Severe Secondary Deficiency of von Willebrand Factor-Cleaving Protease (ADAMTS13) in Patients With Sepsis-induced Disseminated Intravascular Coagulation: Its Correlation to Development of Renal Failure

Short title: Severe ADAMTS13 deficiency in sepsis-induced DIC

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ABSTRACT

Deficiency of ADAMTS13 is found in patients with thrombotic thrombocytopenic purpura (TTP) and the genetic defects in the ADAMTS13 gene or the autoantibody against ADAMTS13 is thought to be responsible for the development of TTP. The clinical correlation and mechanisms of secondary ADAMTS13 deficiency in other disease states were investigated. In addition to TTP, ADAMTS13 levels were severely decreased in patients with sepsis-induced disseminated intravascular coagulation (DIC). The incidence of acute renal failure and serum creatinine levels in patients with ADAMTS13 activity levels less than 20 % (incidence: 41.2 %, creatinine: 1.81±1.70 mg/dL) were significantly higher than those in patients with ADAMTS13 activity levels higher than 20% (incidence: 15.4 %, creatinine: 0.95±0.76 mg/mL) (p<0.05, p< 0.01). Additionally, unusually large von Willebrand factor multimers were detected in 26 out of 51 patients (51.0 %) with ADAMTS13 activity levels less than 20 %. Lower molecular weight forms of ADAMTS13 were found in plasma of sepsis-induce DIC patients, suggesting that deficiency of ADAMTS13 partly was due to its cleavage by proteases in addition to decreased synthesis in the liver. These data suggested that severe secondary ADAMTS13 deficiency can be associated with sepsis-induced DIC and may contribute to development of renal failure.
INTRODUCTION

Deficiency of the von Willebrand factor (vWF)-cleaving protease, ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type 1 repeats) is found in most patients with thrombotic thrombocytopenic purpura (TTP), and this deficiency is thought to be responsible for platelet aggregation and microthrombi formation in the circulation, which in turn cause typical thrombotic microangiopathies (TMA) to develop. Deficiency of ADAMTS13 in TTP is caused by either genetic defects in the ADAMTS13 gene (familial TTP, Upshaw Schulman syndrome) or the presence of autoantibodies against ADAMTS13. Although hemolytic uremic syndrome (HUS) is clinically similar to TTP, the role of ADAMTS13 deficiency in the development of HUS is controversial, since there are conflicting reports about whether ADAMTS13 activity remains unchanged or decreases. It also is possible that secondary deficiency of ADAMTS13 may account for the development of microthrombi formation in other disease states besides TTP. To search for the clinical correlation of secondary ADAMTS13 deficiency in disease states, we measured ADAMTS13 activity levels by the standard method and determined antigen levels by our newly developed monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) for ADAMTS13 in TTP patients and sepsis-induced disseminated intravascular coagulation (DIC) patients. We found that severe secondary ADAMTS13 deficiency could occur in patients with sepsis-induced DIC and that it had a clinical correlation with development of renal failure.

MATERIALS AND METHODS

Blood samples

All samples were obtained with informed consent from patients according to the Declaration of Helsinki. Blood was drawn from 113 patients (65 men, aged 17-83 and 44 women, aged 21-81; idiopathic TTP, 3; Upshaw Schulman syndrome, 1; patients with sepsis-induced DIC, 109). The diagnosis of TTP was made noting the presence of typical clinical features (fever, bleeding
tendency, neurological symptoms); laboratory examinations (thrombocytopenia, hemolytic anemia with the presence of red cell fragmentation, increased levels of LDH, increased levels of serum creatinine); and the effectiveness of plasma exchange treatment. Patients with definite infection such as bacteremia, pneumonia, urinary tract infection, biliary tract infection, or pathogenic E. coli O-157 infection were excluded from the TTP group. The Upshaw Schulman syndrome patient had been suffering from TTP, and plasma transfusion was shown to be effective in preventing the recurrence of TTP.

The diagnosis of DIC was made according to the criteria established by the Japanese Ministry of Health and Welfare in 1988. The criteria for DIC were reported previously. Briefly, the presence of underlying disease such as infection and malignancies; specific clinical conditions (i.e., bleeding symptoms, organ dysfunction); and the results of the laboratory examinations (i.e., platelet counts, prothrombin time, fibrinogen, fibrin degradation products) were quantified on the score basis. If the score was 7 or more, the diagnosis of DIC was made. In the case of patients having a hematological malignancy, scores on the bleeding symptom and platelet counts were excluded and the diagnosis of DIC was made if the total score was 4 or more.

The diagnosis of sepsis was made according to the guidelines of the Society of Critical Care Medicine Consensus Conference Committee. Briefly, patients had to meet at least three of the four criteria for systemic inflammatory response and had to have a known infection or a suspected infection, as evidenced by one or more of the presence of bacteremia, pathological microorganisms or white blood cells in a normally sterile body fluid such as urine and joint fluid; the production of purulent sputum; radiographic evidence of pneumonia; clinical signs associated with a high risk of infection (for examples, cholangitis, peritonitis); or increased levels of endotoxin, β-D-glucan, or Candida antigen.

Thirty-nine patients with DIC were shown to have bacteremia as evidenced by their blood cultures. Twelve patients, whose bacteremia was not evidenced by blood culture, had increased levels of endotoxin, β-D-glucan, or Candida antigen. Of the patients who were negative for bacteria
in blood culture or did not have increased levels of endotoxin, β-D-glucan, or Candida antigen, 28 patients had pneumonia evidenced by radiography, 11 patients had urinary tract infection, 4 patients had wound infection during post operative periods, 1 patient had biliary tract infection, 1 patient had bacterial arthritis, 1 patient had bacterial osteomyelitis, and 12 patients had suspected respiratory infection with the presence of pathogenic microorganisms such as Methicillin-resistant Staphylococcus aureus in sputum cultures.

Citrated platelet-poor plasma samples were prepared and stored at –80 ºC until use. Blood was also drawn from 12 normal volunteers (7 men, aged 25-53 and 5 women, aged 25-48) for preparation of normal pooled plasma. Laboratory analyses of patients’ blood were performed by the standard methods using automated analyzers. Complete blood cell counts, serum creatinine (normal range: 0.4-1.1 mg/dL), serum bilirubin (normal range: 0.2-1.2 mg/dL), aspartate aminotransferase (AST, normal range: 8-35 International Units/L), alanine aminotransferase (ALT, normal range: 5-40 International Units/L), serum albumin (normal range: 3.9-5.1 g/dL), and C-reactive protein (CRP, normal range: < 0.5 mg/dL) were measured in this study.

**Determination of ADAMTS13 antigen and activity levels**

The human ADAMTS13 cDNA used in this study was described previously. Human ADAMTS13 was expressed in human embryo kidney 293 cells stably transfected with pCAG-ADAMTS13 Neo and purified. Murine monoclonal antibodies to human ADAMTS13 (MoAb) were generated by the standard method after immunization of BALB/C mice with recombinant human ADAMTS13. Two MoAbs, WH 10 and WH2-22-1A, were selected for the ELISA, which were shown to bind to the third TSP-1 motif and to the disintegrin domain of ADAMTS13 by the binding study to recombinant ADAMTS13 mutants, respectively. WH10 (2 µg/mL) was used for microtiter plate coating (Maxi Sorp plate, Nalge Nunc International, Rochester, NY). After blocking with 1 % casein, plasma samples from healthy subjects and patients were diluted in phosphate-buffered saline, pH 7.2 / 0.1 % casein and then incubated in WH10-coated plates. ADAMTS13 bound to the microtiter plates was detected by peroxidase-conjugated WH2-22-1A.
Purified recombinant ADAMTS13 was used as the standard to determine ADAMTS13 antigen levels in normal plasma. The ADAMTS13 level in each patient’s plasma was expressed as the percentage of that in normal pooled plasma. ADAMTS13 activity levels in plasma were measured according to the previously described method. Briefly, 10 µL of plasma was mixed with purified vWF (1 µg) in 100 µL of the reaction buffer (5 mM Tris pH 8.0/1.5 M urea/10 mM BaCl2/0.4 mM Pefabloc SC (Roche Diagnostics, Mannheim, Germany)) at 37 °C for 24 h. Reaction was terminated by addition of 10 µL of 500 mM EDTA, pH 8.0. Portions of samples were subjected to 1.4 % SDS-agarose gel electrophoresis to determine the extent of vWF degradation. After electrophoresis, proteins were transferred to PVDF membranes and vWF multimers were detected by peroxidase-labeled rabbit anti human vWF antibodies (Dako, Glostrup, Denmark).

**Quantification of molecular markers of DIC**

Plasma levels of fibrin degradation products (FDP) were quantified with commercial kits (Roche Diagnostics, Tokyo, Japan) used for laboratory examinations. Since quantification of free thrombin concentration in plasma is technically difficult, plasma levels of thrombin/antithrombin III complexes (TAT) are quantified by the ELISA (Sysmex, Inc., Kobe, Japan). Similarly, plasma levels of plasmin/α2 plasmin inhibitor complexes (PIC) were measured by ELISA methods with commercial kits (Sysmex, Inc.) used for laboratory examinations. Plasma plasminogen activator inhibitor 1 (PAI-1) levels were quantified by the latex photometric immunoassay by using a commercial kit (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan) as described previously. The granulocyte elastase digests of cross-linked fibrin (granulocyte elastase-dependent fibrin degradation products, E-XDP) were measured by the automated latex photometric immunoassay using IF-123 monoclonal antibody, which is specific for the fibrin fragment D species generated by granulocyte-elastase digestion. Monoclonal antibody IF-123 bound latex particles (Mitsubishi Kagaku Iatron, Inc.) were used for the assay. A 2.4-µL aliquot of sample plasma was mixed with 32 µL of the latex reagents in 250 µL of Tris-buffered saline, and then absorbance changes were analyzed with an automated analyzer for latex photometric immunoassay (model LPIA-NV7,
Mitsubishi Kagaku Iatron, Inc.). The standard E-XDP was purified according to the method of Kohno et al.\textsuperscript{20} The normal range of plasma E-XDP levels is less than 3 U/mL.

**Effect of granulocyte elastase on ADAMTS13**

Recombinant ADAMTS13 (250 nM) was incubated in 20 µL of Tris-buffered saline, pH 7.4 in the absence or the presence of granulocyte elastase (Elastin Products, Owensville, MO) at 5 nM and 50 nM. Aliquots (5 µL each) were harvested after incubation at 37 °C for 5, 15, and 30 min. The reaction of each aliquot was terminated by addition of the SDS-PAGE sample buffer containing 2% SDS. The samples were then analyzed by SDS-PAGE followed by Western blotting with anti ADAMTS13 monoclonal antibody WH2-22-1A and peroxidase-labeled anti mouse IgG.

**Detection of ADAMTS13 molecular forms in plasma**

The Western blot analysis for ADAMTS13 in plasma by MoAb WH2-22-1A was performed after immunoprecipitation with anti-ADAMTS13 polyclonal antibody immobilized to protein G Sepharose.

**Analysis of vWF multimers in patient plasma**

The vWF multimers in patient plasma was analyzed by SDS-agarose gel electrophoresis according to the method described previously.\textsuperscript{12-14}

**RESULTS**

**ELISA for ADAMTS13**

We generated monoclonal antibodies against recombinant human ADAMTS13 and used them to develop a monoclonal antibody-based ADAMTS13 ELISA. To determine the specificity of this assay, plasma obtained from a patient with Upshaw Schulman syndrome was mixed with normal plasma at various ratios and the ADAMATS13 activity and antigen levels were measured. As shown in Figure 1, the ADAMTS13 activity and the ADAMTS13 antigen levels in Upshaw Schulman syndrome plasma were less than 1 %, and the ADAMTS13 antigen level in the patient plasma increased in parallel with the ADAMTS13 activity in the presence of increasing amounts of...
normal plasma linearly. The correlation coefficient ($r$) value between ADAMTS13 antigen and ADAMTS13 activity was 0.997. The ADAMTS13 level in normal pooled plasma was 1.57 µg/mL when recombinant human ADAMTS13 was used as the standard. The calibration curve was linear ($r=0.999$) and the ELISA could distinguish absorbance changes of ADAMTS13 at 0.3 % of the normal plasma level from ADAMTS13- depleted plasma. The inter-assay variability in samples containing 50 % and 100 % of ADAMTS13 were 7.9% and 5.2%, respectively.

**ADAMTS13 levels in disease states**

ADAMTS13 antigen and activity levels in plasma of patients with sepsis-induced DIC or TTP were studied (Figure 2). The correlation coefficient ($r$) value of ADAMTS13 antigen and ADAMTS13 activity was 0.80. As shown in Figure 2A, discrepancies between ADAMTS13 antigen levels and activity levels were observed in many samples. These discrepancies mainly were caused by the decreased specific ADAMTS13 activity relative to the ADAMTS13 antigen level. There appear to be samples with a higher specific activity of ADAMTS13. To explore the possibility that decreased levels of the ADAMTS13 specific activity correlated with disease states, the western blot analysis of ADAMTS13 molecular forms in patient plasma was performed. Low molecular weight ADAMTS13 species were observed in DIC patient plasma by Western blotting (Figure 2B), indicating proteolytic cleavage of ADAMTS13 could occur under this disease state. The recent report showed that ADAMTS13 could be digested by proteases such as thrombin and plasmin *in vitro*. Since thrombin and plasmin can be generated in DIC, we tested the correlation between ADAMTS13 levels and molecular markers of coagulation and fibrinolysis. There was no correlation of the ADAMTS13 activity, antigen, or the specific activity level with either levels of fibrinogen, FDP, TAT, PIC, PAI-1, or platelet counts (Table 1). We could only find a negative correlation between the activity levels and the antigen levels of ADAMTS13 and the plasma levels of granulocyte elastase digests of fibrin (E-XDP) (Table 1, Figure 3A, B). Based upon these results, we studied effects of granulocyte elastase on ADAMTS13 *in vitro*. In accordance with previous reports, recombinant ADAMTS13 was determined to migrate at approximately 190 kDa by SDS-
PAGE followed by Western blotting.\(^{14,21}\) As shown in Figure 3C, recombinant ADAMTS13 migrating at approximately 190 kDa was converted to the 120 kDa and 100 kDa fragments, and finally to the approximately 40 kDa fragment upon incubation with granulocyte elastase in a dose-dependent and time-dependent manner in vitro. A variety of lower molecular ADAMTS13 fragments were detected in DIC patient plasma by Western blot (Figure 2B). According to the previous report, the ADAMTS13 fragments migrating approximately 150–170 kDa could be generated by thrombin.\(^{21}\) The ADAMTS13 fragments migrating approximately 120 kDa and 100 kDa in patient plasma might correspond to the granulocyte elastase digests of ADAMTS13. However, the 120 kDa ADAMTS13 fragment and the 100 kDa ADAMTS13 fragment could be generated by thrombin and plasmin, respectively.\(^{21}\) It also is possible that thrombin-cleaved ADAMTS13 or plasmin-cleaved ADAMTS13 could be digested by granulocyte elastase or vice versa. These data may suggest that granulocyte elastase plays a role in ADAMTS13 cleavage under certain pathologic conditions together with other proteases (i.e., thrombin and plasmin), and this may partly account for the decrease of the ADAMTS13 specific activity observed in DIC patients.

**ADAMTS13 deficiency in disease states**

ADAMTS13 antigen and activity levels in patient groups and in healthy subjects are shown in Figure 4. The plasma ADAMTS13 antigen and activity levels in untreated patients with TTP (no plasma exchange treatment, no fresh frozen plasma transfusion) were 13.5±7.1% (range: 5.1-19.6%) and 6.3±5.7 (range: 0-12.5 %), respectively(idiopathic TTP 3, Upshaw Schulman syndrome 1). Decreased levels of ADAMTS13 antigen and activity were observed in patients with sepsis-induced DIC compared with healthy subjects (p< 0.01) in this study, and severe decreases of ADAMTS13 activity and antigen levels were observed in quite a few patients with sepsis-induced DIC. Of the 109 sepsis-induced DIC patients, decrease of ADAMTS13 activity levels less than 5 % were found in 17 patients (15.6 %); the clinical features and laboratory data of these patients are summarized in Table 2. Consciousness disturbance, thrombocytopenia, decrease of hemoglobin concentration, and increased LDH levels were commonly found in these patients. These clinical
features were indistinguishable from those of TTP, though these patients had evidence of infection.

Since the highest ADAMTS13 activity level in TTP patients without plasma exchange or blood transfusion in this study was 12.5 %, patients with sepsis-induced DIC were divided into two groups. One included patients with decreased ADAMTS13 activity levels less than 20 % (n=51) and the other included patients with ADAMTS13 activity levels more than 20 % (n=52). Patients with chronic renal failure prior to the infection were excluded from this analysis. Since these patients were each in a severe condition, 25 out of 51 patients (49.0 %) of the former group and 35 out of 52 patients (67.3 %) of the latter group had received blood transfusion of fresh frozen plasma and/or platelet concentrates within 5 days prior to determination of ADAMTS13 levels. This may affect the activity and antigen levels of ADAMTS13.

**Correlation between secondary ADAMTS13 deficiency and organ failure**

Analyses of clinical and laboratory data showed that the patients with severe ADAMTS13 deficiency (ADAMTS13 activity <20 %) had elevated serum creatinine levels (Figure 5), which were significantly higher than those in patients with ADAMTS13 levels higher than 20% (Table 3). The incidence of renal injuries in patients with severe ADAMTS13 deficiency (ADAMTS13 activity < 20 %) was significantly higher than that in patients with ADAMTS13 activity levels higher than 20 % (Table 3). However, there were no differences in the incidence of liver dysfunction or serum levels of bilirubin, AST, and ALT between these groups (Table 3), suggesting that severe ADAMTS13 deficiency in these patients may be linked to development of renal injuries. There was a significant difference in serum albumin levels between these two groups, suggesting that the decrease of ADAMTS13 activity and antigen levels in the patients was at least partly due to reduced synthesis in the liver.

**Analysis of vWF multimers in patients with severe secondary ADAMTS13 deficiency**

Additionally, unusually large vWF multimers were detected in plasma of patients with severe secondary ADAMTS13 deficiency (ADAMTS13 activity < 20 %) as shown in Figure 6. The serum creatinine levels in patients in whom both unusually large vWF multimers and severe ADAMTS13
deficiency were detected were significantly higher than those in patients in whom the unusually
large vWF multimers were absent (Table 4). There was no significant difference in the
ADAMTS13 activity (Table 4) and the ADAMTS13 specific activity between these patient groups
(not shown).

There was a significant difference of CRP levels between the ADAMTS13 activity < 20% group and the ADMATS13 activity > 20% group, but their platelet counts were not significantly
different (not shown), indicating that the decrease of ADAMTS13 may be related to inflammatory
responses. These results are consistent with the data showing a negative correlation between the
activity and antigen levels of ADAMTS13 and the plasma levels of granulocyte elastase digests of
fibrin (E-XDP).

**DISCUSSION**

ADAMTS13 has been shown to play an important role in vWF processing. As shown
previously, ADAMTS13 may cleave the unusually large multimers of vWF on the endothelial cell
surface, preventing entrance of such unusually large multimers into the circulation. Without this
processing of vWF multimers, the unusually large multimers of vWF secreted from endothelial
cells would enter into the circulation and initiate platelet thrombus formation, which in turn will
cause the development of TMA. Patients with primary ADAMTS13 deficiency caused by
defects in the ADAMTS13 gene or having autoantibodies against ADAMTS13 have been shown to
develop TTP, suggesting the important physiological role of ADAMTS13 catalyzed cleavage of
these unusually large vWF multimers in humans. TTP is a fatal thrombotic microangiopathic
disease if the patients are not treated appropriately, but the incidence of TTP is low. While
searching for the role of ADAMTS13 in common thromboembolic diseases, we found severe
secondary ADAMTS13 deficiency in patients with sepsis-induced DIC and showed its clinical
correlation to development of renal failure in this study.

DIC is associated with a variety of disease states such as sepsis, advanced malignancies,
severe tissue damages, and pregnancy-related complications. Sepsis may be the most common pathogenic disease that leads to the development of DIC, and the endotoxemia and the high cytokine levels in the circulation are thought to induce tissue factor expression that in turn initiates fibrin thrombus formation in the circulation. Microthrombi formed in the circulation cause ischemia of a variety of organs and damage them. Lines of evidence have suggested that proteases released from white blood cells may also be involved in development of organ injuries. This study showed that patients with sepsis-induced DIC frequently exhibited decreased antigen and activity levels of ADAMTS13 and that severe ADAMTS13 deficiency was found in these patients at high incidence. Many patients in this study had been transfused with ADAMTS13-containing blood products, such as fresh frozen plasma and platelet concentrates, closely prior to blood sample collection for determination of ADAMTS13 levels, suggesting that the levels of ADAMTS13 in plasma samples of these patients might not reflect the severity of ADAMTS13 deficiency prior to blood transfusion.

Thus, severe secondary ADAMTS13 deficiency in sepsis-induced DIC might be more common. Clinical manifestations and laboratory data of these septic patients with secondary severe ADAMTS13 deficiency were nearly indistinguishable from those of TTP patients, though these patients had evidence of infection (Table 2), indicating that there exist a subset of patients who have secondary severe ADAMTS13 deficiency caused by sepsis and that they not only are clinically similar to TTP but also might have the same pathophysiology of ADAMTS13 deficiency for development of TMA as idiopathic TTP.

Development of organ failure might be caused by both tissue factor-dependent fibrin thrombus formation and platelet aggregation due to severe ADAMTS13 deficiency in the sepsis-induced DIC patients with ADAMTS13 activity levels less than 20%. This notion was supported by the correlation between severe secondary ADAMTS13 deficiency and renal failure in sepsis-induced DIC patients with ADAMTS13 activity levels less than 20%. We could not find any significant difference in the ADAMTS13 specific activity levels between these two groups (not shown). One possibility is that small molecular forms of ADAMTS13 could be lost into urine.
because of the presence of renal injuries. However, we could not determine if this was the case because no urine samples were available for study.

In the previous report by Reife et al., TMA patients who did not have DIC were analyzed for the correlation between ADAMTS13 activity levels and serum creatinine levels without distinguishing TTP from HUS. They found that the creatinine levels in patients with severely decreased ADAMTS13 activity levels were significantly lower than those in patients without severely decreased ADAMTS13 activity levels. These data are opposite to our data that the patients with severe ADAMTS13 deficiency (ADAMTS13 activity <20 %) had significantly higher serum creatinine levels than patients with the ADAMTS13 activity levels higher than 20%. Since HUS patients were not distinguished from TTP patients in the report by Reife et al., it is possible that the patients without severe ADAMTS13 deficiency in that study mainly included HUS patients. Since patients with sepsis-induced DIC were studied in our study, the difference in the patient groups would explain the opposite findings. There was no apparent difference between the levels of platelet counts in patients with the ADAMTS13 activity levels less than 20 % and those with the ADAMTS13 activity levels more than 20 %. The presence of underlying DIC in these patients and platelet transfusion may account for the data.

The presence of the unusually large multimers of vWF in the plasma of the patients with severe secondary ADAMTS13 deficiency and its correlation with the serum creatinine levels would support the notion that severe secondary ADAMTS13 deficiency may correlate with development of renal failure in sepsis-induced DIC. There was no significant correlation of the presence of the unusually large multimers of vWF with ADAMTS13 activity levels. This may be due to the technical difficulties in determining the unusually large vWF multimers and the differences in endothelial cell damage among these patients.

Decreased specific activity of ADAMTS13 presumably caused by its cleavage by proteases would be a mechanism for severe secondary ADAMTS13 deficiency in patients with sepsis-induced DIC. A variety of proteases have been shown to degrade ADAMTS13 in vitro. Thrombin
and plasmin are generated in DIC, and these enzymes may cleave ADAMTS13, resulting in inactivation of ADAMTS13. Our data suggest that granulocyte elastase may be one of the proteases that cleave ADAMTS13 together with thrombin and plasmin under in vivo pathological conditions. In this regard, the case report of chronic relapsing TTP by Galbusera et al., 26 which showed that α1-antitrypsin (the physiological granulocyte elastase inhibitor) therapy was effective for prevention of appearance of unusually large vWF multimers in the circulation but not for prevention of TTP relapse, was interesting and also suggested the link between granulocyte elastase and cleavage of ADAMTS13. Correlation between ADAMTS13 activity and antigen levels and E-XDP levels not only in TTP patients but also in pathogenic E. coli-infection related HUS patients would be the further study to investigate the role of granulocyte elastase in TMA development. Specific inhibitors of these proteases are present at high concentrations in blood, indicating that cleavage of ADAMTS13 by these proteases may depend on the kinetic balance between ADAMTS13, the proteases, and their inhibitors, and thus, cleavage of ADAMTS13 by these proteases may not proceed completely in vivo. It also is possible that other proteases could also digest ADAMTS13 in the disease state. This possibility should be investigated in a future study.

Since serum albumin levels decreased in most patients, liver injuries associated with the underlying disease might be an additional mechanism for decreasing ADAMTS13 antigen levels because this enzyme is synthesized in the liver. Mutations or polymorphisms of the ADAMTS13 gene are another possible cause of a decrease or increase of the ADAMTS13 specific activity. These possibilities also should be explored in future studies.

In conclusion, the precise analysis of ADAMTS13 antigen and activity levels in disease states offers insights into the roles of ADAMTS13 in thromboembolic diseases. Severe ADAMTS13 deficiency takes place secondarily in disease states such as sepsis-induced DIC and it may not be specific for idiopathic TTP and may not have a solo diagnostic value for idiopathic TTP. Though the mechanisms of severe ADAMTS13 deficiency in sepsis are different from those of idiopathic TTP, clinical features of sepsis-induced DIC patients with severe ADAMTS13 deficiency are
similar to those of idiopathic TTP patients and sepsis may partly have the same pathophysiology of severe ADAMTS13 deficiency for TMA development as idiopathic TTP, raising a possibility of novel supportive therapies for septic patients with severe ADAMTS13 deficiency such as ADAMTS13 supplementation, α1-antitrypsin administration, and use of synthetic granulocyte elastase inhibitors. Since severe secondary ADAMTS13 deficiency may correlate with development of organ injuries in sepsis-induced DIC patients, determination of the ADAMTS13 levels of patients in severe conditions upon hospitalization would provide better understanding of the pathological conditions. Current analyses of ADAMTS13 levels in disease states is a retrospective study, and thus, the prospective study for timely execution of ADAMTS13 supplementation for patients with not only TTP, but also ADAMTS13 deficiency-related pathologic states would be needed.
References


10. Loof AH, van Vliet HH, Kappers-Klunne MC. Low activity of von Willebrand factor-cleaving protease is not restricted to patients suffering from thrombotic thrombocytopenic purpura. Br J


Table 1. Correlation between the ADAMTS13 levels and molecular markers of DIC in patients with sepsis-induced DIC

<table>
<thead>
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<th>ADAMTS13</th>
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<tr>
<td></td>
<td>n</td>
<td>Activity (%)</td>
<td>Antigen (%)</td>
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<td></td>
<td></td>
<td>rs*</td>
<td>rs*</td>
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<tr>
<td>Fibrinogen</td>
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<td>FDP</td>
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<td>E-XDP</td>
<td>109</td>
<td>-0.399†</td>
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*rs values determined by Spearman’s correlation coefficient by rank test

† Statistically significant (P<0.01)
Table 2. Clinical profiles of sepsis-induced DIC patients with ADAMTS13 activity levels below 5 %

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<tr>
<th>Parameter</th>
<th>Value</th>
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<tr>
<td>Age</td>
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<tr>
<td>Consciousness disturbance</td>
<td>8/17 (47.1 %)</td>
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<td>Blood transfusion</td>
<td>11/17 (64.7%)</td>
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<tr>
<td>ADAMTS13 Antigen (%)</td>
<td>25.5±13.6</td>
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<tr>
<td>Creatinine (mg/dL)</td>
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<td>Albumin (g/dL)</td>
<td>2.2±0.5</td>
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<td>White blood cell counts (/µL)</td>
<td>11200 ± 7500</td>
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<td>Red blood cell counts (x10⁴/µL)</td>
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<td>Hemoglobin (g/dL)</td>
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<td>Platelet counts (x10⁶/µL)</td>
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<td>LDH (IU/L)</td>
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<tr>
<td>CRP (mg/dL)</td>
<td>18.11±13.41</td>
</tr>
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Values are the mean ± SD.
Table 3. Correlation between ADAMTS13 levels and organ injuries of patients with sepsis-induced DIC

<table>
<thead>
<tr>
<th></th>
<th>ADAMTS13 Activity &lt; 20%</th>
<th>ADAMTS13 Activity &gt; 20%</th>
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<tr>
<td></td>
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<td>n=52</td>
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<td>Creatinine (mg/dL)</td>
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<tr>
<td>ALT (IU/L)</td>
<td>72±109</td>
<td>122±160</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>2.70±3.13</td>
<td>2.20±2.53</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.3±0.4</td>
<td>2.9±0.7</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>13.50±10.51</td>
<td>6.90±8.61</td>
</tr>
</tbody>
</table>

*Statistically significant (Welch’s t-test), † NS: not significant
Values are the mean ± SD.

Incidence of organ injury

<table>
<thead>
<tr>
<th></th>
<th>ADAMTS13 Activity &lt; 20%</th>
<th>ADAMTS13 Activity &gt; 20%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=51</td>
<td>n=52</td>
</tr>
<tr>
<td>Renal Injury</td>
<td>21 (41.2 %)</td>
<td>8 (15.4 %)</td>
</tr>
<tr>
<td>Liver Injury</td>
<td>40 (78.4 %)</td>
<td>38 (73.1 %)</td>
</tr>
</tbody>
</table>

Renal Injury: Serum Creatinine>1.2 mg/dL
Liver Injury: Elevation of one of Bilirubin (>2.0 mg/dL), AST (>40 IU/L), or ALT (>40 IU/L)

*Statistically significant (Fisher’s exact probability test), † NS: not significant
Table 4. Correlation between the presence of unusually large multimers of vWF and the serum creatinine levels of sepsis-induced DIC patients with ADAMTS13 activity levels less than 20 %

<table>
<thead>
<tr>
<th>Unusually Large Multimers of vWF</th>
<th>+</th>
<th>-</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=26</td>
<td>n=25</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>2.39±2.24</td>
<td>1.34±1.35</td>
</tr>
<tr>
<td>ADAMTS13 Activity (%)</td>
<td>6.6±6.8</td>
<td>8.9±6.0</td>
</tr>
</tbody>
</table>

*Statistically significant (Welch’s t-test), † NS: not significant

Values are the mean ± SD.

vWF indicates von Willebrand Factor.
Figure legends

Figure 1. Analysis of ADAMTS13 activity and antigen levels in plasma of patients with Upshaw Schulman syndrome

ADAMTS13 activity and antigen levels in the plasma of an Upshaw Schulman syndrome (USS) patient mixed with normal pooled plasma at various ratios were determined. Panel A shows the result of ADAMTS13 activities in the plasma of the USS patient mixed with normal plasma at various ratios (0:10-10:0). The correlation of ADAMTS13 activity and antigen levels in these samples is shown in panel B.

Figure 2. Analysis of ADAMTS13 activity, antigen, and molecular forms in plasma of patients with sepsis-induced DIC

ADAMTS13 activity and antigen levels in plasma of patients with sepsis-induced DIC were determined as described in METHODS and shown in panel A. The samples indicated by open squares were subjected to immunoprecipitation followed by Western blotting to investigate the cleavage of ADAMTS13 as described in METHODS. Panel B shows a typical Western blot of degraded ADAMTS13 found in the patients’ plasma indicated by the open squares in panel A. Western blotting of ADAMTS13 antigen in normal pooled plasma (N) is shown as the control. ADAMTS13 molecules in normal plasma migrated at approximately 190 kDa.

Figure 3. Correlation between the ADAMTS13 levels and the granulocyte elastase digests of cross-linked fibrin (E-XDP) levels in plasma of patients with sepsis-induced DIC and effect of granulocyte elastase on ADAMTS13 in vitro

The correlations between the activity levels of ADAMTS13 and the plasma levels of granulocyte-elastase digests of fibrin (E-XDP) (panel A), and between the antigen levels of ADAMTS13 and the plasma levels of granulocyte-elastase digests of fibrin (E-XDP) (panel B) in patients with sepsis-induced DIC are shown. Values were analyzed by Spearman’s correlation coefficient by rank test.
Recombinant ADAMTS13 was incubated with granulocyte elastase at 5 nM or 50 nM, and degradation of ADAMTS13 by granulocyte elastase was studied after the indicated time and analyzed as described in METHODS (panel C).

**Figure 4. Plasma ADAMTS13 levels in patients and healthy subjects**

ADAMTS13 activity levels (panel A) and antigen levels (panel B) of healthy subjects, TTP patients (idiopathic TTP, 3; Upshaw Schulman syndrome, 1) before plasma exchange treatment, and patients with sepsis-induced DIC (n=109) are shown. Differences in the mean values (bars) between the healthy subject group and patient groups were statistically significant (Non-repeated measures ANOVA and Dunnett’s test, \( P<0.01 \)).

**Figure 5. Correlation between the plasma ADAMTS13 levels and the serum creatinine levels**

Correlation between serum creatinine levels and ADAMTS13 activity (closed circle) levels or antigen (open circle) levels in patients with sepsis-induced DIC is shown (n=103). Patients with a past history of chronic renal failure were excluded from the study.

**Figure 6. Analysis of vWF multimers of patients with sepsis-induced DIC**

vWF multimers in plasma of sepsis-induced DIC patients with ADAMTS13 activity levels less than 20 % were analyzed by SDS-agarose gel electrophoresis as described in METHODS. The vWF multimer patterns of patients and healthy subjects (N) were analyzed simultaneously. The typical unusually large multimers of vWF found in patients’ plasma with ADAMTS13 activity levels less than 20 % are shown and indicated by the asterisks.
A

Normal plasma standard (%)  
Ratios of USS plasma and normal plasma (v/v)

B

$y = 0.9788x + 0.9388$

$R = 0.997$

Fig.1 Ono et al.
A

\[ Y = 0.7501x - 4.2759 \quad R = 0.80 \]
\[ N = 109 \quad (p<0.01) \]

ADAMTS13 Activity (%) vs ADAMTS13 Antigen (%)

B

Fig. 2 Ono et al.
A

$y = -0.0528x + 35.886, R_s = -0.399, (p<0.01)$

B

$y = -0.0346x + 42.048, R_s = -0.404, (p<0.01)$

Fig3 Ono et al.
Fig4 Ono et al.
Figure 5. Ono et al.
Severe secondary deficiency of von Willebrand factor-cleaving protease (ADAMTS13) in patients with sepsis-induced disseminated intravascular coagulation: Its correlation to development of renal failure

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