Acquired ADAMTS-13 deficiency in pediatric patients with severe sepsis

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ABSTRACT

We studied the state of ultralarge von Willebrand factor (ULVWF) proteolysis in 21 pediatric patients with severe sepsis and found that the overall group of patients had moderately reduced ADAMTS-13 activity, but 31% had severe enzymatic deficiency. The severe deficiency correlated with greater adhesion activity of von Willebrand factor, severity of thrombocytopenia and plasma levels of interleukin-6. It also correlated clinically with severity of illness and organ dysfunction. These results suggest that ULVWF proteolysis is insufficient in septic patients and severely deficient in a subgroup of patients. The deficiency may contribute to the development of thrombocytopenia and ischemic organ failure associated with sepsis.

Key words: sepsis, ADAMTS-13, ULWF, thrombotic microangiopathy.

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Sepsis is a systemic inflammatory response to severe infection[1] and is associated with progressive multiple organ failure. More than 50% of septic patients develop moderate to severe thrombocytopenia, a condition that is correlated with poor prognosis.[2] The underlying mechanisms of sepsis-associated thrombocytopenia and organ failure remain poorly understood. We hypothesize that sepsis-associated thrombocytopenia results from widespread platelet aggregation and thrombotic microangiopathy caused, in part, by deficient proteolysis of ultra-large von Willebrand factor (ULVWF). ULVWF, released from endothelial cells in response to inflammatory stimulation, is hyperactive in its interaction with platelets.[3,4] The hyperactive ULVWF is rapidly converted to less active forms by ADAMTS-13,[5,6] a proteolytic process that is deficient in patients with thrombotic thrombocytopenia purpura (TTP). Some of the clinical presentations of TTP are shared by patients with sepsis. A recent study by Motto *et al.*[7] demonstrated that ADAMTS-13-deficient mice develop TTP-like phenotypes when they are challenged with Shiga toxin from *E. Coli* O157:H7.

von Willebrand factor (VWF) is the most widely used marker of endothelial activation, but its role in sepsis, the utmost condition of wide-spread endothelial cell activation, has been less studied. This is likely due to the belief that VWF circulates in blood without detrimental effects. However, VWF functions differently, depending on its multimeric size and adhesive properties, which are regulated by ADAMTS-13. Indeed, mild-to-moderate ADAMTS-13 deficiency has been detected in systemic inflammation.[8] It is on this background that we developed our hypothesis and tested it in the study reported here.

Design and Methods

This study, approved by the Institutional Review Board of Baylor College of Medicine, recruited patients from one pediatric intensive care unit at Texas Children's Hospital, Houston. Patients were diagnosed as having severe sepsis, defined as suspected or documented infection with the presence of systemic inflammation and signs of acute organ dysfunction using the Organ Failure Index (OFI),[9] the Pediatric Logistic Organ Dysfunction Score (PELOD)[10] and the Pediatric Risk of Mortality Score (PRISM).[11] Exclusion criteria included: (i) blood product transfusion within a week of blood collection, (ii) known thrombotic or bleeding disorders, (iii) hematologic diseases, (iv) >36 hours of meeting the inclusion criteria, (v) non-grafted bone marrow transplantation, (vi) end-stage liver dis-

ease, (vii) warfarin or anti-platelet medication within 3 days of entry into the study, (viii) body weight <8 kg, and (ix) no venous or arterial line access. Controls were recruited from critically ill but not septic, children in the same intensive care unit during the same period.

Blood samples (0.38% of sodium citrate as anticoagulant) were centrifuged at 150×g for 15 min to obtain platelet-rich plasma, which was then centrifuged at 900×g for 10 min at 24°C to obtain platelet-poor plasma. ADAMTS-13 activity in the platelet-poor plasma was measured under flow conditions, as previously described, upon enrollment and during the follow-up period of up to 28 days.[12] For the flow assay, ADAMTS-13 deficiency is defined as severe when its plasma VWF-cleaving activity is less than 30%.[12] ADAMTS-13 antigen was measured by a sandwich enzyme-linked immunosorbent assay (ELISA).[13] We used plasma pooled from ten healthy adult subjects to obtain reference values and the levels of ADAMTS-13 antigen in patients was expressed as a percent of the reference value.

Plasma VWF antigen was measured using a commercial ELISA assay kit (Ramco Laboratories, Houston, TX, USA) according to the manufacturer's instructions. In addition, ristocetin cofactor activity (Rco) was measured, to gauge VWF activity, on a Behring Coagulation System (BCS, Dade Behring, Deerfield, IL, USA) again according to the manufacturer's instructions. Finally, the concentration of the inflammatory cytokine interleukin-6 (IL-6) in plasma was measured using a Quantikine IL-6 Immunoassay kit (R&D Systems Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. The study had a case-control design. Differences in ADAMTS-13 activity between patients and controls were analyzed using the two sample Student's t test and correlated with platelet counts during the follow-up period by regression analysis. Data are presented as mean±SEM and p values less than 0.05 are considered to be statistically significant.

Results and Discussion

We enrolled 21 septic patients (Table 1) with one or more failing organs as determined by the OFI and PELOD scores. The mean platelet count of patients on admission was 157.4±42.7×10^3/μL, which was significantly lower than that of the controls (379.6±37.8×10^3/μL, Student's t test, p<0.005). Nine of the 21 patients (47.6%) had previously been healthy while the rest had underlying medical conditions: encephalopathy (14.3%), juvenile rheumatoid arthritis (4.8%), systemic lupus erythematosus (4.8%), pulmonary hypoplasia (4.8%), acute lymphoblastic leukemia (4.8%), grafted bone marrow transplant (4.8%), Duchenne muscular dystrophy (4.8%), Waardenberg syndrome (4.8%), Aicardi syndrome (4.8%), and Klipple-Trenaunay-Weber syndrome (4.8%).

The mean ADAMTS-13 activity upon admission was significantly lower in patients than in controls (57.4±6.6% vs. 83.0±2.4%, Student's t test, p<0.001). Further analysis showed that while the ADAMTS-13 deficiency was moderate for the group as a whole, a subgroup of seven patients had severe deficiency (9.2-28.7% with a mean activity of 21.4±2.6%). These levels of activity are higher than the 7-10% defined as indicating severe deficiency under static conditions for TTP patients, but may be a threshold for functional deficiency of the metalloprotease *in vivo* as previously demonstrated.[14] ADAMTS-13 activity in patients with platelet counts of less than 100,000/μL was significantly lower than that in patients with higher platelet counts (Figure 1A), consistent with the higher percentage of thrombocytopenia found in patients with severe ADAMTS-13 deficiency (71.4%) than in the rest of patients (30.8%). Four of the seven patients with severe ADAMTS-13 deficiency upon admission (<30% activity) were followed for more than a week and showed steady improvements in enzyme activity (Figure 1B), which was linearly correlated with increase in platelet counts (R^2=0.7054, p<0.001).

In all patients, ADAMTS-13 activity negatively correlated with day-1 PRISM scores (Spearman's rank order correlation, correlation coefficient – 0.434, p<0.05), and with all-days PELOD scores (Spearman's rank order correlation, correlation coefficient – 0.44, p<0.05). The mean PELOD scores were also significantly higher in patients with severe ADAMTS-13 deficiency (<30% activity) than in those with >30% activity (28.5±4.1 vs 12.8±2.9; Student's t- test; p<0.05). The mean level of ADAMTS-13 antigen in the non-septic controls was 75.5±5.6% of adult levels, whereas it was significantly lower in septic patients at 50.5±6.1% (Student's t test, p<0.01). There was no statistically significant difference in antigen levels between patients with platelet counts ≤100,000/μL and those with higher platelet counts.

As expected, plasma VWF was significantly higher in the septic patients than in the controls (179.5±23.2% vs. 103.2±11.3%, Student's t test, p<0.01). VWF levels in patients with <30% ADAMTS-13 activity were marginally higher than those in patients with higher enzymatic activity (204.3±18.1% vs. 147.4±15.4%, Student's t test, p=0.057). During the follow-up period, plasma VWF levels reduced slowly and correlated poorly with platelet counts (R^2 =0.2745). Although Rco was similar in patients and controls (361.1±31.2 vs. 356.9±33.6), it was significantly higher in patients with <30% ADAMTS-13 activity than in the rest of the patients (Figure 2A).

IL-6 levels in patients were significantly higher than those in controls (157.1±47.1, vs.15.4 pg/mL). Furthermore, IL-6 levels in patients with severe ADAMTS-13 deficiency were much higher than the levels in the rest of the patients (Figure 2B) and correlated with changes of ADAMTS-13 activity during the follow-up period (R^2=0.626, p<0.001).

We found that 31% of pediatric patients with septic

Table 1. Demographics and pathogens of patients and controls.

	Septic Children	*Non-septic Children*
n.	21	19
Age (years, mean±SD)	9.5±5.5	11.9±5.2
Sex (male/female)	10/11	8/11
Number of failing organs (OFI) (Median [25th%, 75th%])	3 (1,3.2)	0 (0,0)
PRISM on day 1 (Median [25th%, 75th%])	13 (4.5,18)	0 (0,2)
PELOD score on day 1 (Median [25th%, 75th%])	21 (7,27)	0 (0,5.5)
28-day mortality	1 (4.8%)	0
Organisms grown		
Group A Streptococcus	5	0
Staphylococcus aureus	3	0
Picomavirus	3	0
Streptococcal pneumoniae	2	0
Pseudomonas aeruginosa	2	0
Stenotrophomonas maltophilia	2	0
Klebsiella pneumoniae	2	0
Enterobacter cloacae	2	0
Shigella flexneri	1	0
Escherichia coli	1	0
Citrobacter freundii	1	0
Mycoplasma pneumoniae	1	0
Staphylococcus epidermidis	1	0

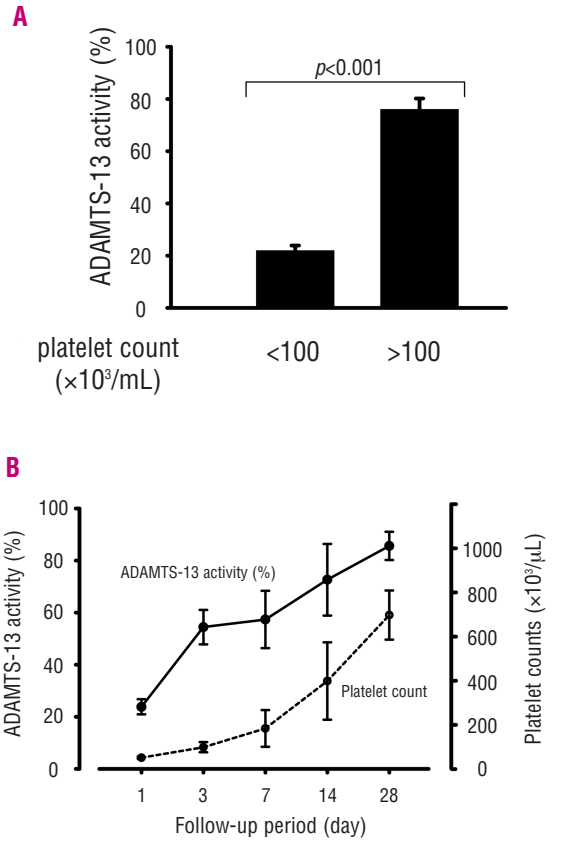

Figure 1. ADAMTS-13 activity was measured under flow by perfusion of patients' plasma over stimulated human umbilical vein endothelial cells. ADAMTS-13 activity was significantly lower in patients with platelet counts ≤100,000/μL than in those with higher platelet counts (A). The enzymatic activity improved during follow-up. The improvement was correlated with an increase in platelet counts (B).

shock had severe ADAMTS-13 deficiency (mean activity of 21.4±2.6%) measured under flow conditions. This level of activity is considered to be sufficient to cleave VWF substrate under static conditions, but is insufficient to cleave ULVWF strings on endothelial cells under flow conditions.[14] Severe ADAMTS-13 deficiency was associated with thrombocytopenia (Figure 1A), higher Rco (Figure 2A), and elevated plasma IL-6 levels (Figure 2B). An improvement in ADAMTS-13 activity during the follow-up period was paralleled by an increase in platelet counts (Figure 1B). Clinically, ADAMTS-13 activity was inversely correlated with the severity of illness (PRISM score) and organ dysfunction (PELOD and OFI scores). Taken together, these results suggest that ADAMTS-13 is severely deficient in a subgroup of patients with septic shock. As a result, hyperactive VWF appears in the plasma (higher Rco), leading to thrombotic microangiopathy that could contribute to the development of

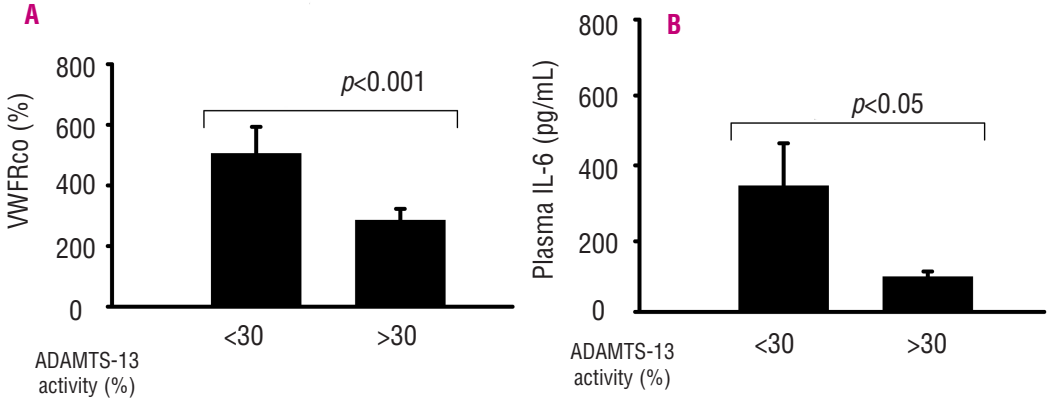

Figure 2. Patients with severe ADAMTS-13 deficiency (<30% activity) had higher ristocetin co-factor activity (A, Student's t test, n=40) and plasma levels of IL-6 (B, Student's t test, n=21).

consumptive thrombocytopenia and ischemic organ failure. Consistent with this hypothesis, microthromboembolism has often been found in autopsies of patients with fatal septic shock.[15]

Multiple factors contribute to the ADAMTS-13 deficiency found in septic patients. First, the synthesis of ADAMTS-13 is deficient, as indicated by reduced ADAMTS-13 antigen, or the enzyme is cleaved by thrombin as previously reported.[16] Second, the systemic and persistent release of ULVWF may consume ADAMTS-13.[17,18] Third, ULVWF proteolysis could be affected by inflammatory mediators such as IL-6.[19] We found that IL-6 levels are significantly elevated in septic patients, especially those with severe ADAMTS-13 deficiency. An improvement in ADAMTS-13 activity is paralleled by a reduction in plasma IL-6, consistent with previous reports that high IL-6 levels are associated with lower platelet counts,[20] higher rates of organ failure[21] and mortality in pediatric patients with severe sepsis.[22] Finally, the underlying conditions present in some patients could also potentially affect ULVWF proteolysis. However, our series of patients was too small to evaluate such mechanisms.

In summary, our results indicate that ADAMTS-13 deficiency may play a role in the development of sepsis-associated thrombocytopenia and organ failure. The notion is consistent with the clinical improvement of septic patients given plasma therapy,[23] but more studies are needed to determine the role of ULVWF proteolysis in the pathology of sepsis. This study also raises the question of whether ADAMTS-13 activity should be assayed in septic patients, especially those with severe thrombocytopenia.

Author Contributions

TCN: recruited patients, data analysis, and manuscript writing; AL: sample testing and analysis (cytokines); LL: sample testing and analysis (flow cytometry); CB: sample analysis (VWF); HC: sample analysis; WSM: patient recruitment; KA: sample analysis (ADAMTS-13 activity); ALB: sample analysis; J-fD: study design, patient recruitment, data analysis, and manuscript writing.

Conflict of Interest

The authors reported no potential conflicts of interest.

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