STA-Owern Koller (Catalog No. 00360); STA Unicalibrator (Catalog No. 00675); STA-Systems Control [N] + [P] (Catalog No. 00678) and Free Protein S (PS) was analyzed using Liatest Free Protein S (Immunoturbidimetric assay, Catalog No. 00561; Diagnostica Stago, S.A.S, France; normal range: 70—130%); STA-Owern Koller (Catalog No. 00360); STA Unicalibrator (Catalog No. 00675); STA-Systems Control [N] + [P] (Catalog No. 00678) on Stago STA Compact Coagulation Analyzer (Diagnostica Stago, Paris, France).

Statistical methods

All analysis was performed using STATA 11.0 (Stata Corp, Texas, USA). Summary statistics were used to describe continuous variables as mean ± standard deviation or median (25th and 75th interquartile range) and categorical data were presented as frequency (%). Univariate analysis of the continuous variables between the study groups was assessed using *t*-Tests/Wilcoxon rank-sum. Chi-square ($\chi 2$) tests or Fisher's exact test was used to compare categorical variables. Correlations between circulating levels of the different biomarkers were investigated by Spearman correlations and presented by rho (ρ) and *p* values.

The relationships between the dependent and the independent variables were analyzed by multiple regression analysis using the stepwise method, including variables that were either found to correlate with or were expected to influence TIC or mortality and the results were reported as regression coefficients and 95% confidence intervals (CI). Survival analysis was done by Cox proportional-hazards regression. The association between the survival time of patients and predictor variables was investigated and results were reported as Hazard ratio (95% CI). Differences in *p* values <0.05 were considered to be statistically significant. Receiver operating characteristic curve analysis was used to generate optimal cut-offs of biomarkers.

Results

Baseline parameters

The study sample was predominantly male 88.3% (106), overall average age of the subjects was 35.2 ± 12.65 years.

Baseline preinjury variables were identical between the two groups. Severe degree of hypoperfusion, anemia, and thrombocytopenia were observed in the TIC group compared with no-TIC.

Sampling was performed within 2 hours (range 1—5) of injury and 29% of the patients were brought to the ED within the 1st hour of injury. Based on the acute trauma induced coagulopathy definition 120 patients were classified into two subgroups: 50 patients (41.6%) who developed TIC and 70 patients (58.4%) who did not develop TIC.

TIC upon ED admission was present in 40% (14/35) of all patients admitted within 60 minutes of injury, 46.3% (19/41) of whom were brought to the ED within 3 hours of injury had developed TIC. A total of 41.7% (10/24) of whom were brought to the ED within 6 hours of injury. 35% (7/20)

Computed tomography (CT) scan, revealed various acute traumatic etiologies, including 1.7% (2) with intraventricular hemorrhage, 45% (54) with SDH, 35.0% (42) SAH and 14.2% (17) EDH. 60.3% patients in the TIC group had acidosis, which was significantly, two times higher, compared with no-TIC group (30%) ($p = .01$). Whilst the groups are statically comparable for CT abnormalities ($p < .05$), the percentage of midline shift and subdural hematoma was higher in patients who developed TIC as compared with no-TIC (4.0% vs. 1.4% and 46.0% vs. 44.3%, respectively). One patient developed hydrocephalus and one had intraventricular hemorrhage in the TIC group. Interestingly the Marshall CT score was significantly high in No-TIC patients compared with TIC group ($p = .02$) (Table 1).

Assessment of subjects on admission by Glasgow Coma Scale (GCS), showed that nearly 57.5% (69) had a GCS score of 7—8, 21.7% (26) had GCS 5—6, and 20.8% (25) had a GCS of 4—3. The frequency of patients having a low GCS score of 3—4 and 5—6 was comparatively higher in TIC group than the no-TIC group [24% (12) vs. 18.6% (13) and 30% (15) vs. 15.7% (11), respectively] ($p = .84$). Inversely the frequency of patients having a GCS score 7-8 was higher in patients without TIC compared to the TIC group [65.7% (46) vs. 46% (23); $p = .07$].

Upon arrival in the ED, elevated PT (>16 s) was observed in 32 (26.7%) patients and elevated aPTT (>39 s) was observed in 17 (14.2%) patients. Ten (1.9%) patients had hypofibrinogenemia. D-dimer levels were <0.5 μg/mL in 27 (48.2%) patients, mild elevations (1—2 μg/mL) were seen in 14 (25%) patients and high D-dimer (hyperfibrinolysis) levels (3—8 μg/mL) was observed in 15 (26.8%) pateints. Hemogram parameters did not significantly associate with the development of TIC. Physiologic and laboratory values were more deranged in patients with TIC, higher frequency of on admission anemia and thrombocytopenia was seen in TIC group compared with no-TIC (Table 1).

Acute coagulopathy, thrombin generation and fibrinolysis of traumatic brain injury

A significant decline in circulatory levels of tissue factor [1.7 (1.02—3.6) ng/mL vs. 3.9 (3.0—4.7) ng/mL, $p = .0002$], tissue factor pathway inhibitor [77.6 (34.6—123.4) ng/mL vs. 108.2 (97.8—114.5) ng/mL; $p = .04$]; EPCR [47.8 (23.3—76.4) ng/mL vs. 85.2 (63.0—103.4) ng/mL, $p = .0005$] were seen following brain injury compared with healthy controls (Fig. 1).

Inversely a significantly marked elevation in the plasma levels of thrombin antithrombin complex [41.3 (17.4—100.2) pg/mL vs. 12.7 (6.8—20.1) pg/mL, $p < .0001$], soluble fibrin monomer [38.4 (23.1—123.8) μg/mL vs. 0.45 (0—5.7) μg/mL, $p < .0001$] were seen following brain injury compared with healthy individuals. A five-fold increase was seen in the plasma levels of tissue plasminogen activator [125.9 (29.2—317.8) ng/mL vs. 25 (11.2—32.4) ng/mL, $p < .0001$] and PAI-1 levels had slight yet insignificant (81.0 ± 35.8 ng/ml vs. 90.8 ± 23.0 ng/ml, $p = .24$) in TBI patients compared with healthy control participants (Fig. 1).

Coagulation activation, anticoagulation, thrombin generation and fibrinolysis with the development of TIC subsequent to isolated severe TBI

Low levels of tissue factor were seen in patients with TIC compared to no-TIC, similar insignificant decline was also seen in TFPI levels. However, severely depleted PC [49.0 (35.5—102.5) % vs. 91.0 (55.0—103.0) %, $p = .04$] and PS [58.5 (34.5—85.5) % vs. 89.0 (73.0—115.0) % $p = <.0001$] was seen in TIC vs. no-TIC patients. Thrombin generation markers TAT [41.3 (19.0—115.6) pg/mL vs. 37.1 (15.3—92.3) pg/mL, $p = .30$] and sFM [64.4 (23.7—138.5) μg/mL vs. 26.7 (21.7—108.7) μg/mL, $p = .12$] both were found to be elevated in TIC compared with no-TIC. Interestingly fibrinolysis markers PAI-1 was slightly lower in TIC than no-TIC group, whereas tPA was elevated in TIC than no-TIC group (Table 2).

ROC analysis depicted an AUC of 0.74 [95% CI 0.63—0.84] with a cut-off of ≥74 of PS (specificity 63.8%, sensitivity 72.7%) (Fig. 2).

Spearman's rank test revealed a significantly positive correlation between TF and PAI-1 levels ($r = 0.61$, $p \leq .01$) and TFPI and PAI-1 ($r = 0.61$, $p \leq .01$) in coagulopathic iSTBI patients (Table 3).

Negative correlation was observed between PT values and levels of PC ($r = -0.023$, $p = .02$), PS ($r = -0.41$, $p = .0001$), TFPI ($r = -0.27$, $p = .01$), and PAI-1 ($r = -0.25$, $p = .01$). Similar negative correlation was found between aPTT and PS ($r = -0.23$, $p = .03$) levels.

Association of acute traumatic coagulopathy accompanying isolated severe traumatic brain injury with unfavorable clinical outcomes

Overall median time taken from injury to sepsis development was 7 (3.5—12.5) days. Incidence of sepsis was higher in TIC patients compared with no-TIC groups (20% vs. 12.9%, $p = .31$). Frequency of hepatic failure was high in TIC patients (10.0% vs. 5.8%, $p = .48$) however, this trend did not attain statistical significance (Table 3). One patient developed multiple organ dysfunction, in the TIC group. Median hospital length stay was elevated in TIC compared with no-TIC patients [9 (5—12) days vs. 7 (2—16) days, $p = .73$). Overall mortality rate was 30%, 14% due to hypovolemic shock, 11% due to brain death, 8% cardiac arrest due to refractory hypertension, 3% due to septic shock, 3% due to posterior cerebral artery stroke, 3% due to ventricular

Table 1 Demographics and clinical pattern of isolated severe TBI patients with and without TIC.

Parameters		Trauma induced coagulopathy (n = 50)	No-Trauma induced coagulopathy (n = 70)	p
Age (y)		34.8 ± 13.35	36.0 ± 12.89	.62
Sex	Male	45 (90.0)	61 (87.1)	.77
	Female	5 (10.0)	9 (12.9)	
Mode of injury	Road traffic accident	35 (70.0)	48 (68.5)	.69
	Fall	9 (18.0)	12 (17.1)	
	Assault	0	2 (2.8)	
	Miscellaneous	6 (12.0)	8 (11.4)	
Time taken from injury to admission (h)*		2 (1—4)	2 (1—5)	.80
Mechanical ventilation	No	21 (42.0)	23 (32.8)	.34
	Yes	29 (58.0)	47 (67.2)	
Systolic BP (mm/Hg)		134.3 ± 3.4	143.1 ± 3.2	.07
GCS		6 (5—7)	7 (5—8)	.09
Hypoperfusion[a] (n = 72)	No	19 (63.3)	33 (78.5)	.18
	Yes	11 (36.4)	9 (21.6)	
Acidosis[a] (n = 68)	No	11 (39.2)	28 (70.0)	**.01**
	Yes	17 (60.3)	12 (30.0)	
Marshall computerized tomography score	Diffuse injury II cisterns present, shift < 5 mm (13.5% mortality)	27 (54.0)	30 (42.9)	**.02**
	Diffuse injury III cistern compressed/absent, shift < 5 mm (34% mortality)	10 (20.0)	19 (27.1)	
	Diffuse injury IV shift > 5 mm (56.2% mortality)	10 (20.0)	6 (8.6)	
	Evacuated mass lesion	3 (6.0)	15 (21.4)	
Hemoglobin (g/dL)		12.3 ± 2.94	12.7 ± 2.50	.35
Hematocrit (%)		38.6 ± 8.67	40.4 ± 7.13	.20
Red blood cell count (10^6/cumm)		4.1 ± 0.93	4.4 ± 0.78	.06
White blood cell count (10^3/cumm)*		14.7 (10.8—18.4)	13.9 (11.0—19.1)	.67
Platelet count (10^3/cumm)*		184.5 (116.0—251.0)	196.5 (138.0—248.0)	.53
Anemia[a]	No	43 (86.0)	61 (87.1)	>.99
	Yes	7 (14.0)	9 (12.9)	
Thrombocytopenia[a]	No	42 (84)	63 (90)	.40
	Yes	8 (16)	7 (10)	

Continuous variables are reported as mean ± standard deviations or *median (interquartile range).Bold font depicts significant p.
BP = blood pressure; GCS = Glasgow Coma Scale; TBI = traumatic brain injury; TIC = trauma induced coagulopathy.
[a] Categorical variables are reported in terms of frequency (percentage).

tachycardia, and 39% suffered cardiac arrest. A total of 19% of the non-survivors had elevated intracranial pressure (ICP).

Unfavorable outcome was significantly associated with elevated systolic blood pressure 147.0 ± 28.71 mm/Hg vs. 136.1 ± 24.80 mm/Hg, p = .03). A total of 36.2% patients had hypertension on admission in patients who had fatal outcome, compared with 19% in the survivor group (p = .04). Glasgow coma scale was significantly low in patients who died (5.1 ± 1.7 vs. 6.5 ± 1.5, p < .0001), compared with those who survived. Mortality was significantly associated with development of TIC, as 61.1% of the patients who died had TIC compared with 33.7% of those who survived (p = .005). Logistic regression analysis revealed a 4.7 (95% CI 1.6—13.3) times higher odds of mortality in patients who developed TIC compared with those who did not. PRP transfusion was also found to be an independent predictor of mortality (adjusted OR 3.9; 95% CI 1.1—14.4), also per unit change in the severity of head injury (GCS) would result in 1.6 (95% CI 1.2—2.2) unit change in the odds for mortality in severe isolated TBI patients. Cox regression analysis hazard ratio showed that TIC and higher severity of injury (low GCS score) were independent predictors of in-hospital mortality (p = .01 and p < .0001, respectively). Risk of in-hospital mortality was 2.8 times higher in TIC patients compared with no-TIC patients (HR 2.8; 95% CI 1.2—6.4) and per unit change in severity of head injury (GCS) would result into 1.5 unit change in the risk for in-hospital mortality in severe isolated TBI patients (HR 1.5; 95% CI 1.2—1.7) (Table 4).

Markers for coagulation activation, anticoagulation, thrombin generation and fibrinolysis did not vary between the survivors and nonsurvivors following iSTBI regardless of the development of TIC.

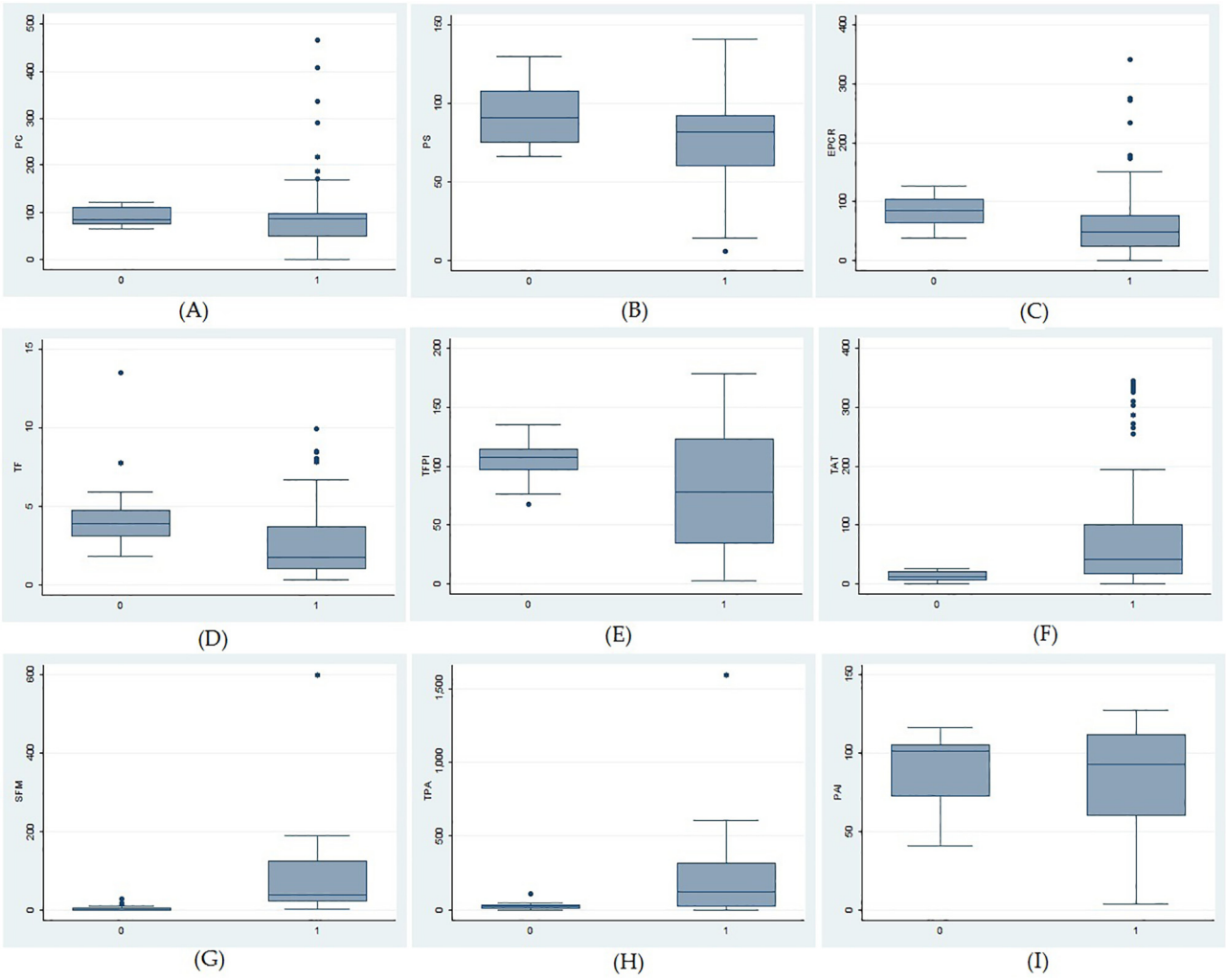


Fig. 1 Box and whisker plots depicting circulating markers of coagulation, anticoagulation, thrombin generation and fibrinolysis in isolated severe TBI patients. (A) Protein C; (B) Protein S; (C) endothelial Protein C receptor; (D) tissue factor; (E) tissue factor pathway inhibitor; (F) thrombin/antithrombin complex; (G) soluble fibrin monomer; (H) tissue plasminogen activator; (I) plasminogen activator inhibitor. 0 = Healthy control (n = 20); 1 = severe isolated TBI (n = 120). EPCR = endothelial protein C receptor; PAI = plasminogen activator inhibitor; PC = protein C; PS = protein S; SFM = soluble fibrin monomer; TAT = thrombin/antithrombin complex; TBI = traumatic brain injury; TF = tissue factor; TFPI = tissue factor pathway inhibitor; TPA = tissue plasminogen activator.

Discussion

We embarked on this study, with the hypothesis that acute coagulopathy of isolated severe TBI is not due to an exclusive dominance of a single protein, instead it is an intricate amalgamation of disorders of multiple distinct pathway specific factors that converge into the TIC phenotype, which we examined to characterize the underlying physiologic mechanisms and ideal treatment of this condition.

TIC was found to occur in 50 (41.6%) isolated severe TBI patients, which was higher than but consistent with the published retrospective and prospective data. Epstein et al., [10] based on their recent meta-analysis reported the pooled incidence of TIC among patients presenting with isolated TBI as 35.2% (95% CI 29.0—41.4), concurrent to the incidence reported by Harhangi et al. [4] in 2008 [32.7 (10—97.5)%].

In the present study, 40% of the patients that were brought to the ED within ≤1 hour of injury developed TIC which increased to 47% for patients who were brought within 3 hours of brain injury. Similarly, Greuters et al. [8] and Franschman et al. [11] previously noted and reported TIC in 25% of TBI patients upon ED arrival and >50% by 48 hours, which may proceed until the 3rd day or even longer. Further, presence of acidosis and hypothermia, which commonly succeed TBI, worsen coagulopathy [12], previously described as a ''bloody vicious cycle". In the present study, development of TIC was also found to be associated with acidosis (p = .01). However, hypothermia was not investigated for the purpose of this study and is therefore, not reported.

Table 2 Correlation of markers of coagulation activation, anticoagulation, thrombin generation, and fibrinolysis in isolated severe TBI patients with and without TIC.

Pathway	Markers	Healthy volunteer	Trauma induced coagulopathy (n = 50)	No-trauma induced coagulopathy (n = 70)	p	*Post hoc* analysis
Coagulation activation	Tissue factor (ng/mL)	3.9 (3.0—4.7)	1.6 (0.8—3.4)	1.9 (1.1—4.3)	**0.0002**	**p = .002[a]; p < .0001[b]; p = .09[c]**
Coagulation inhibition	Tissue factor pathway inhibitor (ng/mL)	108.2 (97.8—114.5)	56.6 (33.3—118.0)	97.1 (34.6—133.4)	**0.02**	*p = .33[a]; **p = .002[b]**; p = .09[c]*
	Protein C (%)	85.5 (76—109)	49.0 (35.5—102.5)	91.0 (55.0—103.0)	**0.04**	*p = .63[a]; **p = .03[b]**; **p = .04[c]***
	Endothelial cell protein C receptor (ng/mL)	85.2 (63.0—103.3)	46.0 (24.3—73.6)	51.0 (13.5—81.0)	**0.002**	**p = .002[a]; p = .0006[b]**; p = .69[c]**
	Protein S (%)	91.5 (76—108.5)	58.5 (34.5—85.5)	89.0 (73.0—115.0)	**0.0001**	**p = .65[a]; p = .0003[b]; p < .0001[c]**
Thrombin generation	Thrombin/antithrombin complex (pg/mL)	12.7 (6.8—20.1)	41.3 (19.0—115.6)	37.1 (15.3—92.3)	**0.0001**	**p < .0001[a]; p < .0001[b]**; p = .30[c]**
	Soluble fibrin monomer (μg/mL)	0.45 (0—5.69)	64.4 (23.7—138.5)	26.7 (21.7—108.7)	**0.0001**	**p < .0001[a]; p < .0001[b]**; p = .12[c]**
Fibrinolysis	Tissue plasminogen activator (ng/mL)	25.6 (11.2—32.3)	133.5 (31.4—453.2)	119.7 (26.0—285.4)	**0.0001**	**p = .0001[a]; p < .0001[b]**; p = .21[c]**
	Plasminogen activator inhibitor (ng/mL)	101.9 (73.2—105.6)	87.5 (56.4—109.6)	98.0 (64.2—113.2)	0.29	*p = .96[a]; p = .22[b]; p = .15[c]*

Continuous variables are reported as median (interquartile range). Bold font depicts significant p-value.
TBI = traumatic brain injury; TIC = trauma induced coagulopathy.
[a] Healthy control vs. No-TIC.
[b] Healthy control vs. TIC
[c] No-TIC vs TI.

Tissue factor pathway activation

Localized activation of extrinsic coagulation pathway and secondary consumption coagulopathy in severe brain injury patients is either catalyzed by the exposure of blood to subendothelial collagen or TF released from the injured brain tissue into the circulation [13].

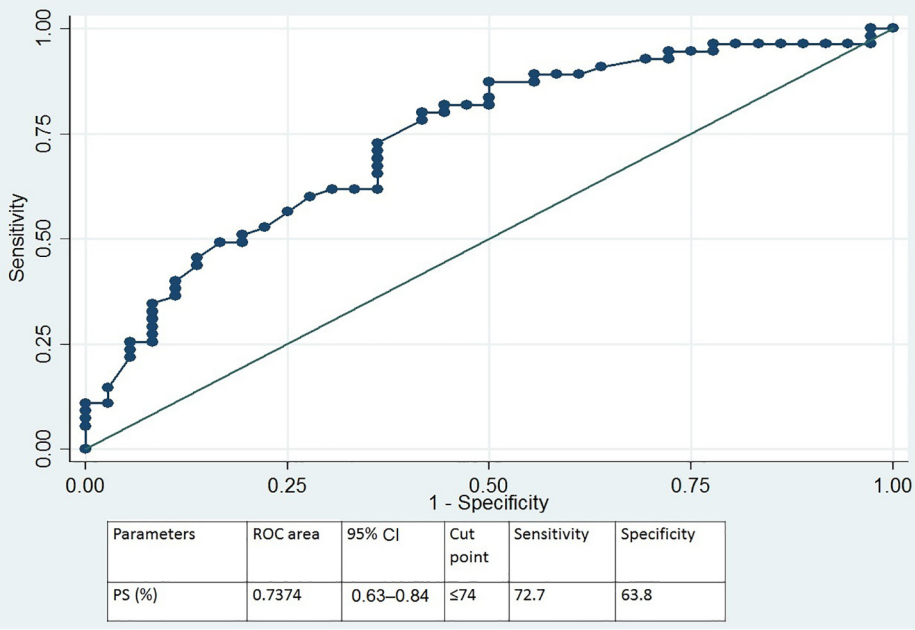
Gando et al. [14] in 1999 reported of markedly elevated plasma TF antigen levels on the day of injury in five patients with isolated TBI, which gradually declined over the subsequent 4 days, confirming TF as the key driver of severe hemostasis failure in TBI. They also found massive thrombin generation and activation as well the release of TF in the healthy controls. Sillesen et al. [15] made a similar observation in porcine model, they found insignificant increase in TF levels [142 (137—148) ng/mL vs. 149 (130—151) ng/mL, p = .71] in TBI/hemorrhage patients compared with healthy control participants.

Contrarily, Allard et al. [16] reported an equally low concentration of soluble TF following TBI. However, they found no statistically significant difference in TF levels between coagulopathic and noncoagulopathic patients. Variation in TF levels was also not associated with poor outcome (OR 1.0; 95% CI 0.89—1.20, p = .65). They concluded that TF may not play a significant role in TBI induced coagulopathy, However, they did not refute the possibility that TF expressed on vascular endothelium, or in brain tissue, or on mononuclear cells may contribute to coagulopathy [17].

TF partakes in the activation of Factor VII (FVII). Wu et al. [18] demonstrated significantly lower activity of plasma FVII among coagulopathic patients compared with noncoagulopathic TBI patients. FVII activity was reported to be an independent risk for coagulopathy following isolated TBI. Two other studies [19,20] demonstrated decreased FVII activity in TIC patients. However, these studies did not focus on isolated TBI patients.

In the present study, sample collection was performed before fluid and/or blood product transfusion and we observed a significant decline in plasma TF levels of isolated severe TBI patients compared with control [1.7 (1.02—3.6) ng/mL vs. 3.9 (3.0—4.7) ng/mL, p = .0002]. Furthermore, plasma TF levels were also observed to be depleted in coagulopathic patients compared with noncoagulopathic patients [1.6 (0.8—3.4) ng/mL vs. 1.9 (1.1—4.3) ng/mL, p = .09). FVII activity was not examined in the present study.

Manoel et al. [21] studied coagulation reaction in patients with isolated severe TBI (n = 48), severe TBI associated with multisystem trauma (n = 137) and multisystem trauma without head injury (n = 160). TF did not vary between isolated TBI and multisystem trauma without head injury patients (0.13 ± 0.04 pg/mL vs. 0.12 ± 0.34 pg/mL) in the entire cohort, whereas TFPI was significantly higher in isolated TBI (90.1 ± 18.6 ng/mL) compared with multisystem trauma with (78.7 ± 19.4 ng/ml) /without head injury (78.4 ± 26 ng/ml) (p < .05). However, in the coagulopathic subgroup, TF and TFPI levels did vary based on site of injury. Notably, severe depletion in TFPI levels were seen in isolated TBI patients compared with control. Furthermore, interestingly TFPI levels in noncoagulopathic isolated TBI



Parameters	ROC area	95% CI	Cut point	Sensitivity	Specificity
PS (%)	0.7374	0.63–0.84	≤74	72.7	63.8

Fig. 2 ROC curve analysis to establish a cut-off of PS levels to clinically identify TBI induced coagulopathy. CI = confidence interval; PS = protein S; ROC = receiver operating characteristic curve; TBI = traumatic brain injury.

Table 3 Effect of TIC development on the clinical course of isolated severe TBI patients.

Clinical parameters		Trauma induced coagulopathy (n = 50)	No-trauma induced coagulopathy (n = 70)	p
Sepsis	No	40 (80.0)	61 (87.1)	.31
	Yes	10 (20.0)	9 (12.9)	
Transfusion requirements	**PRBC (n = 59)**	**2 (0—6)**	**0 (0—3)**	**.002**
	PRP (n = 25)	**0 (0—4)**	**0 (0—0)**	**.001**
	FFP (n = 34)	**0 (0—4)**	**0 (0—0)**	**.007**
Renal failure	No	47 (94.0)	65 (92.8)	.99
	Yes	3 (6.0)	5 (7.2)	
Hepatic failure	No	45 (90.0)	66 (94.2)	.48
	Yes	5 (10.0)	4 (5.8)	

Categorical variables are reported in terms of frequency (percentage). Bold font depicts significant p-value.
FFP = fresh frozen plasma; PRBC = packed red blood cells; PRP = platelet rich plasma; TBI = traumatic brain injury; TIC = trauma induced coagulopathy.

patients did not vary from the TFPI levels in control, whereas in coagulopathic patients TFPI levels were significantly lower than control (p = .02) and noncoagulopathic isolated TBI patients.

Circulating intravascular rather than tissue expressed extravascular TF may play a role in acute trauma induced coagulation.

The activation of anticoagulant protein C pathway

Protein C activation has been hypothesized as a key driver of TIC. Circulating PC is activated by binding to EPCR in the presence of the thrombin-TM complex and PS. Results published by Brohi et al. [22,23] found evidence to implicate decreased levels of PC subsequent to systemic hypoperfusion and TM activity with prolonged coagulation assay levels and fibrinolytic activity in severely injured trauma. In a study of 203 trauma patients, Cohen et al. [24] observed a strong activation of PC which was only present with concomitant hypoperfusion. Activated PC levels were elevated and PC levels depleted in severely injured patients with shock (p < .0001). Similarly, in 39 isolated TBI patients, Cohen et al. [25] found no differences in PC levels (79.4 ± 24.2 vs. 85 ± 36, p = .52), however, in combination with tissue hypoperfusion, a significant decrease in the level of inactivated PC (56.2 ± 32 vs. 85 ± 35, p = .03) was observed.

We examined levels of PC its receptor EPCR and cofactor PS in 120 isolated severe TBI patients and observed no change in comparison to healthy participants (n = 20) [PC: 85 (43—103) % vs. 85.5 (76—109) %, p = .20, PS: 81 (54—102) % vs. 91.5 (76—108.5) %, p = .05, respectively]. Interestingly EPCR levels were significantly low in TBI patients

Table 4 Assessment of clinical and laboratory parameters in iSTBI patients with mortality: results of logistic regression and cox regression analysis.

Variables			Nonsurvivors (n = 36)	Survivors (n = 84)	p	Results of logistic regression outcome: death/survival		Results of Cox regression analysis outcome: time to death	
						Crude OR (95% CI)	Adjusted OR (95% CI)	Unadjusted hazard ratio (95% CI)	Adjusted hazard ratio (95% CI)
Trauma induced coagulopathy		Yes	22 (61.1)	28 (33.7)	.005	3.1 (1.4—7.1)	**4.73 (1.6—13.3)**	**2.2 (1.4—4.2)**	**2.8 (1.2—6.4)**
Hypertension		Yes	13 (36.2)	16 (19.1)	.04	2.4 (1.0—5.7)	—	2.1 (1.0—1.4)	—
GCS		GCS = 8	4 (11.1)	27 (32.1)	.002	1.6 (1.2—2.1)	**1.6 (1.2—2.2)**	1.4 (1.2—1.7)	**1.5 (1.2—1.7)**
		GCS = 7	7 (19.4)	31 (36.9)					
		GCS = 6	5 (13.8)	8 (9.6)					
		GCS = 5	5 (13.8)	8 (9.6)					
		GCS = 4	5 (13.8)	3 (3.5)					
		GCS = 3	10 (27.8)	7 (8.3)					
Transfusion requirement	PRBC	Yes	23 (63.9)	36 (42.9)	.03	2.3 (1.0—5.2)	—	1.5 (0.7—2.9)	—
	PRP	Yes	14 (38.9)	11 (13.1)	.001	4.2 (1.6—10.6)	**3.9 (1.1—14.4)**	2.0 (1.0—4.0)	—
	FFP	Yes	16 (44.4)	18 (21.4)	.01	2.9 (1.2—6.7)	—	1.6 (0.8—3.2)	—
Disseminated Intravascular Coagulation (DIC) The International Society for Thrombosis and Haemostasis (ISTH) guidelines		Nonovert ($\leq$5)	24 (66.7)	47 (55.9)	.03	2.5 (1.2—5.4)		1.9 (1.1—3.4)	—
		Overt (>5)	4 (11.1)	2 (2.4)					

Categorical variables are reported in terms of frequency (percentage). Bold font depicts significant p value.
CI = confidence interval; FFP = fresh frozen plasma; GCS = Glasgow Coma Scale; OR = odds ratio; PRBC = packed red blood cells; PRP = platelet rich plasma.

compared with control [47.8 (23.3—76.4) ng/mL vs. 85.2 (63.0—103.4) ng/mL, $p = .0005$]. Floccard et al. [26] reported an association of low PC activities with prolongation of aPTT ($p < .0001$) and PT ($p < .0001$), and low fibrinogen ($p < .0001$), Factor V ($p < .0001$). Similarly, in the present study we observed a significantly steep fall in PC levels in TIC patients compared with no-TIC patients [49.0 (35.5—102.5) % vs. 91.0 (55.0—103.0) %, $p = .04$], similar decline was also seen in plasma PS levels in TIC patients [58.5 (34.5—85.5) % vs. 89.0 (73.0—115.0) %, $p = .002$]. Interestingly EPCR levels did not significantly vary between TIC and no-TIC patients ($p = .69$). ROC analysis in the present study depicted an AUC of 0.74 [95% CI 0.63—0.84] with a cut-off of ≥ 74 of PS (specificity 63.8%, sensitivity 72.7%) for identifying patients with TIC. Plasma levels of <100IU/dL of PC, PS, and antithrombin have been reported as a risk of venous thromboembolism (VTE) [27].

In pregnant females, PS activity of 21% (10 percentile) yielded (OR 5.9; 95% CI 1.7—18.1) an independent severe pregnancy-induced hypertension (PIH) and severe PIH, whereas free PS level of 23% (5th percentile) (OR 4.4; 95% CI 1.0—14.3) was an independent risk factor for preeclampsia [28].

Brohi et al. [23] reported the activation of PC during hypoperfusion by increased formation of the thrombin—TM complex.

In the present study elevated thrombin generation was observed after brain injury [TAT: 41.3 (17.4—100.2) pg/mL vs. 12.7 (6.8—20.1) pg/mL, $p < .0001$, sFM: 38.4 (23.1—123.8) μg/mL vs. 0.45 (0—5.7) μg/mL, $p < .0001$]. Elevated thrombin generation was also observed in TIC patients, as indicated by increased TAT levels and a three-fold increase in sFM levels compared to no-TIC.

Concurrently, Cohen et al. [25] demonstrated a direct correlation of injury severity with resulted thrombin formation following isolated TBI. However, despite this correlation, there was no increase in PT and PTT with greater AIS (Head) (PT 13.9 ± 0.9 sec vs. 14.3 ± 2.4 sec, $p = .45$; aPTT 26.7 ± 2.48 sec vs. 27.4 ± 3.88 sec, $p = .41$).

Scherer et al. [29] reported elevated sFM, TAT, and the prothrombin fragment F1 + 2 (PF1 + 2) levels to be significantly higher in cerebrovenous blood than in central venous blood ($p < .025$) within 6 hours of isolated TBI, suggestive of an antithrombin mediated early systemic procoagulant overflow from the injured brain microvasculature in isolated TBI patients.

Hyperfibrinolysis (HF)

Recently, the role of the fibrinolytic shutdown has been suggested as another mechanism that makes patients with TBI potentially prone to a hypercoagulable state [30]. We report five-fold higher tPA levels subsequent to head injury (125.9 [29.2—317.8] ng/mL vs. 25 [11.2—32.4] ng/mL, $p < .0001$) and a slight decline in PAI-1 levels compared with control (ng/mL, $p < .0001$).

Activated PC in excess consumes PAI-1 and Brohi et al. [22] identified a dose-dependent reduction in PAI-1 as PC levels reduced, leading to increased fibrinolysis with a rise in tPA levels ($p = .001$) and an associated increase in D-dimers.

Nakae et al. [31] observed on admission abnormal levels of D-dimer (21.6 [8.2—44.8] lg/ml). Higher frequency of abnormal D-dimer levels was observed in patients with TIC compared with no-TIC in the present study (55.4% vs. 29.5%). 22.7% patients had D-dimer levels ranging from 4 to 8 μg/mL in the TIC group compared to 6% in no-TIC group. Nakae et al. [31] also detected the onset of HF in patients with small hemorrhagic foci instead of lesions with the mass effect.

In isolated severe TBI, which is not usually complicated by hypotension, hyperfibrinogenolysis may occur without shock [32—36]. Previously Duvekot et al. [37] refuted this theory and reported an association of tissue perfusion with low plasminogen and PAI-1 levels, and elevated tPA levels.

Stein and Smith in 2004 [38] demonstrated abnormal peak in fibrin degrading product and D-dimer levels within minutes of TBI. In a similar review article, Kuo et al. [39] also found elevated D-dimer levels within 4 hours after TBI. Elevated D-dimer accompanied by increased plasmin activity secondary to enhanced fibrinolytic activity promotes fibrin degradation [36,38,40].

Recently, Chapman et al. [41] reported massive tPA release, and not PAI-1 degradation as the cause of HF in severely injured patients. Total PAI-1 levels did not vary between injured patients and control, providing further evidence that HF was driven by increased tPA and not the PAI-1 loss [41,42].

Battista et al. [43] reported significantly elevated tPA on admission [17.2 (2.3—47.6) ng/ml vs. 1.6 (0.3—5.9) ng/ml, $p < .05$] compared with control and declined subsequently [24 hours, 11.0 (2.0—45.0), $p < .05$]. Unlike the present study, they observed a continuous rise in PAI-1 levels in TBI patients from on admission to 24 hours of injury, compared with control [PAI-1, 43.9 (9.8—208.1) ng/ml and 47.4 (16.9—155.3) ng/ml vs. 17.2 (5.1—26.9) ng/ml, $p < .05$; D-dimer 4911.2 (122.5—13456.2) ng/ml and 1656.9 (144.2—9962.7) ng/ml vs. 115.2 (21.3—251.3) ng/ml, $p < .05$, respectively].

Scherer et al. [29] reported a significant elevation of antithrombin III complex ($p < .025$) and D-dimer ($p < .005$) as well of PF1 + 2 in cerebrovenous blood ($p < .025$) in isolated severe TBI patients compared to control. Furthermore, a recent study reported that endogenous plasminogen activators mediated increase in fibrinolysis after isolated TBI, causes progressive intracerebral hemorrhage [36]. Dekker et al. [44] observed significant association of TBI coagulopathy with low fibrinogen [1.8 (0.8) g/L vs. 2.9 (0.5) g/L, $p < .001$] and high D-dimer levels [17.6 (9.0—67.1) mg/L vs. 6.1 (2.5—11.4) mg/L, $p = .027$].

Effect of TBI induced coagulopathy on clinical outcome

The mortality rate for the study population was 30% (36); 13.3% (16) patients died within 48 hours of injury.

Two-fold higher mortality was observed in TIC compared with no-TIC [22 (44) vs. 14 (20), $p = .005$] TBI patients. The incidence of sepsis was higher in TIC patients compared with no-TIC groups (20% vs. 12.9%, $p = .31$), similarly frequency of hepatic failure was high in TIC patients (10.0% vs. 5.8%, $p = .48$) however, this trend did not attain significance. Development of early coagulopathy also resulted in ele-

vated transfusion requirements (PRBC, $p = .002$; PRP, $p = .001$; FFP, $p = .0007$).

Logistic regression analysis revealed that TIC development in severe isolated TBI was an independent predictor with an increased likelihood of in-hospital mortality (adjusted OR = 4.73; 95% CI 1.68—13.3). Furthermore, adjusted Cox regression analysis revealed that TIC is an independent risk factor for in-hospital mortality was (adjusted) HR 2.8 (95% CI 1.2—6.4).

Brohi et al. [22] report a significant association of low PC ($p = .001$) and high TM levels ($p = .02$) with increased mortality. The incidence of acute renal injury ($p = .002$) and acute lung injury ($p = .09$) was increased with activation of the TM-PC pathway. The results of the present study did not indicate of such a correlation.

Limitation

Serial measurements over the first 48—72 hours of hospital stay were not done in the study, which could have better demonstrated the progression of acute sustained coagulopathy and delayed coagulopathy. Follow-up CT was not done in 6 hours to evaluate for development and/or progression of progressive hemorrhagic injury.

Conclusion

Early coagulopathy was seen to 41% patients with Traumatic Brain Injury as early as ~3 hours of injury, without influence of fluid or blood product administration or clinical evidence of shock, hypoperfusion, hypotension and despite the absence of extracranial injury. This study demostrates that TBI is an independent risk factor for the development of early coagulopathy. This study also disproves TF as the key mediator of TIC in severe isolated TBI patients and suggest a substantial role of PC anticoagulant pathway. TIC was found to be an independent predictor of poor outcome, with a five times high odds for mortality. Lastly, we recommend that the cut-off generated in the present study ($\geq$ 74 of PS; specificity 63.8%, sensitivity 72.7%) for the early prognostication and identification of TIC following brain injury, may be used by the clinician for diagnosis and management of the patients.

Conflicts of interest

The authors have no conflicts of interest to declare.

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