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Low Plasma ADAMTS13 Activity is Associated with Coagulopathy, Endothelial Cell Damage and Mortality after Severe Pediatric Trauma

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Summary

Decrease of plasma activity of ADAMTS13, a metalloenzyme that cleaves von Willebrand factor (VWF) and prevents adhesion and aggregation of platelets, has been reported early after onset of systemic inflammation resulting from infections and after severe trauma. Here, we determined whether trauma-induced systemic (sterile) inflammation would be associated with a reduction of plasma ADAMTS13 activity in pediatric patients and its association with disease severity and outcome. Pediatric patients (n=106) with severe trauma at a level 1 pediatric trauma center between 2014 and 2016 were prospectively enrolled. Blood samples were collected upon arrival and at 24 hours and analyzed for plasma levels of ADAMTS13 activity, VWF antigen, collagen binding activity, human neutrophil peptides (HNP) 1-3, coagulation abnormalities, endothelial glycocalyx damage, and clinical outcome. Plasma samples were also collected for similar measurements from 52 healthy pediatric controls patients who underwent elective minor surgery. The median patient age was 9 years with 81% sustaining blunt trauma. The median injury severity score was 22 and the mortality rate was 11%. Plasma levels of ADAMTS13 activity were significantly lower and plasma levels of VWF antigen and HNP1-3 proteins were significantly higher for pediatric trauma patients on admission and at 24 hours when compared to controls. Finally, the lowest plasma ADAMTS13 activity was found in patients who died from their injuries. We conclude that relative plasma deficiency of ADAMTS13 activity may be associated with more

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Authorship statement

RR, XLZ, and J-FP contributed to study design and wrote manuscript. RR, J-FP, JM, WC, and XLZ contributed to experiments, data collection, and data interpretation. MS performed statistical analysis. All other authors contributed to revising and finalizing the manuscript.

Disclosure of conflict of interest

XLZ serves in the speaker's bureau of Alexion and is a consultant for Ablynx and BioMedica. All other authors declare no relevant conflict of interest associated with this work.

R.R., X.L.Z., and J.F.P. designed study, interpreted results, and wrote manuscript. J.M., W.J.C., M.S., B.M.W., and M.K.C. performed experiments, analyzed and interpreted data, and revised manuscript.

severe traumatic injury, significant endothelial glycocalyx damage, coagulation abnormalities and mortality after severe trauma in pediatric patients.

Keywords

Pediatric; Trauma; Injury; Coagulopathy; ADAMTS-13; von Willebrand Factor; human neutrophil peptide

Introduction

Trauma is the leading cause of pediatric mortality, potential years of life lost, and accounts for a significant medical cost in the developed world.(1, 2) Pediatric trauma places a significant emotional and economic burden upon families. Prior estimates of annual costs related to pediatric trauma, in the US alone, are over \$200 billion.(3) The predominate causes of mortality among pediatric trauma patients are traumatic brain injury and hemorrhage; in addition, blunt injury is more common in children with over 40% of deaths attributable to motor vehicle accidents. (4, 5) Mechanisms of coagulation abnormalities following severe pediatric trauma are not fully understood. Recent reports have estimated the incidence of coagulation abnormalities in children to range from 21% to 57%.(5-9) Children have a markedly different injury profile and response to trauma than adults with much lower overall trauma-related mortality rates and lower mortality rates related to organ failure or infection.(10, 11)

There are multiple pediatric trauma studies demonstrating a distinct difference in mortality between children with and without coagulation abnormalities.(5, 6, 8, 9) Elucidating key individual factors in development of these abnormalities will be important and could direct development of targeted therapies to lessen the effects of early post-traumatic coagulation abnormalities. In addition, identifying why some patients experience a hypocoagulable response to trauma and others do not may allow individualized therapy for these groups. To our knowledge, prospective studies on the proposed mechanisms of post-traumatic coagulation abnormalities in children are limited. Our current understanding of these mechanisms is predicated mostly on data interpretation from adult samples.

Recently, there has been an increased interest on the vascular endothelium, its microenvironment and how changes in this milieu following traumatic injury may contribute to coagulation abnormalities.(12-14) The endothelium and its glycocalyx layer, under normal conditions, inhibits coagulation, prevents platelet aggregation, adherence and migration of blood cells, and regulates vascular tone and permeability.(15, 16) Endothelial activation occurs in the setting of trauma or sepsis and can induce a procoagulant state that is associated with microvascular injury and thrombosis.(15, 17) Syndecan-1, a heparan-sulfate proteoglycan, has been widely studied as the main marker of endothelial glycocalyx breakdown following traumatic injury. (18, 19) A recent adult study has demonstrated that syndecan-1 has a role not only as a biomarker of endothelial glycocalyx breakdown, but also as a qualitative index for the endotheliopathy of trauma. (20)

Immediately following severe trauma, systemic inflammation occurs, which leads to neutrophil recruitment, activation, and degranulation at the site of injury (21, 22). This process results in the release of major neutrophil granular contents, including a group of small antibiotic peptide, referred to human neutrophil peptides (HNP) 1-3 or alpha-defensins. HNP1-3 kills various bacteria, fungi, and viruses (23). It also activates platelets and coagulation (24), inhibits fibrinolysis, and blocks the cleavage of VWF by ADAMTS13 (25). Therefore, the release of HNP1-3 may be associated with thrombosis. However, plasma levels of HNP1-3 and its role in patients with severe trauma are not yet known.

The present study is to determine the plasma levels of ADAMTS13 activity. VWF antigen and activity, and HNP1-3, in pediatric patients with severe trauma and healthy controls and to determine their association with endothelial glycocalyx damage, coagulation abnormalities and mortality.

Material and Methods

The Internal Review Board of the University of Alabama at Birmingham approved this prospective observational cohort study performed between 2014 and 2016. Delayed written consent was obtained from the patient legally authorized representative.

Participants

Consecutive pediatric trauma patients admitted to the Children's Hospital of Alabama, the only level 1 pediatric trauma center in the state of Alabama, were studied. 106 pediatric trauma patients, under the age of 18, met level 1 trauma criteria (Table 1) were eligible for enrollment. Exclusion criteria included patients admitted > 6 hours after their injury, patients with burns >20% of the total body surface area, patients admitted for primary asphyxiation, patients with known or expected pregnancy, known liver disease, and/or known coagulation disorders and suspected non-accidental trauma. The control cohort includes 52 pediatric volunteers (mean age, 6.20 ± 6.5 years). The numbers of trauma and control patients were determined based on those patients enrolled from March 2014 through March 2016.

Sample collection and measurements

Blood samples were collected within 20 minutes of arrival to the trauma room and at 24 hours after hospital admission. Blood samples were placed in sodium citrated tubes (one part 0.106 mol L⁻¹ sodium citrate and nine parts venous blood) and transported to the laboratory for immediate processing. The plasma from each sample was extracted and stored in -80 C° freezer until analysis. Prothrombin times (PT), platelet count (PC) and base deficit (BD) determination were analyzed as part of the standard clinical tests by the hospital laboratory. Prothrombin ratio (PT ratio) was calculated as observed PT divided by mean normal PT for the reagent used. Control samples were collected from healthy children with no history of coagulopathy who were undergoing minor elective surgery. Blood was collected at the time of intravenous catheter insertion prior to induction of general anesthesia. The blood was processed in an identical fashion as described above for trauma patients.

Measurement of plasma levels of ADAMTS13 activity, VWF antigen, VWF activity, and HNP1-3

Assay for plasma ADAMTS13 activity.—Plasma ADAMTS13 activity in each sample was determined by a recombinant FRETs-VWF73 assay, as previously described.(26, 27) Samples were measured against a standard reference curve using pooled normal human plasma in serial dilutions. Normal ADAMTS13 activity is 1 U/mL (0.5-1.5 U/mL) correlating to 80%-120%. ADAMTS13 activity for the samples is expressed as a percentage.

Assay for VWF antigen and collagen binding activity.—Plasma VWF antigen and activity were measured by enzyme-linked immunosorbent assay (ELISA). Ninety-six-well plates were coated with either rabbit polyclonal anti-human VWF antibodies (Dako, Denmark) for antigen at 4 °C overnight or type III collagen (Southern Research, Birmingham, AL) for activity. The plates were blocked for 30 minutes at room temperature with PBS containing 2.5% bovine serum albumin (BSA) and 0.05% Tween 20. Block buffer was decanted then plasma samples added and plates incubated for 2 h at 37 °C. The samples were decanted and wells then washed 3 times with PBS. Rabbit polyclonal anti-human VWF conjugated with horseradish peroxidase (Dako, Denmark) diluted to 1:3000 in PBST with 0.5% BSA was then added to wells and incubated at 37 °C for 1 h. The wash step was repeated and tetramethylbenzidine (TMB) was added to wells.

Color change reaction observed and stopped with 0.5 M H₂SO₄ after 5-10 minutes. The optical density was measured at 450 nm using a spectrophotometer. Samples were measured against a standard reference curve using pooled normal human plasma in serial dilutions. Normal VWF antigen is assumed to be 10 µg/mL (5-20 µg/mL) correlating to 50%-200%. Plasma VWF antigen and activity for the samples are expressed as the percentage of normal.

Assay for HNP1-3 proteins.—An ELISA was used to detect total plasma levels of HNP1-3 according to the manufacturer's recommendation (Hycult Biotech Inc, Plymouth Meeting PA) as previously described.(28) Briefly, standards at varying dilutions or plasma samples (1:10-1:80) were added to a microtiter plate pre-coated with a monoclonal anti-human antibody that recognizes HNP1-3. These reaction mixtures were incubated for 60 minutes at 25°C then washed three times. A streptavidin-peroxidase conjugate was added to the wells and the reaction mixtures were incubated for 60 minutes at 25°C and then washed three times. This was followed by incubation with TMB (ThermoFisher, Waltham, MA). The reaction was stopped by addition of 1 M HCl and the absorbance in each well was measured at a wavelength of 450 nm. The concentration of HNP1-3 in the plasma samples was determined from the calibration curve and expressed as nanograms per milliliter.

Measurements of plasma syndecan-1

Plasma samples were measured in duplicate for Syndecan-1 using an ELISA kit. All measurements were performed in accordance with the manufactures' instructions (Cell Sciences, MA, USA).

Clinical data collection and outcome measures

Data were prospectively collected on patient demographics, time of injury, the mechanism of injury (blunt or penetrating), Injury Severity Score (ISS), baseline vital signs, transfusion requirements, and pre-hospital fluid administration. A blood gas was obtained on patient arrival per protocol for the management of high-level pediatric trauma patients. BD was used as a measure of the degree of tissue hypoperfusion. Patients were followed until discharge or death.

Statistical analysis

For all trauma patients (n=106), medians with interquartile ranges or means with 95% confidence intervals were calculated for demographic (age and gender), injury (ISS, mechanism type, and AIS), and physiology (base deficit, PT ratio, and platelet count) characteristics. Median values of plasma ADAMTS13 activity, VWF antigen, ADAMTS13 activity/VWF antigen ratio and HNP1-3 proteins were calculated for samples collected at admission to the hospital and 24 hours following that admission for both trauma and before surgery for control patients using the nonparametric, independent sample test of medians. Controls were then compared to trauma patients at admission with ISS values < 15 and ≥ 15 and to trauma patients based on survival using the nonparametric, independent sample test of medians. Plasma ADAMTS13 activity, VWF antigen, and ADAMTS13 activity/VWF antigen ratio median values at admission were then compared to the plasma syndecan-1 values using the nonparametric, independent sample test of medians. Prothrombin time (PT) ratios were grouped as < 1.2, 1.2 to 1.5 and ≥ 1.5. Plasma levels of ADAMTS13 activity, VWF antigen, ADAMTS13 activity/VWF antigen, and HNP1-3 protein median values collected at admission to the hospital were compared to controls and PT ratio groups using the nonparametric, independent sample test of medians. Dot plot plots with the median ± 95% confidential interval (CI) were prepared using Graphpad Prism7 (GraphPad Software, La Jolla, California). Kruskal-Wallis test (non-parametric) was performed to determine the statistical significance of the differences in various groups. One (*), two (**), three (***), and four stars (****) indicate the *p* values less than 0.05, 0.01, 0.005, and 0.0001, respectively.

Results

Patient population

During the study period, 143 patients requiring full trauma team activation were evaluated. A total of 37 patients were deemed ineligible by study exclusion criteria. Patients were excluded for the following reasons: age (n=2), downgrade to level 2 (n=2), unable to obtain blood (n=5), dead on arrival (n=5), missed (n=6), arrival >6 hours after injury (n=8), burns or asphyxiation (n=9). In all, 106 consecutive pediatric trauma patients were enrolled in the study with a median age of 9 years (IQR: 4-12). Median ISS was 22 (IQR: 12, 34) and 81% sustained blunt trauma. Clinical characteristics of the children included are shown in Table 2. Coagulopathy (defined as PT ratio > 1.2) was present in 27% of patients measured on arrival to the hospital, and 25% had a base deficit ≥ 6 mmol/L. Overall mortality rate was 11%. The median time from injury to blood sample collection was 90 minutes (IQR: 60, 160).

Plasma levels of ADAMTS13 activity, VWF antigen, VWF activity, and HNP1-3 proteins in trauma patients and controls

Pediatric trauma patients had a significantly lower plasma median levels of ADAMTS13 activity (%) on admission (83.0%, $p<0.0001$) and at 24 hours (74.3%, $p<0.0001$) when compared to control patients (112.5%) (Fig. 1A). Plasma median levels of VWF antigen (106.5%) and activity (114.7%) were significantly elevated in trauma patients on admission as compared with those (VWF_{Ag}=75.1% and VWF_{Ac}=83.8%) in the controls ($p<0.0001$) (Fig. 1B & 1C). To evaluate the relationship between plasma levels of ADAMTS13 activity and VWF antigen, we calculated the ADAMTS13 activity/VWF antigen ratio. The median ratios of ADAMTS13/VWF antigen and ADAMTS13/VWF activity were markedly decreased in trauma patients on admission (0.8 and 0.8) ($p<0.0001$) and at 24 hours after admission (1.01 and 1.5) compared to controls (1.5 and 1.8) ($p<0.0001$) (Fig. 1D & 1E). Finally, plasma median levels of HNP1-3 that is believed to competitively bind to VWF antigen, thus preventing its cleavage by ADAMTS13 (25), were significantly elevated on admission (15.8 ng/mL $p<0.0001$) and remained high 24 hours later (8.4 ng/mL, $p<0.005$) compared with that in the control (3.6 ng/mL) (Fig. 1F).

Plasma levels of ADAMTS13 activity, VWF antigen, and HNP1-3 proteins and injury severity score

To characterize how plasma levels of ADAMTS13 activity, VWF antigen, and HNP1-3 respond to injury after admission, we utilized Injury Severity Score (ISS) to categorize patients into those with low ISS (<15 , $n=34$), those with moderate ISS (15-25, $n=26$), and those with high ISS (≥ 25 , $n=46$). Pediatric trauma patients with low ISS had median plasma levels ADAMTS13 activity (100.4%) equivalent to the control population (116.9%) ($p>0.05$). However, those patients with moderate (80.2%) and high ISS (74.9%) had significantly lower levels of plasma ADAMTS13 activity when compared with the control (116.9%) ($p<0.0001$) (Fig. 2A). The median plasma VWF antigen levels in patients with low ISS (112.8.7%) ($p<0.05$), moderate ISS (145.2%, $p<0.0001$), and high ISS populations (112.7%, $p<0.05$) were significantly higher than those in the control (79.6%) (Fig. 2B). Strikingly, the ADAMTS13 activity to VWF antigen ratios were progressively reduced from low ISS (<15) (median 0.95, $p<0.01$), moderate ISS (15-25) (median 0.55, $p<0.0001$) to high ISS (≥ 25) (median 0.70, $p<0.0001$) compared with the control (median 1.5) (Fig. 2C), suggesting the relative deficiency of plasma ADAMTS13 activity as a function of disease severity. Finally, plasma HNP1-3 levels were significantly elevated in all groups. In low, moderate, and high ISS groups, the median plasma HNP1-3 levels were 8.1, 17.7, and 22.4 ng/ml were significantly increased compared with the control (3.1 ng/ml) (Fig. 2D), indicative of neutrophil activation and degranulation in trauma patients.

Plasma levels of ADAMTS13 activity, VWF antigen, and HNP1-3 protein and outcome from severe trauma

We compared the plasma levels of these biomarkers in patients based to their clinical outcome. There was a significant decrease in plasma ADAMTS13 activity between the controls (median 116.9%) and all trauma patient groups ($p<0.0001$). However, there was no statistically significant difference in the plasma ADAMTS13 activity when comparing the

survivors (median 85.4% and non-survivors (median 61.6%) ($p>0.05$) (Fig. 3A). Furthermore, plasma levels of VWF antigen were also significantly increased in trauma survivors (median 117.9%) compared to controls (median 76.7%) ($p<0.0001$) compared with the control (76.7%) ($p<0.0001$) and a significant difference in these levels was noted between trauma survivors (median 117.9%) and non-survivors (median 72.5%) ($p<0.05$). However, no difference in plasma levels of VWF antigen was noted between the control and non-survivor group ($p>0.05$) (Fig. 3B). When evaluating the plasma ADAMTS13 activity to VWF antigen ratio upon admission, the median ratio of the control group (1.5) was significantly higher than the patients who survived (0.7, $p<0.0001$) or died (0.8, $p<0.05$), but there was again no difference between median values of survivors and non-survivors ($p>0.05$) (Fig. 3C). Finally, plasma levels of HNP1-3 showed a similar relationship to the median values of plasma ADAMTS13 activity and VWF antigen with statistical differences between controls (3.1 ng/ml) vs. survivors (14.3 ng/ml, $p<0.005$) or non-survivors (19.2 ng/ml, $p<0.05$) but no difference between the survivors and non-survivors ($p>0.05$) (Fig. 3D).

Plasma levels of ADAMTS13 activity, VWF antigen, and HNP1-3 proteins and endothelial glycocalyx damage after severe trauma

Plasma levels of circulating syndecan-1 were measured to examine the relationship between trauma-induced endothelial cell glycocalyx damage and changes in plasma levels ADAMTS13 activity, VWF antigen, and HNP1-3 protein release in pediatric patients. Pediatric trauma patients were divided into four groups based on their plasma values of syndecan-1 levels (median values <20 ng/ml for group 1, <40 ng/ml for group 2, <80 ng/ml for group 3, and those >80 ng/ml for group 4). These specific ranges were chosen based on a recent adult study characterizing those patients with syndecan-1 level of >40 ng/mL as having increased transfusion requirements, longer hospital stays, and lower survival rates. (20) Median plasma levels of syndecan-1 for all trauma groups were significantly higher than the median syndecan-1 value in controls (not shown). The four groups of pediatric trauma patients had significantly lower levels of plasma ADAMTS13 activity compared to control values irrespective of their median value of plasma syndecan-1 (Fig. 4A). Furthermore, only the trauma patient group with the median plasma level of syndecan-1 between 40 and 80 ng/ml had significantly higher plasma levels of VWF antigen compared to controls ($p<0.05$) (Fig. 4B). Interestingly, the median ADAMTS13 activity to VWF antigen ratio for each trauma group was significantly lower than the median ADAMTS13 activity to VWF antigen ratio measured in controls irrespective of their median value of syndecan-1 ($p=0.0001-0.01$) (Fig. 4C). Finally, HNP1-3 protein levels and syndecan-1 levels were significantly higher in all trauma patients with elevated syndecan-1 levels than the control group ($p=0.0001-0.01$) (Fig. 4D). Plasma HNP1-3 levels in patients with syndecan-1 levels >80 ng/ml (28.9 ng/ml) were ~10 fold higher than those in the controls (3.1 ng/ml) ($p<0.0001$).

Plasma levels of ADAMTS13 activity, VWF antigen, and HNP1-3 proteins and severity of post-traumatic coagulation abnormalities

To evaluate how the plasma levels of ADAMTS13 activity, VWF antigen, and HNP1-3 proteins may differ in the setting of worsening traumatic coagulopathy, we divided the

trauma population into in tertiles based on the value of their PT ratio. These groups were also compared to our control group. Regarding plasma ADAMTS13 activity, each of median value of the three groups of trauma patients was significantly different than control plasma ADAMTS13 activity (all p values <0.0005) (Fig. 5A). Interestingly, there was a significant difference in plasma ADAMTS13 activity between control patients and those trauma patients even without coagulopathy (PT ratio < 1.2) ($p<0.0001$). The median plasma levels of VWF antigen were significantly increased in the trauma group without coagulopathy (PT ratio < 1.2) ($p<0.0001$) and with PT ratio of 1.2-1.5 ($p<0.05$) compared with the median control values. As coagulopathy became more severe (PT ratio > 1.5) median plasma levels of VWF antigen were not significantly different from the median control values (Fig. 5B). The median plasma ADAMTS13 to VWF ratio for control patients was significantly higher than those in trauma patient groups with PT ratio < 1.2 , PT ratio 1.2-1.5, and PT ratio >1.5 (all p values <0.0001). The ratio of the patient group with severe coagulopathy (PT ratio 1.5) was not statistically different from the one measured in controls. There was no significant difference in this ratio between the three trauma groups, as coagulopathy became more severe (Fig. 5C). All three trauma patient groups (11.0, 22.2, and 36.2 ng/ml) had a significantly higher median plasma value of HNP1-3 proteins compared to the median control value (3.1 ng/ml) (all p values <0.0001). However, none of the median plasma value of HNP1-3 proteins of each individual trauma group was significantly different from one another ($p>0.05$) (Fig. 5D).

Discussion

The main results of this prospective study showed that plasma levels of ADAMTS13 activity were significantly lower and plasma levels of VWF antigen/activity and HNP1-3 proteins were significantly higher for pediatric trauma patients on admission and at 24 hours when compared to controls. Furthermore, the lowest plasma ADAMTS13 activity was found in patients who died from their injuries. These results demonstrate that early after severe trauma, there is a relative deficiency in plasma ADAMTS13 activity that is associated with more severe traumatic injury, significant endothelial glycocalyx damage, coagulation abnormalities, and mortality in pediatric patients.

The first important result of the present study shows early abnormalities in the activity of circulating ADAMTS13 and a corresponding extracellular increase in the release of VWF antigen after severe trauma in pediatric patients. This finding is associated with a higher median plasma level of VWF antigen and more importantly a significantly low ratio of ADAMTS13 activity/VWF antigen or ADAMTS13/VWF activity ratio at admission to the hospital that persisted for the first 24 hours after trauma. The mechanisms that explain the significant decrease in plasma ADAMTS13 activity early after severe trauma are not fully understood. It may first be caused by consumption of ADAMTS13 activity when the supply of its substrate, VWF, exceeds ADAMTS13 proteolytic activity, as found in our study and previously reported in mice after *E. coli* endotoxin challenge (29) and in human after desmopressin challenge (30). Interestingly, the sepsis-induced increase in plasma levels of VWF antigen and the corresponding decrease in the plasma ADAMTS13 activity correlated with respective changes in hepatic mRNA expression for both proteins.(29) Second, the release of inflammatory cytokines by neutrophils and endothelial cells associated with the

systemic inflammatory response to severe trauma stimulates the synthesis of ultra large VWF multimers (31) and inhibits ADAMTS13 biosynthesis (32). Third, elastase release by activated neutrophils can cause proteolytic cleavage of ADAMTS13, thus significantly decreasing plasma ADAMTS13 activity in systemic inflammation.(29) Fourth, coagulation products, such as tissue factor and thrombin, may directly inhibit the secretion of ADAMTS13, but increase the release of VWF antigen by endothelial cells.(33) Finally, plasma level of thrombospondin-1, a matrix glycoprotein (34) and HNP1-3 (28) are significantly elevated after severe trauma. This glycoprotein and cationic peptides can compete with ADAMTS13 for the interaction with the A2 domain of VWF antigen.(25, 35). In addition, thrombospondin-1 protects reactive ultra large VWF antigen against degradation by ADAMTS13, thus securing platelet and thrombus adhesion to the injured endothelium. (36)

The second important result shows that pediatric trauma patients with high ISS have plasma ADAMTS13 activity levels significantly lower than both the controls and low ISS group. Furthermore, the ADAMTS13 activity/VWF antigen ratio was also significantly lower in the high ISS group compared with the low ISS group and the control patients. These results suggest that plasma ADAMTS13 activity may be associated with disease severity. Interestingly, although the ADAMTS13 plasma levels were lower in patients who died from their injuries than in those who survived, the ADAMTS13 activity/VWF antigen ratio was similar between both groups of trauma patients. This result is explained by the lower plasma level of VWF antigen in patients who died than in those who survived. Our pediatric data are in accordance with the results of a recent adult trauma study that found that plasma levels of VWF antigen were above normal level in 85% of the patients admitted to the ICU after initial resuscitation. However, early plasma levels of VWF antigen were lower in non-survivors than in survivors.(37) In contrast, plasma levels of VWF antigen correlated with poor outcome in patients with subarachnoid hemorrhage(38) or traumatic brain injury.(39) The difference between these studies may be explained by the fact that lower plasma levels of VWF measured in non-survivors from multiple trauma may be caused by a consumption of this protein secondary to the massive systemic inflammatory response, activation of the coagulation cascade, and endotheliopathy of trauma observed in those patients.

The third important result shows that plasma levels of syndecan-1 for all trauma patients who were divided in quartiles based on based on their initial plasma values of syndecan-1 levels were significantly higher than the median syndecan-1 value measured in controls. The four groups of pediatric trauma patients had also significantly lower levels of plasma ADAMTS13 activity compared to control values irrespective of their median value of plasma syndecan-1. These results indicate that there is no direct correlation between the severity of endotheliopathy measured by the plasma levels of syndecan-1 and the inhibition of ADAMTS13 activity in plasma after severe pediatric trauma. Recent clinical studies have established the measurement of circulating syndecan-1 as an index of the severity of endotheliopathy in adult trauma patients.(20, 40) Syndecan-1 plasma levels of ≥ 40 ng/ml identified adult trauma patients with significantly worse outcomes.(20) We have previously shown that there is also a correlation between the plasma levels of syndecan-1 measured at the admission to the hospital and the outcome of severely traumatized pediatric patients.(41) This lack of correlation between the severity of endotheliopathy and the insufficient cleavage

capacity of ADAMTS13 after severe pediatric trauma may be explained by the fact that the trauma-induced inhibition of plasma ADAMTS13 activity is multifactorial and may be caused by reduced liver synthesis, consumption, direct inhibition, proteolytic cleavage and increased clearance.(42) One additional mechanism that affects the cleavage of VWF antigen by ADAMTS13 is mediated by the trauma-dependent activation of neutrophils. Recent work from our group has demonstrated that activated neutrophils release of HNP1-3 proteins that not only induce direct bacterial killing, but also prevent the cleavage of VWF multimers by ADAMTS13 by their high-affinity binding to the central A2 domain of that protein.(25) Interestingly, we also reported that HNP1-3 may contribute to the onset of an acquired thrombotic thrombocytopenic purpura (TTP) when the plasma level of ADAMTS13 activity is already reduced by IgG autoantibodies against ADAMTS13.(28) Our results show that plasma levels of HNP1-3 are significantly increased after major trauma in pediatric patients and correlate with the severity of injury measured by the injury severity score. As the circulating ADAMTS13 activity is likely to be reduced after severe trauma, it is possible that the release of HNP1-3 further reduces the activity of this protease. As only a small fraction of HNP1-3 may be released in the bloodstream from the sites of neutrophil activation, the ten-fold increase above controls of the plasma levels of HNP1-3 measured in pediatric patients with an elevated injury score may reflect the massive local release of HNP1-3 from activated neutrophils. Finally, we found that the release of HNP1-3 into the bloodstream of patients with acquired TTP was associated with a major activation of the alternative pathway of the complement, suggesting a potential causative role of complement activation in acquired TTP.(28) Although we did not measure plasma levels of complement species in our pediatric trauma population, we have previously reported that severe trauma is associated with a massive activation of the alternative complement pathway in adult patients.(43) Thus, additional studies will be needed to determine how important is the synergism between the activation of alternative complement pathway and the release of HNP1-3 on the activity of plasma ADAMTS13 after severe trauma.

The last important result of this study is that patients with the lowest plasma ADAMTS13 activity developed a coagulopathy shown by a PT ratio of more than 1.2 at the admission to the hospital. Several previously published have shown a similar correlation in non-trauma patients with hemorrhagic events. For example, two studies have reported that adult patients who presented with disseminated intravascular coagulation associated with bleeding had significantly lower plasma levels of ADAMTS13, which were between 40 and 60% of normal values.(44, 45) Furthermore, low plasma ADAMTS13 activity has been found to be a predictor of postoperative bleeding in pediatric patients with cyanotic congenital heart disease.(46) The mechanism(s) for this correlation is not fully understood. Our results also show a low ADAMTS13 activity to VWF antigen ratio, suggesting that a potential for accrual of large VWF antigen multimers at the surface of the vascular endothelium causing platelet clumping and formation of thrombin. We and others have previously shown that the massive activation of the anticoagulant protein C plays a mechanistic role in the development of the early coagulopathy in adult patients with the most severe traumatic injuries by deactivating activated Factors V and VIII and causing an activation of the fibrinolytic pathway. (47) It is known that the activation of protein C requires the binding of thrombin to thrombomodulin at the surface of the vascular endothelium.(48) Thus, the

trauma-induced systemic activation of the coagulation cascade observed in patients with a low activity of circulating ADAMTS13 may result in a maladaptive activation of the protein C pathway that causes a short-lived, but clinically important, hypocoagulable state after severe trauma. Additional studies will be needed to demonstrate the presence of a causal link between inhibition of plasma ADAMTS13 activity, activation of the protein C after severe trauma, and to further determine the utility of ADAMTS13 as a prognostic marker of outcome/mortality.

The results presented here are subject to the limitations related to the fact that it is a single center observational study. Although there were many trauma patients and controls included in the study, care must be taken in interpreting the data as there were limited numbers in some stratified groups utilized in the analysis. Furthermore, the correlations that we observed are not necessarily mechanistic. However, this study represents a severely injured pediatric trauma population in which these observations have not been described to date. This will require further confirmatory studies on larger pediatric trauma populations. A second limitation of this study is that while hepatic stellate cells are the primary source for active plasma ADAMTS13, we have not taken in account the fact that platelets (49) and endothelial cells (50) also contain small amount, but functionally active ADAMTS13 that may be an important source of ADAMTS13 after severe trauma. Future preclinical and clinical studies are therefore needed to determine the importance of that second pool of active ADAMTS13 after severe trauma.

In summary, this study demonstrates that severe trauma induces a significant and early inhibition of plasma ADAMTS13 activity that is associated with more severe traumatic injury, significant endothelial glycocalyx damage, coagulation abnormalities and mortality in pediatric patients. Further studies will be needed to determine the importance of the imbalance between ADAMTS13 plasma activity and the release of VWF antigen at the surface of the vascular endothelium and the development of early and late post-traumatic coagulation abnormalities in pediatric patients. Our ongoing studies are to determine whether supplementation of an active recombinant ADAMTS13 in animal models of polytrauma and in patients with severe trauma will impact their mortality and morbidity.

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What is known about this topic?

- Increased von Willebrand factor (VWF) and reduced ADAMTS13 activity are risk factors for various thromboses.
- However, it is not known whether plasma levels of ADAMTS13 activity and VWF predict severity and outcome in pediatric trauma patients.

What does this paper add?

- This study demonstrates that plasma levels of ADAMTS13 activity are lower and plasma VWF and human neutrophil peptides (HNP) 1-3 are higher in pediatric trauma patients on admission and at 24 hours when compared to healthy controls.
- The lowest plasma ADAMTS13 activity is found in patients who died from their injuries.

Fig. 1.

Plasma levels of ADAMTS13, VWF antigen, ADAMTS13/VWF ratio, and HNP1-3 protein following severe trauma in pediatric patients. Plasma ADAMTS13 activity (A13) (A), VWF antigen (B), VWF activity (C), A13/VWF antigen ratio (D) or A13/VWF activity ratio (E), and HNP1-3 (F) in trauma patients on admission (0 h) and at 24 hours (24 h) when compared to controls (Con). Each dot represents a value from each individual patient. The lines within the dots are the median $\pm$ 95% confidential interval (CI). Sign * indicates the p value < 0.05 , ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.0001$. Kruskal-Wallis (non-parametric) tests were used to determine the statistical significance of the differences among three groups. n.s. indicates no statistically significant difference.

Fig. 2.

Association of plasma levels of ADAMTS13, VWF antigen, A13/VWF ratio, and HNP1-3 protein with ISS score in pediatric patients following severe trauma. The relationship between trauma patients' ISS score and their plasma ADAMTS13 activity (A), VWF antigen (B), A13/VWF ratio (C), and HNP1-3 concentrations (D). Each dot represents a value from each individual patient. The lines within the dots are median $\pm$ 95% confidential interval (CI). Kruskal-Wallis (non-parametric) test was performed to determine the statistical significance among three groups. Signs *, **, ***, and **** indicate the p values <0.05 , <0.01 , <0.005 , and <0.0001 , respectively. n.s. indicates no statistically significant difference.

Fig. 3.

Association of plasma levels of ADAMTS13, VWF antigen, A13/VWF ratio, and HNP1-3 protein with outcome following severe trauma in pediatric patients. The plasma levels of ADAMTS13 activity (A), VWF antigen (B), A13/VWF ratio (C), and HNP1-3 (D) are shown for pediatric trauma patients who survived and died in comparison with the healthy controls. Each dot represents a value from each individual patient. The lines within the dots are median $\pm$ 95% confidential interval (CI). Kruskal-Wallis (non-parametric) test was performed to determine the statistical significance among three groups. Signs *, **, ***, and **** indicate the p values <0.05 , <0.01 , <0.005 , and <0.0001 , respectively. n.s. indicates no statistically significant difference.

Fig. 4.

Association between endothelial glycocalyx damage and plasma levels of ADAMTS13, VWF antigen, A13/VWF ratio, and HNP1-3 protein following severe trauma. Plasma ADAMTS13 activity (A), VWF antigen (B), A13/VWF ratio (C), and HNP1-3 (D) in relation to the median or quartile values of plasma syndecan-1 were determined. Each dot represents a value from each individual patient. The lines within the dots are median $\pm$ 95% confidential interval (CI). Kruskal-Wallis (non-parametric) test was performed to determine the statistical significance among three groups. Signs *, **, ***, and **** indicate the p values <0.05 , <0.01 , <0.005 , and <0.0001 , respectively. n.s. indicates no statistically significant difference.

Fig. 5.

Association of PT ratio with plasma levels of ADAMTS13 activity, VWF antigen, and HNP1-3 protein in patients with severe trauma. Plasma ADAMTS13 activity (A), VWF antigen (B), A13/VWF ratio (C), and HNP1-3 (D) in relation to the PT ratios in patients with severe trauma were determined. Each dot represents a value from each individual patient. The lines within the dots are median $\pm$ 95% confidential interval (CI). Kruskal-Wallis (non-parametric) test was performed to determine the statistical significance among three groups. Signs *, **, ***, and **** indicate the p values <0.05, <0.01, <0.005, and <0.0001, respectively. n.s. indicates no statistically significant difference.

Table 1:**Children's of Alabama Level 1 Trauma Triage Criteria**

Intubation prior to arrival
Airway compromise
Hemodynamic Instability
Glasgow Coma Score (GCS) less than 10
Deteriorating level of consciousness
Focal neurologic findings
Deep penetrating wounds to torso, head, or neck
Amputations, partial amputations, and/or crush injuries proximal to ankle/wrist
Pelvic ring fracture with associated long bone fractures
Multi-System injury of 2 or more organ systems
Emergency Physician Discretion

Table 2

Clinical Characteristics of trauma patients (n=106)

Demographic data	n (%)
Age	
Median [IQR]	9.0 [4.0, 12.0]
Gender	
Male	77 (72.6)
Female	29 (27.4)
Characteristics of injury	
ISS	
Median [IQR]	22 [12, 34]
ISS 15	72 (67.9)
ISS 25	46 (43.4)
Mechanism Type	
Blunt	86 (81.1)
Penetrating	15 (14.2)
Other	5 (4.7)
Severe head injury (AIS head 3)	62 (58.5)
Physiology	
Base deficit 6 mmol/L	27 (25.5)
PT ratio > 1.2	29 (27.4)
aPTT (seconds)	27.6 [25.2, 31.9]
Platelet Count 10 ³ /μL	283 [224, 337]
Mortality	11%