



## Activation of coagulation and endothelium with concurrent impairment of anticoagulant mechanisms in patients with typhoid fever



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### SUMMARY

**Objectives:** Typhoid fever caused by *Salmonella* Typhi remains a major burden worldwide. Gastrointestinal bleeding can be seen in up to 10 percent of patients and may be fatal. The coagulopathy, which may be the driver of this severe complication in patients with typhoid fever, however is ill defined. The aim of this study was to evaluate the activation of coagulation, anticoagulation, and fibrinolysis in patients with acute typhoid fever.

**Methods:** Parameters of coagulation and fibrinolysis were measured in 28 hospitalized patients with culture-confirmed or PCR-confirmed typhoid fever and compared to 38 age- and sex-matched healthy volunteers.

**Results:** Patients demonstrated activation of the coagulation system, as reflected by elevated in vitro thrombin generation and high plasma levels of fibrinogen, D-dimer and prothrombin fragment F1 + 2 in concert with consumption of coagulation factors resulting in a prolonged prothrombin-time and activated-partial-thromboplastin-time. Concurrently, the anticoagulant proteins, protein C and antithrombin, were significantly lower in comparison to healthy controls. Patients also demonstrated evidence of activation and inhibition of fibrinolysis and a marked activation of endothelial cells. The extent of coagulation activation was associated with the course of the disease, repeated testing during convalescence showed a return toward normal values.

**Conclusions:** Activation of coagulation is an important clinical feature of typhoid fever and is associated with severity of disease.

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## Introduction

Typhoid fever is a systemic disease caused by the Gram-negative bacteria *Salmonella (S.) enterica* serovar Typhi that constitutes a global problem with an estimated 21 million cases annually resulting in over 220,000 deaths.<sup>1–3</sup> Patients with typhoid fever usually present with protracted high grade fever, and characteristically a normal to low white blood cell and platelet count.<sup>4</sup> Gastrointestinal bleeding has been previously described as the most common complication in typhoid fever, occurring in up to 10 percent of patients.<sup>5</sup> In the majority of cases, the bleeding is minimal and resolves without the need for intervention.<sup>6</sup> In a small percentage of cases however bleeding is significant and can become fatal,<sup>7</sup> however more recent data is lacking on the actual incidence on intestinal hemorrhage and the case fatality rate.

Our understanding of the underlying mechanisms responsible for gastrointestinal bleeding and the role of the coagulation system and fibrinolysis in the pathogenesis of typhoid fever is limited. The extent of disseminated intravascular coagulation (DIC), which is characterized by widespread activation of coagulation resulting in intravascular formation of fibrin,<sup>8</sup> has been proposed as a marker of disease severity in typhoid fever.<sup>9,10</sup> Conversely, others have reported that DIC is mostly a subclinical event and found bleeding complications in typhoid fever to be uncommon. Notably, in terms of blood coagulation, it has been suggested that circulating bacteria and endotoxin do not play an important role in typhoid fever pathogenesis.<sup>11</sup> Interestingly, preclinical work has recently identified a new virulence factor of *S. Typhi* - called Typhi chimaeric A<sub>2</sub>B<sub>5</sub> typhoid toxin or exotoxin - which is thought to contribute to the acute symptomatology in patients with typhoid fever by activation of, among other factors, vascular endothelial cells.<sup>12</sup>

The aim of this study was to obtain new insights into the pathogenesis of typhoid fever by defining the involvement of the coagulation, endothelium and fibrinolytic systems in patients hospitalized with this infection. In addition to the endothelium, we focused on the three major pathways – procoagulant, anticoagulant, and fibrinolytic – that together determine the overall balance of clot formation and resolution. Furthermore, we investigated whether the extent of coagulation activation correlated with a positive blood culture and the presence of disease complications.

## Materials and methods

### Ethical statement

The study protocol was approved by the National Research Ethics Committee (NREC) of Bangladesh (BMRC/NREC/2010-2013/1543) and the Oxford Tropical Research Ethics committee (OXTREC-reference 25-11).

### Patients and study design

In order to include patients hospitalized with typhoid fever, we performed a prospective all-cause fever study from January 2012 through June 2012 in Chittagong Medical College Hospital in Chittagong, Bangladesh as previously described.<sup>13–15</sup> Consecutive patients ( $\geq 16$  years of age) who were admitted with an acute febrile illness (documented fever  $\geq 38^\circ\text{C}$  axillary, up to 48 hours after admission, history of fever less than 2 weeks) were recruited after giving informed written consent. History and physical examination findings were recorded; baseline complete blood counts, renal and liver functions tests were analyzed. Blood serum, urine, and feces samples were obtained at admission and day 10 post fever onset or at hospital discharge and stored at  $-20^\circ\text{C}$ . Plasma was prepared from blood collected in EDTA and citrate tubes (BD vacutainer®) and stored immediately at  $-70^\circ\text{C}$  pending analysis. Blood cultures

at admission were performed by inoculating 8–10 mL of blood into BacT/ALERT FA blood culture bottles and incubated in the BacT/ALERT automated system (Biomérieux) for 5 days. Positive bottles were processed by standard microbiological methods. If the Gram-stain showed Gram-negative bacilli, blood culture fluid was tested for *S. Typhi* and was identified using API biochemical testing (Biomérieux) and specific antisera (BioRad). Bacterial isolates were stored on agar slopes and couriered to Oxford University Clinical Research Unit (OUCRU) in Ho Chi Minh City, Vietnam for confirmation of identification. Real-time PCR was performed on stored blood, urine and feces samples targeting *STY0201* (Putative fimbrial adhesion in *S. Typhi* CT18) or *SSPA2308* (hypothetical protein in SPA AKU-12601) as described.<sup>16</sup>

A diagnosis of typhoid fever was confirmed if *S. Typhi* was isolated from the blood culture and/or the real-time PCR indicated the presence of *S. Typhi* targets in blood, urine or feces. Complicated typhoid fever was defined if one or more of the following were present: gastrointestinal bleeding (the presence of visible blood in the stool); intestinal perforation; encephalopathy (delirium, obtundation or coma); hemodynamic shock; renal impairment (creatinine level  $> 2.0\text{ mg/dL}$ ); hepatitis (as indicated by AST or ALT level  $\geq 1000\text{ U/L}$ ); pneumonia (respiratory symptoms with abnormal chest X-ray shadowing) or pleural effusion; the presence of a focal septic complication; severe anemia (hematocrit  $\leq 20\%$ ), the need for a blood transfusion and death in hospital.<sup>5,7</sup> Thrombocytopenia was defined as a platelet count of  $< 100 \times 10^9/\text{L}$ .

38 Bangladeshi volunteers who were known to have no illness that could affect hemostasis and were not currently receiving any medication served as healthy controls.

### Assays

Stored EDTA and citrated plasma were stored at  $-80^\circ\text{C}$  except when transferred on dry ice to the Academic Medical Center, Amsterdam for the coagulation related assays. Prothrombin time (PT), activated partial thromboplastin time (APTT), antithrombin (AT) and D-dimer were assayed on an automated blood coagulation analyzer (BCS® XP, Siemens). Fibrinogen levels were derived from the change in optical signal in the PT. Prothrombin fragment F1 + 2 (F1 + 2) (Enzygnost®, Siemens), plasmin-alpha2-antiplasmin complexes (PAPc; DRG Diagnostics), soluble form of the urokinase-type plasminogen activator receptor (suPAR; suPARgnostics®, Virogates), endothelial cell specific molecule (ESM)–1/Endocan (Lunginnov), plasminogen activator inhibitor (PAI)–1 and tissue-type plasminogen activator (tPA) (Biomed) were determined by enzyme-linked immunosorbent assays (ELISA).<sup>17,18</sup> Von Willebrand factor (VWF, percentages) and total protein S levels were determined with in-house ELISAs (using antibodies from Dako).<sup>19</sup> Free protein S was measured by precipitating the C4b-binding protein-bound fraction with polyethylene glycol 8000, then measuring the concentration of free protein S in the supernatant. Protein C was assayed by kinetic assay (Coamatic®, Chromogenix™). Of note, AT and tPA were only measured in the blood culture-positive sub-set of patients due to limited volumes of plasma collected on day of enrolment. In vitro thrombin generation with 5 pM tissue factor and associated measures (lag time, peak, time-to-peak, area-under-the-curve (or endogenous thrombin potential (ETP), and velocity index) were assayed with the Calibrated Automated Thrombogram® (Fluoroskan Ascent, ThermoLab systems) and Thrombinoscope® software (Thrombinoscope BV).<sup>18,20</sup> Resistance to activated protein C (APC) was determined by testing the effect of APC (Enzyme Research Laboratories) on the endogenous thrombin potential (ETP) with the CAT assay. APC-sensitivity of each plasma sample was determined in both the presence and absence of  $\sim 4\text{ nM}$  APC. APC concentrations used were adjusted to maintain a residual thrombin generation activity of  $\sim 10\%$  in normal pooled plasma. The

**Table 1**  
Baseline information for 28 patients with typhoid fever, comparing culture-positive and culture-negative groups.

Characteristic	Healthy controls (n = 38)	All (n = 28)	P value <sup>a</sup>	Patients with typhoid fever		
				Culture negative for S. Typhi (n = 17)	Culture positive for S. Typhi (n = 11)	P value <sup>b</sup>
Age, years	33.5 (19–55)	28 (20–45)	NS	31 (20–45)	25 (20–45)	NS
Male sex, no. (%) of patients	19 (50)	16 (57)	NS	10 (59)	6 (55)	NS
Fever duration before admission, days	0 (0–1)	7 (1–15)	<0.0001	5 (1–15)	7 (4–14)	NS
Duration of admission, days	0 (0–0)	5 (0–12)	<0.0001	4 (0–10)	5 (0–12)	NS
Temperature (Celsius, axillary)	36.1 (33.8–37.4)	38.8 (38–40.1)	<0.0001	38.9 (38–39.9)	38.6 (38.3–40.1)	NS
Systolic blood pressure, mmHg	120 (106–169)	116 (85–159)	.01	118 (90–140)	111 (85–195)	NS
Pulse, beats/min	72.5 (40–120)	104 (70–150)	<0.0001	96 (70–120)	110 (89–150)	.04
Blood urea nitrogen, mg/dL	6–20 <sup>c</sup>	12.5 (9.3–76)	–	12.8 (10–76)	12 (9.3–15)	NS
Serum creatinine level, mg/dL	0.1–0.5 <sup>c</sup>	0.9 (0.6–2.2)	–	0.9 (0.7–2.2)	0.9 (0.6–1.3)	NS
Aspartate aminotransferase level, U/L	<40 <sup>c</sup>	40 (13–1430)	–	31 (13–1430)	80 (37–450)	NS
Alanine aminotransferase level, U/L	<45 <sup>c</sup>	49 (15–1199)	–	33 (15–1199)	60 (24–220)	NS
Hemoglobin level, g/dl	13.3 (8.4–15.5)	12.1 (3.5–14.5)	.002	12 (3.5–14.4)	12.4 (7.5–14.5)	NS
WBC count, x10 <sup>9</sup> cells/L	7.5 (4.5–10)	9 (1.5–29)	NS	10.8 (1.5–29)	6.2 (4–14)	.03
Neutrophil percentage	57 (42.9–76)	76.5 (3–92.1)	.0001	72 (3–92.1)	78 (62–90)	NS
Monocyte percentage	3 (2–9.3)	2 (0–7)	NS	2 (0–7)	2 (1–4)	NS
Lymphocyte percentage	35.5 (20–50)	19 (1–36)	.0001	1 (1–36)	20 (8–35)	NS
Death, no. (%) of patients	0 (0)	1 (3.7)	–	1 (6.3)	0 (0)	NS

All values are reported as median value mean (range) unless stated otherwise. NS: not significant; WBC: white blood cell count.

<sup>a</sup> comparison between healthy controls and typhoid fever patients.

<sup>b</sup> comparison between culture negative/PCR+ and culture positive patients.

<sup>c</sup> Normal range, not measured in the control group.

normalized ratio (APCsr) was determined by dividing the APCsr of an individual by the APCsr of the pooled plasma (a ratio > 1.6 reflects an APC resistant phenotype). DIC scores were calculated using the International Society on Thrombosis and Hemostasis (ISTH) standardized method.<sup>21</sup>

### Statistical analysis

A primary analysis was performed in which results for control subjects and patients with typhoid fever were compared. Subgroup analysis was performed to compare results from patients with blood culture-positive for *S. Typhi* species with those from culture-negative but PCR positive patients and to compare results from patients with complicated disease with those from patients with uncomplicated disease.

Data on patient's characteristics and laboratory results were compared between different groups using student's t-test or Mann-Whitney U for continuous variables, and the  $\chi^2$  test or Fisher's exact test for categorical variables as appropriate. Correlations were calculated using the Spearman rho test. All analyses were performed using Graphpad prism, version 6 for Mac (Graphpad Software).

## Results

### Patients

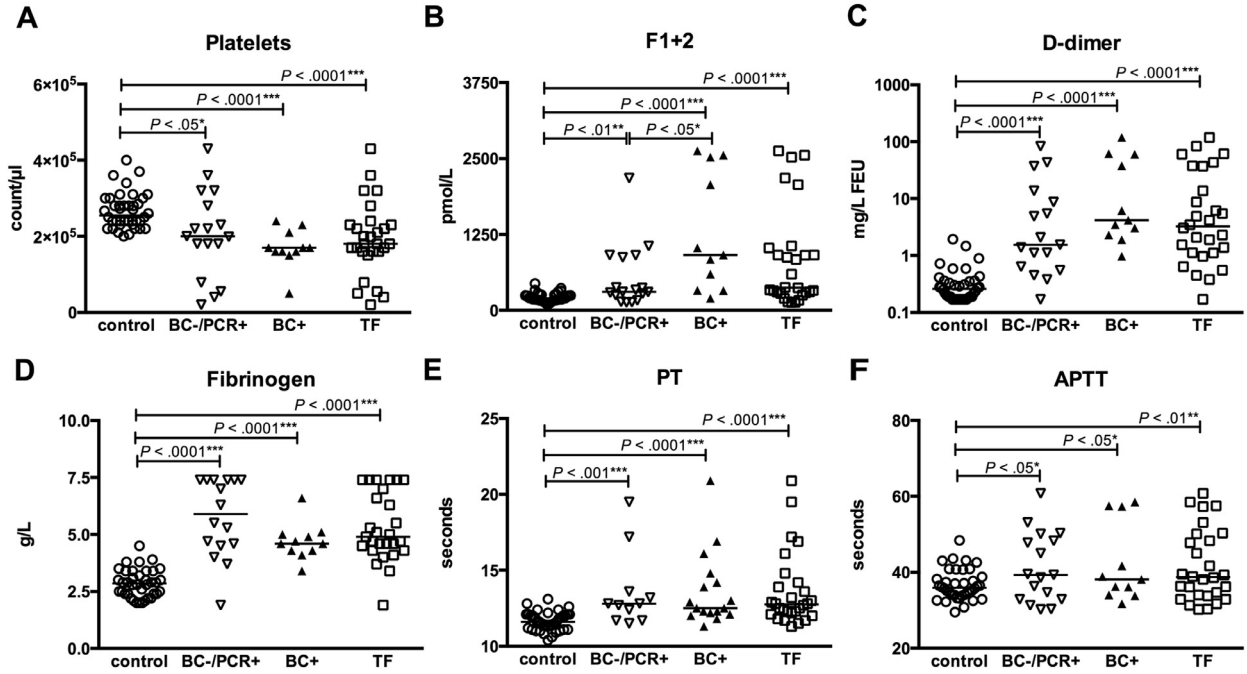
A total of 144 consecutive patients with fever admitted to the hospital were enrolled in this study, of which 28 had confirmed typhoid fever (19.4%). Of these confirmed typhoid fever cases, 11 (39%) were diagnosed by isolation of *S. Typhi* from blood, and 17 (61%) by positive *S. Typhi* PCR in blood (15), urine (2) and/or feces (1). Thirty-eight healthy local volunteers served as controls. Baseline clinical features are shown in Table 1. Patients with typhoid fever had significantly lower median platelet counts than the control group ( $P < .0001$ ; Fig. 1A). There was one death (3.7%) due to septic shock in the group of patients who were *S. Typhi* culture-negative but *S. Typhi* blood PCR-positive.

### Activation of coagulation in typhoid fever

In patients with typhoid fever, coagulation was significantly enhanced as reflected by elevated plasma concentrations of F1+2 and D-dimer when compared to healthy controls ( $P < .0001$  for both comparisons; Fig. 1B and C). Fibrinogen levels were strongly elevated in patients versus controls ( $P < .0001$ ; Fig. 1D). In addition, both PT and APTT were prolonged in patients compared to controls, suggesting an increased consumption of clotting factors ( $P < .01$ – $0.0001$ ; Fig. 1E and F). The observed differences in F1+2, D-dimer, fibrinogen, PT and APTT were preserved in subgroup analyses, in which culture-negative but PCR-positive and culture-positive groups were compared separately against the controls (Fig. 1). To further investigate the functionality of the detected coagulation activation, we analyzed the coagulation potential of plasma induced by tissue factor by calibrated automated thrombinography (CAT, Fig. 2A).<sup>18,20</sup> In typhoid fever patients, the lag-time was significantly prolonged in comparison to the controls, which is in line with the observed prolonged PTs ( $P < .0001$ ; Fig. 2B). The peak thrombin (Fig. 2C) and velocity index (data not shown) had higher median levels in patients which could be a direct result of affected anticoagulant pathways, since antithrombin is an important determinant of CAT.<sup>20</sup> The area-under-the-curve (or ETP) did not differ between typhoid and controls suggesting that despite delayed coagulation initiation, the amount of thrombin and thus clots that can be formed during typhoid fever is similar between groups (Fig. 2D). In the subgroup analyses, culture-negative and PCR-positive patients had higher peak thrombin levels and higher median ETP compared to controls, implying that this subgroup have a greater ability to generate thrombin ( $P < .05$ ; Fig. 2).

### Activation of the vascular endothelium

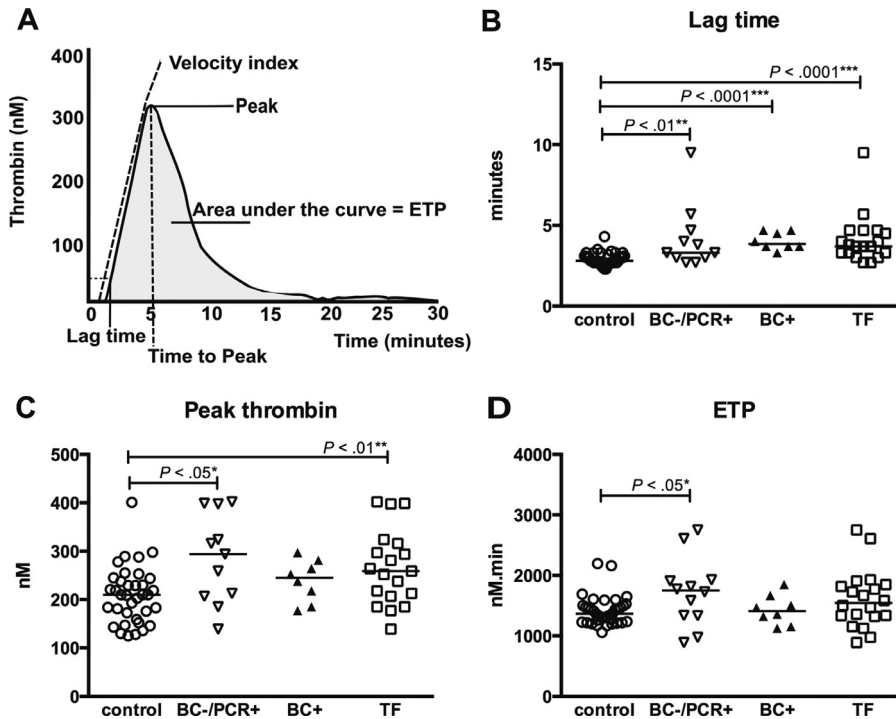
During acute inflammation, the endothelium becomes activated, which contributes to the attraction of leukocytes and formation of a thrombus.<sup>22,23</sup> Recent preclinical work has shown that *S. Typhi* exotoxin interacts with podocalyxin-like protein-1 which localizes to the apical side of epithelial cells and is expressed in vascular endothelial cells.<sup>12</sup> We found strong endothelium



**Fig. 1.** Activation of coagulation.

Coagulation factors (Platelets, A; Prothrombin fragment 1 + 2, B; D-dimer, C; Fibrinogen, D; Prothrombin time, E; Activated partial thromboplastin time, F) in patients with typhoid fever (empty squares) who were culture positive (black triangles) or culture negative (empty triangles) for *S. Typhi* and in healthy Bangladeshi control subjects (empty circles). Data are expressed as dot plots with medians.

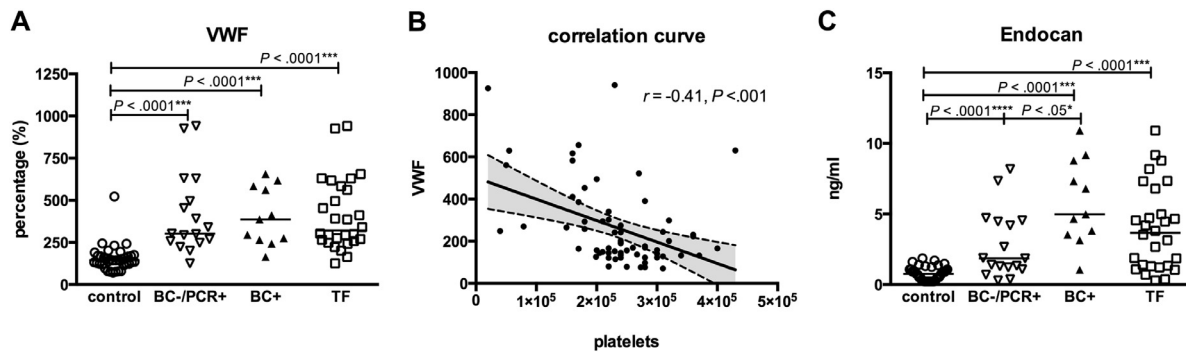
F1 + 2: prothrombin fragment 1 + 2; PT: prothrombin time; APTT: activated partial thromboplastin time.



**Fig. 2.** In vitro thrombin generation.

In vitro thrombin generation (thrombin generation assay, A; Lag time, B; Peak thrombin, C; Endogenous thrombin potential, D) assessed in typhoid fever patients (empty squares) who were culture positive (black triangles) or culture negative (empty triangles) for *S. Typhi* and of healthy Bangladeshi control subjects (empty circles). Data are expressed as dot plots with medians.

ETP: endogenous thrombin potential.



**Fig. 3.** Activation of vascular endothelium.

Activation of the endothelium (von Willebrand factor, A; Endothelial cell specific molecule-1, C) in patients with typhoid fever (empty squares) who were culture positive (black triangles) or culture negative (empty triangles) for *S. Typhi* and in healthy Bangladeshi control subjects (empty circles). Data are expressed as dot plots with medians. Correlation curve platelet count versus von Willebrand factor (B).  $P$ -value and Spearman Rho, linear regression analysis.

VWF: von Willebrand factor; ESM-1/Endocan: endothelial cell specific molecule-1.

activation in typhoid fever patients when compared to controls as reflected by increased levels of VWF, a large multimer secreted by endothelial cells following stimulation by among others endotoxin, cytokines or thrombin ( $P < .0001$ ; Fig. 3A).<sup>18,24</sup> VWF plays a key role in platelet adhesion and aggregation and observed low platelet counts in typhoid fever patients strongly correlated with VWF concentrations ( $r = -0.41, P < .001$ ; Fig. 3B). Further evidence of activation of vascular endothelium was provided by measurements of endocan, a newly described soluble proteoglycan expressed by endothelial cells and which expression relates with severity of illness in patients with sepsis.<sup>25</sup> Endocan was elevated in patients when compared to controls; subgroup analyses showed that endocan levels in the culture-positive group were higher than in the culture-negative PCR-positive patients ( $P < .0001$ ; Fig. 3C).

#### Downregulation of anticoagulant pathways

Coagulation activation in patients with typhoid fever was paralleled with a depression of anticoagulation pathways: AT and protein C concentrations were decreased and APC-sensitivity ratios were increased in patients compared to healthy controls ( $P < .05, P < .0001$  and  $P < .01$  respectively; Fig. 4A–C). Total (and free, data not shown) protein S levels were not different in patients compared to controls (Fig. 4D).

#### Activation and inhibition of fibrinolysis

Patients with typhoid fever demonstrated evidence of activation and inhibition of fibrinolysis, as reflected by elevated plasma levels of tPA and PAI-1 ( $P < .05$ , and  $P < .01$  respectively; Fig. 5A and B). In addition, patients showed strongly increased PAPc when compared to healthy subjects ( $P < .001$ ; Fig. 5C). Levels of suPAR, an important soluble mediator of fibrinolysis that is proposed as a potential prognostic biomarker for sepsis,<sup>26,27</sup> were significantly higher in patients compared to controls ( $P < .0001$ ; Fig. 5D). To assess coagulation–fibrinolysis (im)balance we assessed DIC scores which were strongly increased in patients compared to controls, with 5 patients (18%) having a DIC score  $\geq 5$ . These differences were preserved in the subgroup analysis, in which culture-negative and culture-positives were compared separately with controls ( $P < .0001$ ; Fig. 5E).

#### Association between coagulation abnormalities and severity of disease

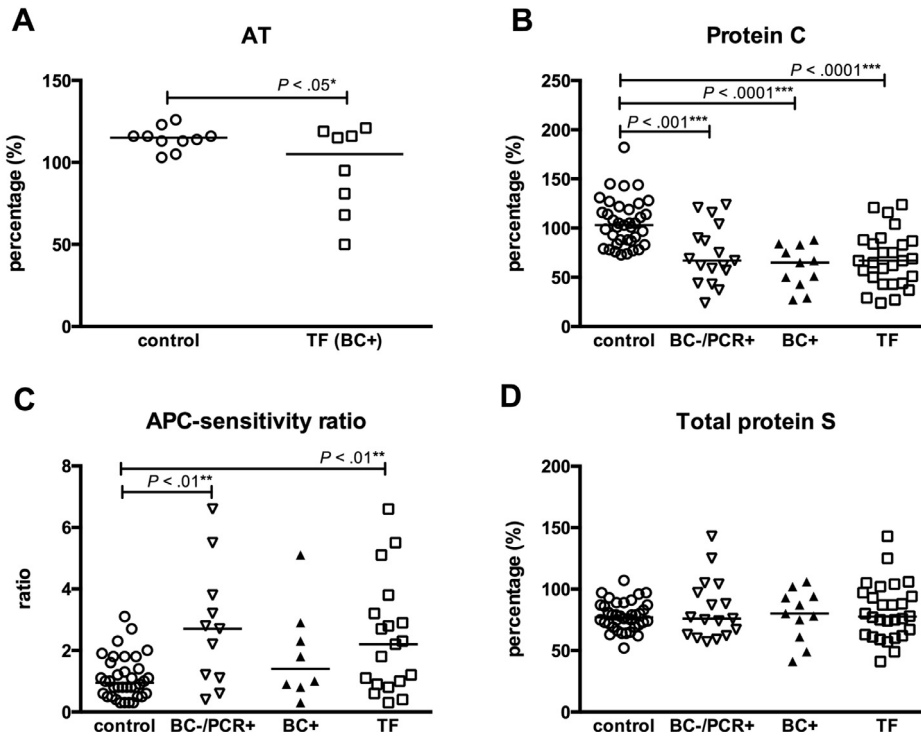
Having characterized the spectrum of coagulation activation in typhoid fever, we next sought to examine associations between the coagulation profile and clinical severity. Eleven (39%) patients were

classified as having complicated typhoid fever. Specific complications were: pneumonia (3); encephalopathy (2); gastrointestinal bleeding (1); severe anemia requiring blood transfusion (1); acute hepatitis (1); hepatic abscess (1); septic arthritis (1); and renal failure, cardiopulmonary arrest and death (1). Having a positive blood culture did not result in a higher risk of developing a complication in this study, only 2 out of 8 patients with a positive blood culture developed a complication. Fibrinogen levels were significantly elevated in patients with complicated typhoid fever when compared to patients with uncomplicated typhoid fever (median (IQR); 7.4 (5–7) vs 4.7 (4–5),  $P < .01$ ). Other measured abnormalities in coagulation, anti-coagulation and fibrinolysis or endothelium activation were not associated with the presence of complications (data not shown). The extent of coagulation activation correlated with the stage of the disease; most measured markers showed complete (APTT, endocan, protein C;  $P < .01$ – $0.05$ ; Fig. 6 (mean with 95% CI) or near complete normalization (F1 + 2, D-dimer, fibrinogen, PT, PAI-1 and suPAR; data not shown) in patients from whom follow-up samples were available at discharge. Plasma levels of VWF and PAPc were not yet normalized at time of discharge (data not shown). Furthermore, in patients the platelet count – but not white blood cell count – negatively correlated with almost all coagulation and fibrinolysis markers ( $P < .05$ ; except for fibrinogen,  $P = .06$ ) and positively correlated with the natural anticoagulant protein C ( $P < .0001$ ).

#### Discussion

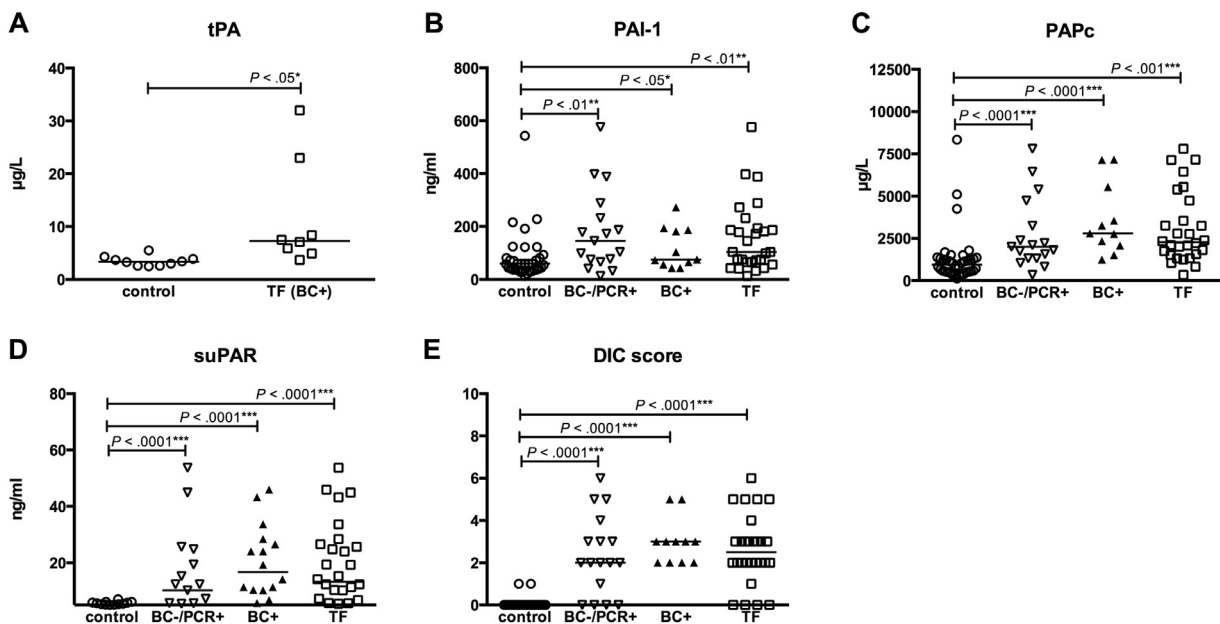
Different forms of bleeding have been described in typhoid fever, either self-limiting local hemorrhage in the gastrointestinal tract due to inflammation or generalized bleeding as a result of imbalance in the activation of coagulation.<sup>28</sup> In the present study, we demonstrate that the coagulation system is markedly activated in almost all patients involving all three major pathways: procoagulant, anticoagulant, and fibrinolytic. These results shed light on the potential mechanisms responsible for the different forms of bleeding observed in these patients.

Our data point to an overall prothrombotic state in typhoid fever. Patients demonstrated increased plasma prothrombin fragments as well as fibrin degradation products and lower protein C and AT concentrations than healthy controls. The ability to generate thrombin in vitro was partially delayed in patients confirming the prolonged PT, but patients were capable of generating higher amounts of thrombin, which is most likely the result of the affected anticoagulation pathways, as these are important determinants of in vitro thrombin generation.<sup>20</sup> Moreover, DIC scores, which provide insight into the coagulation–fibrinolysis



**Fig. 4.** Anticoagulation.

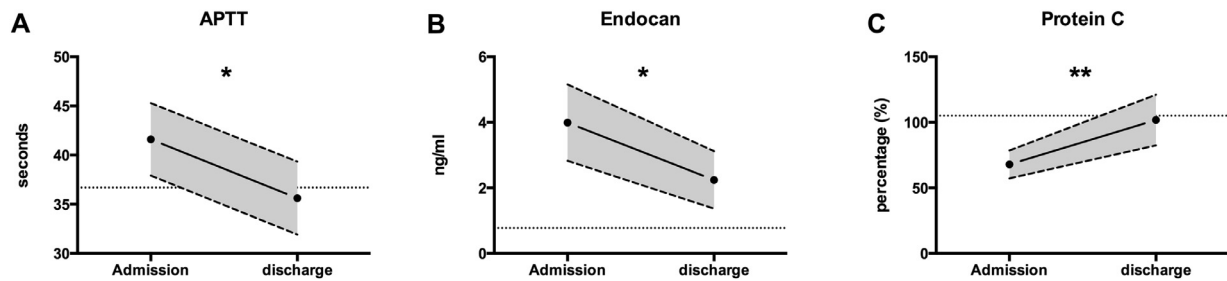
Anticoagulant factors (Antithrombin, A; Protein C, B; Activated protein C sensitivity ratio, C; Total protein S, D) in patients with typhoid fever (empty squares) who were culture positive (black triangles) or culture negative (empty triangles) for *S. Typhi* and in healthy Bangladeshi control subjects (empty circles). Data are expressed as dot plots with medians. AT: antithrombin; APC: activated protein C.



**Fig. 5.** Activation and inhibition of fibrinolysis.

Activation and inhibition (Tissue-type plasminogen activator, A; Plasminogen activator inhibitor type, B; Plasmin-alpha2-antiplasmin complexes, C; Soluble form of the urokinase-type plasminogen activator receptor, D; Disseminated intravascular coagulation, E) of fibrinolysis in patients with typhoid fever (empty squares) who were culture positive (black triangles) or culture negative (empty triangles) for *S. Typhi* and in healthy Bangladeshi control subjects (empty circles). Data are expressed as dot plots with medians.

tPA: tissue-type plasminogen activator; PAI-1: plasminogen activator inhibitor type; PAPc: plasmin-alpha2-antiplasmin complexes; suPAR: soluble form of the urokinase-type plasminogen activator receptor; DIC: disseminated intravascular coagulation.



**Fig. 6.** Association between coagulation abnormalities and severity of disease.

The normalization of three parameters (Activated partial thromboplastin time, A; Endothelial cell specific molecule-1, B; Protein C, C) over time in typhoid fever patients ( $n = 16$  patients). The time points are day of enrollment, and day of discharge (mean with 95% CI). Dotted line represents reference values of healthy controls. APTT: activated partial thromboplastin time; ESM-1/Endocan: endothelial cell specific molecule-1.

(im)balance,<sup>8</sup> were markedly elevated in the majority of typhoid fever patients. Furthermore, in this study we were able to collect samples from patients on day of admission and on day of discharge, which gave us a further insight into the course of disease. The extent of coagulation activation correlated with the course of disease, most measured markers showed complete or near normalization during convalescence. However, samples were only collected acutely and at time of hospital discharge - there are multiple other factors apart from disease progression, which may account for these differences such as use of antibiotics.

Fibrinolysis was grossly activated in our cohort of patients as shown by increased tPA and PAPc levels. In addition, our data on reduced expression of anticoagulant mechanisms are consistent with previous investigations reporting low circulating levels of protein C and AT in typhoid fever,<sup>28,29</sup> which could, in part, contribute to the development of DIC. Of note, protein C becomes activated by the thrombin-thrombomodulin complex. In a previous study, an increase in soluble thrombomodulin was found in patients with typhoid fever.<sup>30</sup> One would hypothesise that increased levels of soluble thrombomodulin would lead to elevated levels of thrombin-thrombomodulin complexes and enhanced consumption of protein C. In addition, we found an APC resistant phenotype. APC is an anticoagulant as it inactivates factors Va and VIIIa. Reduced sensitivity for APC, caused by for instance a factor V Leiden mutation, is a well-established risk factor for the development of venous thrombosis.<sup>31</sup> We now demonstrate that patients with typhoid fever patients have decreased plasma protein C concentrations together with an APC-resistant phenotype.

Our study design uniquely enabled us to compare markers of coagulation and fibrinolysis in blood culture-positive and culture-negative/PCR-positive patients in the same population in order to generate future hypotheses. Therefore no adjustments were made for multiple comparisons. We demonstrate that virtually all measured markers are significantly affected by typhoid fever and there was a trend to more pronounced activation of coagulation, fibrinolysis and endothelium with concurrent impaired of anticoagulant pathways in the blood culture-positive group when compared to the culture-negative/PCR-positive patients. The presence of bacteria in the systemic compartment could contribute to this phenomenon due to increased shedding of endotoxin and/or the recently discovered *S. Typhi* exotoxin.<sup>12</sup> Endotoxin has previously been suggested to be the prime mediator of the hematologic changes observed in typhoid patients<sup>11,32</sup> and indeed is a known potent inducer of coagulation.<sup>22,24</sup> Its role as a virulence factor in typhoid fever however is debatable as *S. Typhi*'s Vi capsule can prevent endotoxin recognition by the host.<sup>2,33</sup> In addition, endotoxins could not be detected in typhoid fever patients with severe DIC.<sup>11</sup> The exotoxin of *S. Typhi* can, however, interact with the vascular endothelium<sup>12</sup> and perhaps contributes to the signs and symptoms of typhoid fever. We now observe that markers of endothelial ac-

tivation, most notably VWF and endocan, are sharply increased in the blood culture-positive group when compared to the culture-negative/PCR-positive group. However, the significance of the activation of coagulation, fibrinolysis, and concurrent downregulation of the anticoagulation, is limited by the lack of a non-typhoid febrile control group, which calls into question whether this is a typhoid-specific phenomenon.

Acute infection can result in systemic activation of coagulation.<sup>22,24</sup> One could postulate that in typhoid fever the activation of coagulation and deposition of fibrin as a consequence of inflammation can be considered instrumental in containing inflammatory activity. Immunologic functions of thrombi are critically dependent on platelets.<sup>23</sup> In our study, 18% of typhoid fever patients had marked thrombocytopenia and most measured markers for coagulation negatively correlated with platelet count. The mechanism of thrombocytopenia in typhoid fever remains ill defined. It has been postulated that defects in the production of platelets are caused by a direct toxic effect of *Salmonella* on the bone marrow,<sup>34</sup> while others have suggested non-immune platelet destruction to be an effect of DIC.<sup>23,35</sup> More recent insights indicate that the abundant presence of hemophagocytes during *Salmonella* infections, which are important for intracellular replication and thus maintenance of chronic infection, could in part explain the low platelet - as well as leukocyte - counts commonly seen in typhoid fever.<sup>36</sup>

In summary, our data show that acute typhoid fever is characterized by activation of coagulation and endothelium, activation and inhibition of fibrinolysis and concurrent impairment of anticoagulant mechanisms. The clinical importance of our findings is underscored by the association between the observed disturbances in the coagulation cascade and the course of disease. Repeated testing during convalescence showed a return toward normal values.

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