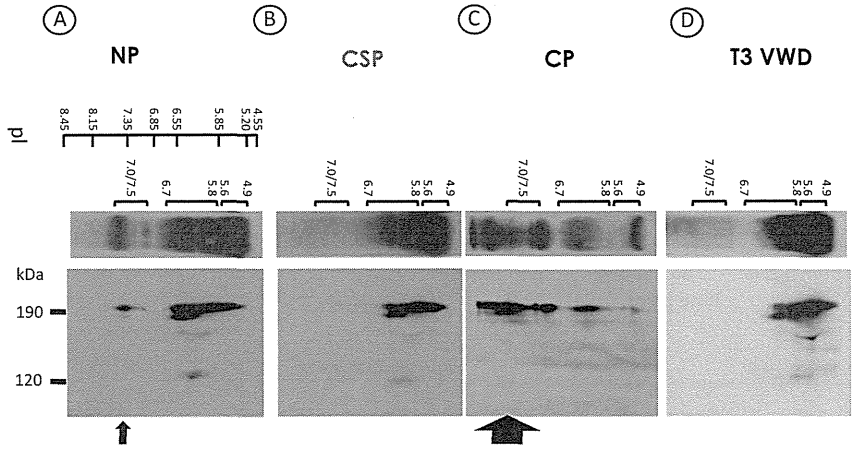

Fig. 5. ADAMTS13 in plasma fractions separated by IEF followed by SDS-PAGE. Normal plasma (NP), CSP, CP, and T3-VWD plasmas were subjected to IEF (upper panel). ADAMTS13 separated by IEF was subjected to a second dimension of electrophoresis by SDS-5% PAGE under reducing conditions and then to immunoblotting with anti-ADAMTS13 MoAb (WH2-11-1). (A) All three groups of ADAMTS13 bands (4.9-5.6, 5.8-6.7, and 7.0/7.5) in NP appeared as a 190-kDa by SDS-5% PAGE. Arrow indicates the VWF-ADAMTS13 complex. (B, D) The ADAMTS13 band at pI 7.0 or 7.5 was completely absent in CSP, almost indistinguishable to the case in T3-VWD plasma. (C) In CP, two faint bands with pI ranges of 4.9 to 5.6 and 5.8 to 6.7 and several strong bands with pI beyond 7.0 were detected. Arrow indicates the VWF-ADAMTS13 complex.

FFP ($r = 0.646$, $p < 0.01$; Fig. 4, right). These results indicate that the decreased level of ADAMTS13 activity in CP of blood group O was correlated with the low level of VWF antigen.

Cryoprecipitation efficiently removes the VWF-ADAMTS13 complex from plasma

To determine whether cryoprecipitation can remove the VWF-ADAMTS13 complex from plasma, we performed two-dimensional analysis (IEF followed by SDS-5% PAGE) under reducing conditions. As shown in Fig. 5A, all three groups of ADAMTS13 bands (pI 4.9-5.6, 5.8-6.7, and 7.0/7.5) in normal plasma migrated mainly as a 190-kDa band in SDS-5% PAGE, indicating that all three groups of bands included ADAMTS13. In CSP, however, the pI 7.0 or 7.5 band of ADAMTS13 was totally absent, almost indistinguishable from the case of T3-VWD plasma (Figs. 5B and 5D). Furthermore, when CSP was spiked with purified pd-VWF, a new band with pI 7.5 was generated (data not shown), indicating that ADAMTS13 in CSP can bind to higher molecular weight VWFMs and form a complex, as is the case in FFP.

In CP, we observed two faint bands with pI ranges of 4.9 to 5.6 and 5.8 to 6.7, and also several strong bands with pI greater than 7.0 (Fig. 5C).

Down regulation of H-SIPA with purified pd-ADAMTS13, CP, and CSP

In H-SIPA using a mixture of normal washed PLTs, ADAMTS13-dp plasma, and purified pd-VWF, maximum PLT aggregation (approx. 70% light transmittance) was achieved in the absence of ADAMTS13 (Fig. 6A). Under this condition, purified pd-ADAMTS13 spiked into the mixture inhibited H-SIPA in a dose-dependent manner at ranges of 5% to 20% of ADAMTS13 activity, but this effect reached a plateau (approx. 20% light transmittance) at the ranges from 50% to 500% of ADAMTS13 activity (Fig. 6A). Addition of NMC-4 almost totally blocked the PLT aggregation (approx. 3% light transmittance).

Further, ADAMTS13 in both FFP and CSP from normal individuals inhibited H-SIPA in a dose-dependent manner at the ranges of 5% to 20% of ADAMTS13 activity (Figs. 6B and 6C), comparable to the effect of purified pd-ADAMTS13.

On the other hand, ADAMTS13 in CP did not clearly inhibit H-SIPA at the initial phase before 140 seconds, even at 20% of ADAMTS13 activity. However, at the later phase of H-SIPA, the aggregation curves were uniformly reversed at a final concentration of 5% to 20% of ADAMTS13 activity. Consequently, the maximum PLT aggregation at the endpoint at 340 seconds was almost indistinguishable from that of FFP or CSP. Thus, the inhibition rates (%) in CP at two time points (140 and 340 sec) with two different final concentrations (5 and 20%) of ADAMTS13 activity were measured in each three times at the same occasion, and the results were the following: $20.5 \pm 14.0\%$ (at 140 sec) versus $46.9 \pm 11.3\%$ (at 340 sec; $p = 0.012$) in the presence of 5% ADAMTS13 activity and $57.7 \pm 5.9\%$ (at 140 sec) versus $85.7 \pm 2.7\%$ (at 340 sec; $p = 0.004$) in the presence of 20% ADAMTS13 activity (figure not shown).

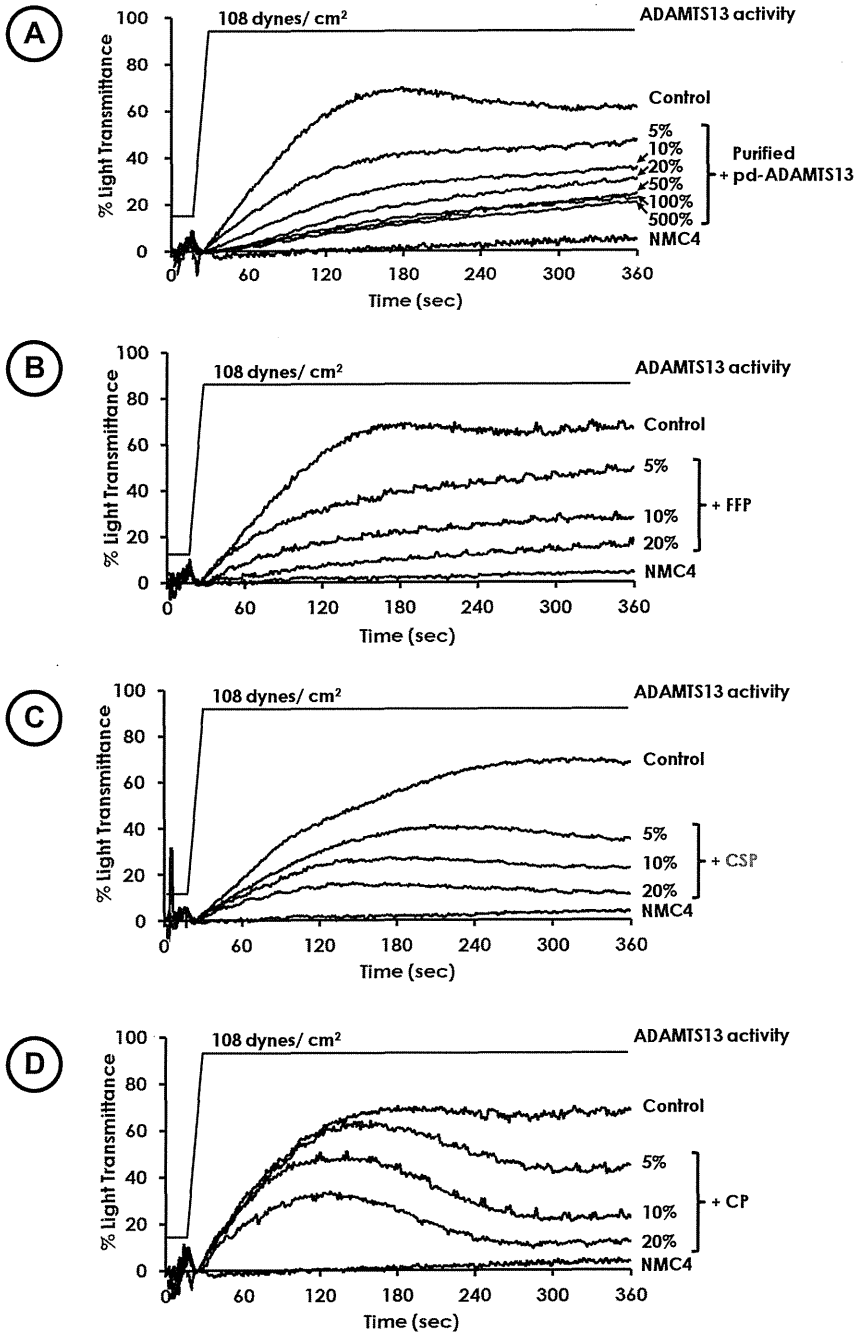

Fig. 6. Inhibitory effect of ADAMTS13 on H-SIPA. (A) The purified pd-ADAMTS13 inhibits H-SIPA in a dose-dependent manner at ranges of 5% to 20% of ADAMTS13 activity in the mixture. The inhibition reaches a plateau (approx. 20% light transmittance) at ranges of 50% to 500% of ADAMTS13 activity. (B, C) ADAMTS13 in both FFP and CSP from normal individuals exhibited dose-dependent inhibition of H-SIPA at the ranges of 5% to 20% of ADAMTS13 activity. (D) ADAMTS13 in CP did not clearly inhibit H-SIPA at the initial phase at less than 10% of ADAMTS13 activity. The inhibition of PLT aggregation was found at the ranges of 5% to 20% of ADAMTS13 activity; at the later phase of H-SIPA the maximum aggregation at the endpoint was almost the same as in FFP and CSP.

DISCUSSION

Using IEF analysis with a large-pore agarose-acrylamide composite gel, we have shown that ADAMTS13 in the plasma milieu is present in a complex with larger VWFMs, but is less likely to form complexes with smaller VWFMs (dimers and tetramers). Thus, cryoprecipitation followed by centrifugation could efficiently separate the two forms of ADAMTS13, with the VWF-bound ADAMTS13 in CP and the free ADAMTS13 and ADAMTS13 bound to smaller VWFMs in CSP. In support of these results regarding coprecipitation of ADAMTS13 and VWF, the ADAMTS13 activity levels we observed were closely correlated with VWF antigen levels in CP ($r = 0.646$), but not in either CSP ($r = -0.055$) or FFP ($r = 0.002$; Fig. 4). In addition, we observed no difference among blood groups with respect to the recovery rate of VWF antigen in CP, but the VWF antigen level in CP was lower in blood group O than in the other blood groups. As a result, both the ADAMTS13 activity and the VWF antigen levels in CP were significantly lower in blood group O than in non-O blood groups (Table 1). Further, we determined that approximately 95% of the original ADAMTS13 activity in FFP is recovered after cryoprecipitation; approximately 93% of the recovered ADAMTS13 activity remained in CSP, whereas 7% was found in CP. This relative distribution of ADAMTS13 in FFP and CSP was consistent with previous reports.[24-26]

Evidence that the pI 7.0 or 7.5 band is a complex of VWF and ADAMTS13 is clearly provided by the following observations: 1) plasmas from both VWF antigen–defective T3-VWD and ADAMTS13 antigen–defective USS patients lacked the bands at pI 7.0 or 7.5; 2) an equal mixture of plasmas from T3-VWD and USS generated the bands at pI 7.0 or 7.5; and 3) CSP prepared from normal plasma lacked the bands at pI 7.0 or 7.5, whereas CSP spiked with purified VWF regenerated these bands. On the other hand, we assume that the proteins in the two other band groups (pI 4.9-5.6 and 5.8-6.7) are less involved

in complex formation with VWF, because both band groups are present in T3-VWD plasma. Furthermore, because pd-ADAMTS13 purified from pooled normal plasmas has only one band with pI 4.9 to 5.6,[14] the pI 5.8 to 6.7 band might represent a complex with proteins other than VWF. This speculation originates from the observation that ADAMTS13 can bind in vitro to a soluble form of CD36[27] and Lys-plasminogen.[28]

In a previous study, immunoprecipitation method using anti-VWF was used to show that approximately 3% of the total in plasma ADAMTS13 is bound to VWF.[12] By contrast, in our IEF gel analysis, coupled with densitometry, we observed that the amount of VWF-bound ADAMTS13 in plasma appeared to be much lower than the amount of unbound ADAMTS13, but greater than the 3% of total ADAMTS13 (Fig. 1).[12] This discrepancy might be attributable to differences in the experimental designs employed in these studies.

The mechanism by which ADAMTS13 binds to VWF in the plasma milieu is a critical issue that remains to be addressed. Because Fujikawa and coworkers[29] succeeded in purifying ADAMTS13 from a commercial concentrate of Factor VIII and VWF, prepared from CP, such concentrates might contain the VWF-ADAMTS13 complex itself. After extensive fractionation, including fibrin-clot formation, ammonium sulfate precipitation, and sequential chromatography, the purified ADAMTS13 described in that study was free of VWF. However, in our experience, the VWF-ADAMTS13 complex in CP is not readily dissociated by size-exclusion chromatography in the presence of either 0.15 or 1 mol/L NaCl (data not shown). In fact, when we treated CP with 1 mol/L NaCl for 1 hour at room temperature before IEF, the bands at pI 7.0 or 7.5 persisted, indicating that no dissociation of VWF-ADAMTS13 complex had taken place under conditions of high ionic strength. These results may indicate that VWF binding to ADAMTS13 is independent of ionic strength. In this regard, McKinnon and colleagues[30] reported that the N-linked glycans of VWF exert a modulatory effect on the interaction with ADAMTS13 and that removal of the N-linked glycans from VWF increased its affinity for ADAMTS13 under static conditions. Furthermore, Yeh and coworkers[19] recently reported that ADAMTS13 possesses a disulfide bond–reducing activity that regulates shear-induced thiol-disulfide exchange. Therefore, one of the mechanisms underlying formation of VWF-ADAMTS13 complexes might involve disulfide-bond formation between ADAMTS13 and VWF. To address this issue, we investigated whether IAA, a blocker of free thiols, might prevent the formation of a disulfide bond–mediated covalent complex under high shear stress. We observed, however, that VWF-ADAMTS13 complex formation was unaffected by IAA treatment, suggesting that in CP, the amount of VWF-ADAMTS13 complex formed in a thiol-dependent fashion is marginal. This finding rules out a major role for disulfide bonds, but otherwise we have not elucidated the binding mechanism of VWF and ADAMTS13; this issue remains to be addressed in future studies.

PE is a first-line treatment for acquired TTP. For this purpose, either FFP or CSP is commonly used,[31] but the results regarding CP have been controversial.[32] At least one case of congenital TTP (USS) has been successfully treated with CP.[9] Therefore, it is important to determine whether there is a functional difference between bound and unbound ADAMTS13 and whether any such difference has physiologic relevance. Here we have clearly shown that CSP contains the unbound or less bound ADAMTS13, whereas CP contains much more bound ADAMTS13 and lower levels of its unbound counterpart. An authoritative determination regarding which form of ADAMTS13 more efficiently down regulates H-SIPA will be crucial in establishing the optimal treatment modality for TTP patients.

In most acquired TTP patients, plasma ADAMTS13 activity is less than 5% of normal. As a consequence, UL-VWFMs are not cleaved after secretion from endothelial cells and remain anchored to the cell surface in long strings.[33] Circulating PLTs adhere to these long strings, resulting in occlusive PLT thrombi. However, smaller VWFMs do not induce this spontaneous adhesion and aggregation of PLTs. Consequently, increased fluid shear stress is required to induce PLT aggregation in vitro.[2]

To reproduce the PLT aggregation generated in the microvasculature of ADAMTS13 activity–deficient TTP patients, here we employed an H-SIPA assay system that uses a mixture of washed normal PLTs and ADAMTS13-dp plasma spiked with purified pd-VWF to mimic TTP plasmas. In this assay, the purified pd-ADAMTS13 inhibited H-SIPA in a dose-dependent manner, reaching a plateau of 20% ADAMTS13 activity at final pd-ADAMTS13 concentrations up to 500%. Under the same experimental conditions, FFP, CSP, and CP inhibited H-SIPA in a dose-dependent manner to the same extent at the end points; in CP, however, the aggregation inhibition curves were different, and in fact no distinct inhibition was observed at the initial phase of PLT aggregation. This might be simply explained by the fact that the VWF concentration in the H-SIPA reaction mixtures was much higher than in CSP or FFP. Alternatively, the binary complex of ADAMTS13 and larger VWFMs might modulate a different phase of H-SIPA than unbound ADAMTS13, because CSP spiked with purified VWF readily generates the ADAMTS13–larger VWFM complex with pI 7.0 or 7.5. The larger VWFM is required in the earliest phase of PLT thrombi formation and high shear stress, but in the later phase the ADAMTS13–larger VWF complex embedded in the thrombi may play a role in regulating the size of the thrombi to prevent microvascular occlusion. Further studies are required to determine the functional differences between ADAMTS13 in CSP and CP.

In conclusion, our results indicated that both plasma products of FFP and CSP are effective in treatment of TTP. However, CSP may be more favorable for PE in acquired TTP patients: relative to FFP, CSP has a lower level of VWF and a comparable ADAMTS13 activity, but lower amounts of ADAMTS13–larger VWFM complex.

ACKNOWLEDGMENTS

Plasma of T3-VWD patient was provided by Dr Midori Shima, Department of Pediatrics, Nara Medical University. YH performed the research, analyzed and interpreted data, and wrote the manuscript; MH and AI performed research; KS contributed vital reagent; MM analyzed data and wrote the manuscript; and YF designed the research, interpreted data, and wrote the manuscript.

CONFLICT OF INTEREST

YH, MH, AI, and KS have no conflict of interest. MM is a member of the clinical advisory board for Alexion Pharma. YF is a member of the clinical advisory boards for Baxter BioScience and Alexion Pharma.

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SHOCK, Vol. 39, No. 5, pp. 409–414, 2013

RATIO OF VON WILLEBRAND FACTOR PROPEPTIDE TO ADAMTS13 IS ASSOCIATED WITH SEVERITY OF SEPSIS

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Received 5 Jan 2013; first review completed 29 Jan 2013; accepted in final form 5 Mar 2013

ABSTRACT—Von Willebrand factor (VWF)–cleaving protease (ADAMTS13) cleaves ultralarge VWF (ULVWF) secreted from endothelium and by which is regulating its physiologic function. An imbalance between ULVWF secretion and ADAMTS13 level occurs in sepsis and may cause multiple organ dysfunction. We evaluated the association between the VWF-propeptide (VWF-pp)/ADAMTS13 ratio and disease severity in patients with severe sepsis or septic shock. In 27 patients with severe sepsis or septic shock and platelet count less than 120 000/μL, we measured plasma VWF, VWF-pp, and ADAMTS13 levels on hospital days 1, 3, 5, and 7. The VWF-pp/ADAMTS13 ratio was increased greater than 12-fold in patients with severe sepsis or septic shock on day 1 and remained markedly high on days 3, 5, and 7 compared with normal control subjects. The VWF-pp/ADAMTS13 ratio significantly correlated with Acute Physiology and Chronic Health Evaluation II score on days 1 and 5; Sepsis-related Organ Failure Assessment score on days 1, 3, and 5; maximum Sepsis-related Organ Failure Assessment score and tumor necrosis factor α level on days 1, 3, 5, and 7; and creatinine level on days 1, 5, and 7. Patients with greater than stage 1 acute kidney injury had significantly higher VWF-pp/ADAMTS13 ratio than patients without acute kidney injury. In summary, the VWF-pp/ADAMTS13 ratio was associated with disease severity in patients with severe sepsis or septic shock and may help identify patients at risk for multiple organ dysfunction by detecting severe imbalance between ULVWF secretion and ADAMTS13 level.

KEYWORDS—Sepsis, von Willebrand factor propeptide, ADAMTS13, multiple organ dysfunction

INTRODUCTION

Severe sepsis and septic shock result from the systemic host response to infection, including inflammation, coagulation, and changes in the vascular endothelium. Vascular endothelial activation, dysfunction, and injury facilitate leukocyte and platelet aggregation and aggravate inflammation and thrombosis (1). Von Willebrand factor (VWF) is a key marker of endothelial changes (2).

Von Willebrand factor is a multimeric glycoprotein that circulates in plasma and functions as a bridge between the subendothelial matrix and platelets. The subunit precursor proVWF (350 kd) is synthesized in the endothelium and contains signal peptide, VWF propeptide (VWF-pp), and VWF subunit. The proVWF is dimerized through disulfide bonds after removal of signal peptide in the endoplasmic reticulum. The proVWF dimers are transported to the Golgi apparatus, VWF-pp is cleaved, and additional disulfide bonds form between proVWF dimers to yield ultralarge VWF (ULVWF; size, >20,000 kd). Ultralarge VWF condenses into tubules and forms Weibel-Palade bodies. Ultralarge VWF and VWF-pp are stored in Weibel-Palade bodies in equimolar amounts on a subunit basis (3, 4).

Several inflammatory mediators, such as thrombin, histamine, and proinflammatory cytokines, including tumor necrosis factor α (TNF-α) and interleukin 8 (IL-8), activate endothelial cells and induce Weibel-Palade body exocytosis (5, 6), causing cell surface expression of ULVWF and release of VWF-pp into the bloodstream. Because longer VWF is more active, and ULVWF causes spontaneous platelet aggregation and thrombosis, it is immediately cleaved by VWF cleaving protease after secretion, which is also known as a disintegrin-like and metalloprotease with thrombospondin type 1 motif, member 13 (ADAMTS13). This cleavage results in smaller and less adhesive plasma forms of VWF (7). In the absence of ADAMTS13, secreted ULVWF strings that are bound to endothelium are not cleaved but adhere to platelets, which bind to leukocytes and cause thrombosis and inflammation (8, 9).

Because an appearance of ULVWF in plasma has been demonstrated in patients with inadequate function of ADAMTS13, as in thrombotic thrombocytopenic purpura (TTP) or sepsis (10, 11), it may suggest an imbalance between ULVWF secretion and ADAMTS13 function. Plasma ULVWF may be a good marker to detect this imbalance, but it is technically difficult to determine ULVWF and quantify it. Furthermore, plasma ULVWF often cannot be detected in patients having

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This study was supported in part by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan to H.F. (no. 21791774) and K.N. (no. 21592313).

M.M. and Y.F. are members of the advisory board of Alexion Pharma. Y.F. is a member of the advisory board of Baxter BioScience. The other authors have no conflict of interest to declare.

This work was presented at the annual meetings of the American Society of Hematology, San Diego, California, on December 11, 2011, and International Society on Thrombosis and Haemostasis, Kyoto, Japan, on July 24, 2011.

Statement of authorship: H.F. performed most of the experiments, data analysis, and manuscript preparation. H.F., K.N., H.A., T.W., T.S., H.M., and M.M. participated in the acquisition of blood samples and measurement of several parameters. H.F., K.N., M.S., Y.F., and K.O. participated in analysis and interpretation of data. K.N. and K.O. made the overall experimental designs and direction of this work and prepared the draft of the manuscript. All authors read and approved this version of the manuscript.

DOI: 10.1097/SHK.0b013e3182908ea7

this imbalance and developing organ failure, as in chronic relapsing TTP (12).

The mean or median levels of ADAMTS13 are decreased to 20% to 43% normal in sepsis (13–15). However, ADAMTS13 level less than 10% normal is enough to prevent the clinical manifestation of primary thrombotic microangiopathy in patients with congenital ADAMTS13 deficiency (16). This suggests that patients with sepsis have a high enough ADAMTS13 level to prevent thrombotic microangiopathy, but it may not be high enough to cleave all ULVWF secreted from endothelium during sepsis. Furthermore, multiple organ dysfunction in children with thrombocytopenia was resolved by restoring ADAMTS13 activity by plasma exchange (17). Therefore, both decreased ADAMTS13 level and the imbalance between ULVWF secretion and ADAMTS13 activity may cause microvascular thrombosis formation in sepsis. If so, it may be clinically relevant to measure the imbalance between ULVWF secretion and ADAMTS13 activity.

The VWF-pp is secreted in equimolar amounts to the total subunits of secreted ULVWF and more rapidly cleared from the circulation than VWF (half-life: VWF-pp, 3 h; VWF, 12 h) (5). Therefore, we hypothesized that VWF-pp level may reflect ULVWF secretion and that the VWF-pp/ADAMTS13 ratio may be a sensitive and real-time measure of imbalance between ULVWF secretion and plasma ADAMTS13 level. Higher VWF-pp/ADAMTS13 ratio may reflect insufficient control of VWF multimer size, and this may accelerate microvascular thrombus formation, inflammation, and organ failure.

The purpose of this study was to investigate whether the VWF-pp/ADAMTS13 ratio is associated with disease severity in patients with severe sepsis or septic shock. Although there have been several previous studies about VWF, VWF-pp, and ADAMTS13 levels in patients with severe sepsis or septic shock, limited information is available about the time course of these levels simultaneously measured. We determined the time course of the levels of VWF, VWF-pp, and ADAMTS13 during the early phase of sepsis.

MATERIALS AND METHODS

Patients

From January 2008 to December 2009, all patients treated at the intensive care unit of the Department of Emergency and Critical Care Medicine, Nara Medical University Hospital, was considered for the study. Inclusion criteria for the study were (i) severe sepsis or septic shock as defined by published guidelines (18) and (ii) platelet count less than 120 000/μL. Exclusion criteria were (i) patients younger than 18 years, (ii) pregnancy, (iii) medical history of chronic renal failure (stage 5 chronic kidney disease) (19) or chronic liver disease (20), (iv) cardiopulmonary arrest, (v) other hematologic disorders that may lower the platelet count such as TTP, and (vi) malignancy. There were 27 patients included in the study. This study protocol was approved by the institutional review board of Nara Medical University Hospital. Written informed consent was obtained from enrolled patients or family members.

Evaluation

Clinical information was collected including age, sex, diagnosis, serum creatinine level, and survival status at 28 days after admission. Survivors were defined as patients who were alive 28 days after admission, and nonsurvivors were patients who died within 28 days after admission. The severity of disease and organ failure were assessed with Acute Physiology and Chronic Health Evaluation II (APACHE II) score (21) and Sepsis-related Organ Failure Assessment (SOFA) score (22) at days 1 (on admission), 3, 5, and 7 after admission. Maximum SOFA (Max SOFA) score was defined as the Max SOFA score during the clinical course at any time on day 28 or less. Acute kidney injury (AKI) stage was assessed by the criteria of the Acute Kidney Injury Network Working Group (23).

Assays

Citrated blood samples were obtained from patients who met the inclusion criteria on admission to the intensive care unit (day 1) and days 3, 5, and 7. Blood samples were centrifuged at 1,500*g* for 10 min in a cooled centrifuge immediately after drawing, and aliquots of plasma were stored at −80°C until assayed. Blood samples were obtained from 15 healthy volunteers (nine men and six women; age range, 23–55 years [mean, 40 years]) and pooled for ADAMTS13, VWF-pp, and VWF assays as the normal controls being 100%.

Activity of ADAMTS13 was assayed using a commercial kit (Kainos Laboratories, Inc, Tokyo, Japan). The plasma level of VWF-pp was measured with an enzyme-linked immunosorbent assay kit (Sanquin, Amsterdam, the Netherlands). Levels of IL-6 (R&D Systems Inc, Minneapolis, Minn), TNF-α (R&D Systems Inc), and VWF (Dako, Glostrup, Denmark) were measured.

Data analysis

Data analysis was performed with statistical software (SPSS, Inc, Armonk, NY; and GraphPad, San Diego, Calif). Data are reported as mean ± SD or median with interquartile range. The Shapiro-Wilk test was used to evaluate normality of data. Groups were compared with *t* test or Mann-Whitney *U* test, and the relation between two variables was evaluated with Spearman rank correlation. Statistical significance was defined by $P \leq 0.05$ for 2-sided tests.

RESULTS

Most patients were men, and the most common diagnosis was intra-abdominal infection (Table 1). Most patients (20 of 27 patients) were survivors; one patient with acute abdomen died on day 6, and the other 26 patients completed blood collection until day 7. All measurements did not differ between male and female patients. There were no differences between survivors and nonsurvivors in APACHE II score, SOFA score, serum creatinine level, platelet count, and fibrin degradation product level (data not shown).

In patients with severe sepsis and septic shock, the mean VWF level was high on day 1, and there was no significant change in mean VWF level from day 1 to day 7 (Table 2). The VWF level did not differ between survivors and nonsurvivors (data not shown). The level of VWF did not correlate with any clinical scores or laboratory markers (data not shown).

The mean VWF-pp level was high on day 1, remained high but decreased significantly from day 1 to day 3, and remained high from day 3 to day 7 (Table 2). There were no differences in mean VWF-pp level between survivors and nonsurvivors (data not shown). The levels of VWF-pp were correlated significantly with SOFA score on days 1, 3, and 5; with Max SOFA at days 5 and 7; and with TNF-α level on day 1 (Table 3).

The mean level of ADAMTS13 was significantly lower in patients on day 1 than normal controls, and the mean level of ADAMTS13 increased in patients significantly from day 1 to day 3 and from day 3 to day 5 (no difference between values on days 5 and 7) (Table 2). The mean level of ADAMTS13 was significantly higher in survivors than in nonsurvivors on days 1, 5, and 7 but not on day 3 (Table 2). The levels of ADAMTS13 correlated negatively with APACHE II score on days 1 and 5; SOFA score on day 5; Max SOFA score on days 1, 3, and 5; TNF-α on day 5; and IL-6 and creatinine levels on day 7 (Table 3).

The mean VWF-pp/ADAMTS13 ratio was 12-fold greater in patients on day 1 than normal control subjects, and the mean ratio decreased significantly in patients from day 1 to day 3

TABLE 1. **Clinical and laboratory findings on admission in patients with severe sepsis or septic shock***

	Total	Male (n = 16)	Female (n = 11)
Age, y	70 ± 16	71± 14	68 ± 19
APACHE-II score	21.0 ± 7.3	22.0 ± 7.4	24.0 ± 4.6
SOFA score	11.1 ± 3.3	12.3 ± 3.2	11.3 ± 2.8
SIRS score†	20	12	8
Survivors	20 (74)	13 (81)	7 (64)
Diagnosis			
Intra-abdominal infection	18 (67)	11 (69)	7 (64)
Urinary tract infection	3 (11)	1 (6.3)	2 (18)
Pneumonia	2 (7)	2 (13)	0
Burn wound sepsis	2 (7)	0	2 (18)
Necrotizing fasciitis	1 (4)	1 (6.3)	0
Descending mediastinitis	1 (4)	1 (6.3)	0
Acute Kidney Injury > stage 1	19 (70)	11 (69)	8 (73)
Platelet count, /μL	8.3 ± 3.0	9.1 ± 3.3	7.2 ± 2.2
Creatinine, mg/dL	1.7 ± 1.0	1.9 ± 1.0	1.4 ± 1.0
ADAMTS13, %	24.9 ± 8.5	25.9 ± 9.5	23.5 ± 7.4
von Willebrand factor propeptide, %	293.8 ± 153.8	294.6 ± 142.8	292.7 ± 175.7
von Willebrand factor, %	212.3 ± 86.3	225.2 ± 81.4	194.7 ± 83.8

ADAMTS13, von Willebrand factor propeptide, and von Willebrand factor are expressed as a percentage of normal controls. Data are reported as mean ± SD or number (%).
*n = 27 patients.
†Systemic inflammatory response syndrome score >3.

and remained markedly increased compared with controls at days 5 and 7 (Table 2). The VWF-pp/ADAMTS13 ratio correlated significantly with APACHE II score on days 1 and 5; with SOFA score on days 1, 3, and 5; and with Max SOFA score and TNF-α level on days 1, 3, 5, and 7 (Table 3). The IL-6 and TNF-α levels in patients on days 1 and 3 were markedly greater than the upper limit of normal (Table 2).

Nineteen patients with severe sepsis or septic shock developed AKI of greater than stage 1 within 48 h after admission (Table 1). The mean levels of VWF and ADAMTS13 did not differ between patients with or without AKI, but patients with AKI had significantly greater mean levels of VWF-pp (AKI, 338% ± 143%; no AKI, 190% ± 134%; $P \leq 0.02$) and VWF-pp/ADAMTS13 ratio (AKI, 15% ± 7%; no AKI, 7% ± 6%; $P \leq 0.001$) on day 1. The VWF-pp level correlated significantly with serum creatinine level on day 1, and the VWF-pp/ADAMTS13 ratio correlated significantly with serum creatinine level on days 1, 5, and 7 (Table 3).

DISCUSSION

A decreased level of ADAMTS13 on admission had been described previously in patients with sepsis (24) and correlated with AKI (11), APACHE II score, and poor prognosis (13). The present results confirmed that decreased ADAMTS13 levels correlated with disease severity scores including APACHE II and Max SOFA on the same days of observation including the day on admission (Table 3). The finding that means ADAMTS13 level was significantly lower in nonsurvivors than survivors on days 1, 5, and 7 (Table 2) suggests that ADAMTS13 level may be a prognostic marker for survival during the early phase of sepsis.

The cause of the decreased ADAMTS13 levels in sepsis is controversial. Possible mechanisms for the decrease include consumption because of excess substrate and proteolytic degradation by thrombin, plasmin, and neutrophil protease (11, 25). In addition, infusion of endotoxin or desmopressin into healthy volunteers may increase plasma VWF and VWF-pp levels and may decrease ADAMTS13 activity (26, 27); this suggests that ADAMTS13 may be consumed mainly by excessive ULVWF released by endotoxin or desmopressin, or secretion of ADAMTS13 may be inhibited. Greater duration or intensity of stimulation to endothelium, causing ULVWF secretion with proinflammatory cytokines such as TNF-α (28), may induce greater imbalance between ULVWF secretion and plasma ADAMTS13 level, resulting in larger VWF molecules in plasma and a prothrombotic condition.

What can be used to estimate the extent of the imbalance between ULVWF secretion and plasma ADAMTS13 level? The appearance of ULVWF in plasma may be a good marker for the imbalance between ULVWF secretion and plasma ADAMTS13 level (14). However, ULVWF can be detected only by time-consuming immunoblotting after electrophoresis, and it is difficult to quantify ULVWF reproducibly (11, 15). Furthermore, ULVWF is very adhesive to platelets and can cause spontaneous platelet aggregation, associated consumption, and decreased levels of ULVWF. The disappearance of ULVWF may be observed in some patients with chronic TTP during acute episodes (12). In addition, some studies show no correlation between ULVWF and decreased levels of ADAMTS13 (11, 29).

The ratio of VWF level to ADAMTS13 activity is reported to be more useful than VWF multimer analysis (ULVWF detection) alone for the diagnosis of highly prothrombotic states induced by the imbalance between VWF secretion and ADAMTS13 (15). However, plasma VWF level may not reflect ULVWF secretion accurately because VWF may be affected by ABO blood group antigens; in addition, secreted plasma VWF can be consumed at the endothelial injury site, especially during inflammation, by binding to the subendothelial matrix, endothelium, platelets, or white blood cells (9). An increased plasma level of VWF on admission is reported to be associated with an increased risk of death from severe sepsis (30); yet, the present study showed that markedly increased VWF levels in patients with severe sepsis or septic shock were not associated with disease severity during the first 7 days and showed increasing tendency despite resolution of clinical symptoms, consistent with other studies (15, 24). Thus, plasma VWF level did not likely reflect ULVWF secretion rate in the present study.

In contrast with VWF, the VWF-pp is not affected by ABO antigen and does not bind to the vascular wall; consequently, plasma level of VWF-pp may more accurately reflect ULVWF

TABLE 2. **Levels of VWF, VWF-pp, ADAMTS13, and inflammatory markers in patients with severe sepsis or septic shock***

		Patients with severe sepsis or septic shock			
		Day			
Variables	Control subjects	1	3	5	7
VWF, %	96 ± 14	212 ± 86	228 ± 85	240 ± 85	252 ± 112
VWF-pp, %	96 ± 16	294 ± 154	240 ± 115†	219 ± 117	228 ± 162
ADAMTS13, %					
All patients	100 ± 10	25 ± 8.5‡	30 ± 9‡	33 ± 11‡	33 ± 11
Survivors	NA	27 ± 8.6	31 ± 8.7	35 ± 9.4	36 ± 10
Nonsurvivors	NA	19 ± 5.4	27 ± 8.2	25 ± 10	24 ± 9.4
P	NA	0.03§	NS	0.03§	0.02§
VWF-pp/ADAMTS13 ratio	0.97 ± 0.18	12.9 ± 7.2‖	8.9 ± 5.1‖	7.7 ± 6.0	7.9 ± 7.1
IL-6,¶ pg/mL	<2.4	1,220 (362–3,610)	206 (58–1,050)	115 (29–338)	75 (20–446)
TNF α (pg/mL)**	<1.8	5.8 (3.3–21.3)	3.4 (2.4–5.8)	2.5 (1.2–4.0)	2.0 (1.4–3.3)

Data are reported as mean ± SD or median (interquartile range). VWF, VWF-pp, and ADAMTS13 are expressed as a percentage of normal controls.
*n = 27 patients (day 1) or 26 patients (days 3, 5, and 7) with sepsis or septic shock; 15 normal control subjects.
†VWF-pp: difference between days 1 and 3, $P \leq 0.05$.
‡ADAMTS13: difference between normal controls and patients on day 1, $P \leq 0.001$; difference between days 1 and 3, $P \leq 0.01$; difference between days 3 and 5, $P \leq 0.05$.
§ADAMTS13: difference between survivors and nonsurvivors, $P \leq 0.05$.
‖VWF-pp/ADAMTS13 ratio: difference between normal controls and patients on day 1, $P \leq 0.001$; difference between days 1 and 3, $P \leq 0.01$.
¶Upper limit of normal, 2.41 pg/mL.
**Upper limit of normal, 1.79 pg/mL.
NS indicates not significant ($P > 0.05$); NA, not applicable.

secretion induced by endothelial activation than VWF (5). In the present study, increased plasma VWF-pp level was associated with SOFA score and TNF-α on day 1 (Table 3), suggesting that VWF-pp may be a better marker of acute endothelial activation than VWF in the early phase of sepsis. The marked increase in VWF-pp level on admission significantly

TABLE 3. **Relation between VWF-pp, ADAMTS13, and clinical scores and markers in patients with severe sepsis or septic shock**

	Day 1		Day 3		Day 5		Day 7	
	*r**	*P* ≤	*r**	*P* ≤	*r**	*P* ≤	*r**	*P* ≤
VWF-pp								
APACHE II	0.32	NS	0.16	NS	0.45	0.05	0.07	NS
SOFA	0.51	0.007	0.47	0.02	0.55	0.01	−0.62	NS
Max SOFA	0.25	NS	0.38	NS	0.44	0.03	0.59	0.003
TNF-α	0.47	0.02	0.54	NS	0.38	NS	0.44	NS
IL-6	0.20	NS	−0.11	NS	0.25	NS	0.42	NS
Creatinine	0.59	0.001	0.34	NS	0.32	NS	0.18	NS
ADAMTS-13								
APACHE II	−0.54	0.004	−0.30	NS	−0.68	0.001	−0.34	NS
SOFA	−0.32	NS	−0.30	NS	−0.57	0.007	−0.13	NS
Max SOFA	−0.53	0.005	−0.42	0.03	−0.47	0.02	−0.29	NS
TNF-α	−0.07	NS	−0.37	NS	−0.40	0.05	−0.43	NS
IL-6	−0.04	NS	−0.36	NS	−0.39	NS	−0.45	0.05
Creatinine	−0.33	NS	−0.22	NS	−0.35	NS	−0.47	0.02
VWF-pp/ADAMTS-13 ratio								
APACHE II	0.45	0.03	0.15	NS	0.69	0.001	0.19	NS
SOFA	0.65	0.001	0.41	0.04	0.68	0.001	−0.61	NS
Max SOFA	0.45	0.03	0.41	0.04	0.52	0.007	0.63	0.001
TNF-α	0.44	0.03	0.57	0.002	0.44	0.03	0.59	0.007
IL-6	0.12	NS	0.04	NS	0.48	0.02	0.70	0.001
Creatinine	0.76	0.001	0.29	NS	0.49	0.02	0.48	0.02

*Spearman rank correlation (ρ).
NS indicates not significant ($P > 0.05$).

decreased by day 3, but remained more than 2-fold greater than normal for at least 7 days (Table 2), and this is evidence of persistent endothelial activation in sepsis. This also is consistent with previous studies that showed increased plasma VWF-pp level in sepsis and association with SOFA score and creatinine level but not with prognosis (24, 31).

The VWF-pp/ADAMTS13 ratio significantly correlated with disease severity including APACHE II score, SOFA score, the proinflammatory cytokine TNF-α, and creatinine during the period of observation (Table 3). Marked increase in the VWF-pp/ADAMTS13 ratio seemed to correlate with disease severity better than VWF-pp or ADAMTS13 level alone in patients with severe sepsis or septic shock. These results suggest that an imbalance between ULVWF secretion and ADAMTS13 level induced by endothelial activation or dysfunction may cause microthrombi and inflammation that lead to organ failure. In a porcine model of *Escherichia coli* sepsis, observations included decreased ADAMTS13 level, increased proportion of large-molecular-weight VWF multimers, glomerular microthrombi enriched with platelets and VWF, and acute renal failure (28). Therefore, the imbalance between VWF secretion and ADAMTS13 may induce platelet-VWF thrombosis in the kidney without appearance of ULVWF in plasma (29). Correcting this imbalance may help prevent or treat acute renal failure in sepsis.

We have recently found that ADAMTS13 may suppress intravascular growth of thrombus (32) and may control thrombosis and inflammation in the microcirculation in brain ischemia, brain reperfusion injury, and myocardial infarction (33–35). These suggested that administration of recombinant ADAMTS13 may correct the imbalance between ULVWF secretion and ADAMTS13 level and may help treat patients with severe imbalance who are at risk for multiple organ dysfunction. In children with thrombocytopenia, multiple organ dysfunction was resolved by restoring ADAMTS13 activity by plasma exchange (17). The VWF-pp/ADAMTS13 ratio may help identify patients with severe sepsis or septic shock at high risk for organ dysfunction because of imbalance between ULVWF secretion and ADAMTS13. Furthermore, this ratio may help identify patients susceptible for organ failure due to endothelial dysfunction in other diseases. Although the present prospective study was limited to few patients who had sepsis and thrombocytopenia, some trends were observed, and larger, controlled, prospective studies are necessary to evaluate and validate these findings.

CONCLUSION

The present study showed simultaneous changes in the levels of ADAMTS13, VWF-pp, VWF, and VWF-pp/ADAMTS13 ratio in patients during the first week of severe sepsis or septic shock. The ratio of VWF-pp/ADAMTS13 was associated with disease severity more than isolated VWF-pp or ADAMTS13 levels. Further studies may show whether organ failure may be prevented by identifying patients with abnormal VWF-pp/ADAMTS13 ratio and restoring the balance between VWF-pp and ADAMTS13 with plasma exchange or recombinant ADAMTS13.

ACKNOWLEDGMENTS

The authors thank Ms. Akiko Kitaoka, Ms. Ayami Isonishi, and Seiji Kato for their valuable help to measure VWF-pp and ADAMTS13 level.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Therapeutic Modality of 11 Patients with TTP in a Single Institution in Miyazaki from 2000 to 2011

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Abstract

Objective Thrombotic thrombocytopenic purpura (TTP) is a life-threatening generalized disease with pathological features that are termed thrombotic microangiopathies. Since the discovery of the von Willebrand factor-cleaving protease [a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13)], it is widely known that approximately two-thirds of TTP patients have a severe deficiency of ADAMTS13 activity due to gene mutations or acquired autoantibodies to this enzyme. However, the remaining one-third of TTP patients have only moderately reduced or almost normal ADAMTS13 activity. To elucidate the clinical characteristics and outcomes of these two types of TTP, we have retrospectively analyzed the cases of acquired TTP patients treated in a single institution from 2000 to 2011.

Methods Our case studies include 11 TTP patients, of which 5 were considered idiopathic and 6 had cases of TTP associated with underlying diseases such as non-Hodgkin lymphoma or connective tissue diseases.

Results These patients were treated with a combination therapy of plasma exchange and steroids and with several adjunctive therapeutic regimens including the on-label use of cyclophosphamide and cyclosporine and the off-label use of high-dose steroid or immunoglobulin with rituximab. Splenectomies were not performed. As a result of these treatments, 6 out of the 7 patients with ADAMTS13 activity deficient TTP achieved a complete remission without relapse, but the remaining 4 patients with non-ADAMTS13 activity deficient TTP all died without complete remission.

Conclusion We present herein the detailed clinical courses of 11 patients with TTP and address our experiences with the efficacy of various therapeutic regimens. This case-oriented study should be helpful to the physicians who directly care for TTP patients, and may provide a future direction for developing a more efficient treatment modality.

Key words: thrombotic thrombocytopenic purpura, ADAMTS13, plasma exchange, adjunctive therapeutic regimen, rituximab

(Intern Med 52: 1883-1891, 2013)
(DOI: 10.2169/internalmedicine.52.8253)

Introduction

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening generalized disease with pathological features that are termed thrombotic microangiopathies (TMAs). These features are characterized by the triad of microangiopathic hemolytic anemia, destructive thrombocytopenia, and organ (renal) failure due to platelet thrombi (1-5).

In 1996, Amorosi and Ultmann (2) defined the classic "pentad" of clinical features of TTP which included the aforementioned triad plus fluctuating neurological signs and

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Received for publication May 28, 2012; Accepted for publication March 6, 2013
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fever. Regarding TTP treatment, Rock et al. (6) published a breakthrough report in 1991 showing that plasma exchange (PE) therapy saved the lives of 90% of TTP patients. However, the pathogenesis of TTP was not addressed until the discovery of the von Willebrand factor (VWF)-cleaving protease, now known as ADAMTS13-a disintegrin-like and metalloproteinase with a thrombospondin type I motifs 13 (7, 8). Subsequent studies have indicated that TTP is caused by severe deficiency of ADAMTS13 activity in approximately two-thirds of patients. Of these patients, a minor population (circa 5%) have the gene mutations which define Upshaw-Schulman syndrome (9, 10) and a major population (circa 95%) have acquired autoantibodies to this enzyme (11, 12). Therefore, the pathogenesis of TTP is not well defined and the efficacy of PE therapy has been controversial in the remaining one-third of acquired TTP patients who do not show severe deficiency of ADAMTS13 activity. Furthermore, recent studies indicate that some populations of acquired TTP patients with severe deficiency of ADAMTS13 activity do not respond well to PE therapy, and the reasons for this must be explored (13).

We treated 11 patients with acquired TTP from 2000 to 2011 in Miyazaki Prefectural Hospital, a regional referral hospital in Japan. In these patients, the basic PE and steroid therapy was supplemented with several adjunctive therapeutic regimens. These included the on-label use of cyclophosphamide and cyclosporine and the off-label use of high-dose steroid or immunoglobulin and rituximab, but did not include splenectomy. We analyzed the detailed clinical courses of our 11 TTP patients and evaluated the efficacy of the therapeutic regimens. This case-oriented study should be helpful to the physicians who directly care for TTP patients and may provide a future direction for the development of new treatment modalities.

Materials and Methods

Patients: From January 2000 to December 2011, 11 patients with acquired TTP were diagnosed at the Miyazaki Prefectural Hospital (Table 1). The diagnosis of TTP was made by the classic pentad with the following laboratory markers (14): (i) microangiopathic hemolytic anemia (hemoglobin [Hb] ≤12 g/dL), Coombs test negative, non-detectable serum haptoglobin (<10 mg/dL), more than 2 fragmented red cells (schistocytes) in a microscopic field with a magnification of 100, and the concurrent increase of serum lactate dehydrogenase (LDH) above the institutional baseline; (ii) thrombocytopenia (platelet count ≤100×109/L); (iii) fever ≥ 37℃; (iv) central nervous system (CNS) involvement ranging from headache to coma and including neurological dysfunction, convulsion, or clouding of consciousness; and (v) renal involvement including abnormal urinalysis in addition to the elevation of the serum creatinine level.

Among the 11 patients with TTP, 5 patients had no underlying disease and were classified as having acquired idiopathic TTP (ai-TTP) and 5 patients had TTP associated with connective tissue diseases. In this patient group, 2 had systemic lupus erythematosus (SLE), 2 had systemic sclerosis (SSc), and 1 had overlapping diseases (OS: SLE + SSc). The remaining patient had TTP associated with non-Hodgkin lymphoma (NHL) (Table 1). This retrospective study was conducted in compliance with good clinical practices and the ethical principles of the Declaration of Helsinki.

ADAMTS13 assays: Through March 2005, the ADAMTS 13 activity was measured at Nara Medical University by the classic von Willebrand factor multimer (VWFM) assay with a detection limit of 3% of the normal control (9). Thereafter, the ADAMTS13 activity was determined by a chromogenic ADAMTS13-act-ELISA (Chr-act-ELISA) with a detection limit of 0.5% of the normal (15). For consistency, all the samples tested prior to 2005 were re-evaluated by Chr-act-ELISA using the plasmas that had been stored at -80℃.

The plasma ADAMTS13 inhibitor titers were analyzed using patient plasmas that had been heat-inactivated at 56℃ for 30 minutes (15) according to the original method established for the measurement of factor VIII inhibitor (16). The results were expressed in Bethesda units (BU) where one unit was defined as the amount necessary to reduce the ADAMTS13 activity to 50% of the control levels. The ADAMTS13 inhibitor titers were considered negative for values of less than 0.5 BU/mL, marginal for values between 0.5 and 1 BU/mL, and positive for values greater than 1 BU/mL (14).

Plasma exchange therapy: We performed plasma exchange (PE) therapy with fresh frozen plasma (FFP) at 60 mL/kg body weight until we observed the recovery of the following variables: increased platelet count (>150×10^9/L), decreased lactate dehydrogenase (LDH) levels, and decreased neurological abnormalities (4, 8). The PE therapy was performed for 3 consecutive days from the diagnosis of TTP and then the frequency was gradually tapered off. A diagnosis of complete remission (CR) was considered when a normalization of both the physical and the laboratory findings were achieved as previously described (4). The PE therapy was usually accompanied by high-dose methylprednisolone (mPSL) pulse therapy with an intravenous drip infusion rate of 1 g mPSL/day for 3 consecutive days.

Case series: The clinical and laboratory findings at admission for 11 patients with acquired TTP are shown in Table 1 and their treatment regimens and therapeutic outcomes are summarized in Table 2.

Case 1: In 2003, a 56-year-old man developed petechiae and a fever, then fell into a coma prior to his admission to our hospital. Based on the clinical diagnosis of TTP, PE therapy was immediately initiated with high-dose mPSL pulse therapy under sedation and with intubation. The patient was extubated on hospital day 13 but the thrombocytopenia remained, and, thus, a second course of mPSL pulse therapy was initiated on hospital day 14. On hospital day 16, the laboratory results showed that a low plasma level of ADAMTS13 activity (<3%) and positivity for an ADAMTS

Table 1. Clinical and Laboratory Findings on Admission of Eleven Patients with Acquired TTP

Case			1	2	3	4	5	6	7	8	9	10	11
Gender			M	F	M	M	F	F	F	F	F	M	F
Age			56	50	48	56	50	17	71	68	56	59	51
Body Weight (kg)			65	45	70	70	55	50	40	48	55	50	71
Etiology			Idiopathic	Idiopathic	Idiopathic	NHL	OS (SLE+SSc)	SLE	Idiopathic	SSc	SSc	Idiopathic	SLE
Year			2003	2005	2007	2007	2007	2008	2008	2008	2008	2009	2009
Initial clinical signs													
Fever (°C)			39.0	38.6	37.2	36.0	38.1	37.8	38.6	38.8	38.0	38.8	38.1
Anemia			+	+	+	+	+	+	+	+	+	+	+
Purpura			+	+	+	+	+	+	+	+	+	+	+
Renal disfunction			-	-	-	+	+	-	+	+	+	+	-
Neurologic signs			Coma (JCS 200)	Coma (JCS 200)	Right Hemiplegia, eye deviation to the right	(JCS 30)	Tonic-clonic convulsion	Tonic-clonic convulsion	(JCS 200)	(JCS 30)	Right hemiplegia	Coma (JCS 200)	Coma (JCS 200)
Peripheral blood		Normal ranges											
Platelets	($\times10^9$/L)	137-378	11.0	11.0	7.0	7.0	32.0	6.0	8.0	86.0	29.0	14.0	15.0
WBC	($\times10^9$/L)	3.0-8.7	6.3	10.4	10.2	5.2	7.6	3.8	7.4	8.1	12.4	12.9	5.9
RBC	($\times10^{12}$/L)	3.7-4.9	2.5	2.0	2.5	2.0	3.4	2.2	2.3	3.6	2.6	2.7	3.4
Hb	(g/dL)	10.7-15.3	8.6	6.6	7.9	6.4	9.1	6.7	7.6	9.1	7.6	6.2	9.1
Reticulocyte	(‰)	0.4-1.6	6.1	12.6	9.4	3.4	8.2	7.5	4.1	1.1	3.2	6.2	4.7
Schistocytes on blood film		-	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Blood chemistry													
Total protein	(g/dL)	6.7-8.3	6.6	6.2	7.6	5.5	6.9	8.3	5.8	6.1	4.2	7.2	7.6
Total bilirubin	(mg /dL)	0.2-1.2	3.5	2.8	4.3	0.6	0.7	2.4	6.5	0.7	1.7	2.1	4.2
Direct bilirubin	(mg /dL)	0-0.2	0.5	0.2	0.4	0.2	0.2	0.4	1.1	0.3	0.4	0.4	0.4
AST	(IU/L)	7-38	47	36	43	17	217	33	364	42	80	37	41
ALT	(IU/L)	4-43	30	15	29	8	63	15	220	20	20	21	20
LDH	(IU/L)	119-229	910	753	1,295	415	972	756	1,730	1,035	3,750	1,138	947
BUN	(mg /dL)	8.0-20.0	16	14	24	18	69	9	36	33	60	36	29
Creatinine	(mg /dL)	0.4-0.8	1.1	1.2	1.3	1.6	2.6	0.6	2.0	2.1	1.6	1.4	1.0
CRP	(mg /dL)	0-0.3	1.5	0.1	0.8	0.1	2.5	0.2	4.2	2.9	0.6	3.0	0.3
Haptoglobin	(mg /dL)	19-170	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10
Hemostatic test													
PT	(sec)	9.3-12.0	12.0	12.0	14.0	10.8	9.7	13.0	13.6	12.7	13.7	12.8	12.3
A-PTT	(sec)	24.0-40.0	34.3	38.3	35.1	30.3	36.3	32.3	33.0	35.3	35.3	34.6	25.4
Fibrinogen	(mg /dL)	150-360	168	174	327	361	300	194	167	230	210	154	247
Antithrombin III	(%)	70.0-130.0	81	105	96	87	88	92	78	82	92	102	94
FDP-P	(μg/mL)	0-5.0	4.0	6.0	3.2	1.3	7.0	2.0	4.8	6.0	7.0	4.9	7.7
D-dimer	(μg/mL)	0-1.0	2.0	3.1	2.0	0.3	4.1	1.0	2.1	3.2	3.6	2.2	4.0
PA IgG	(ng/10^7cells)	<46	96.3	220.8	53.7	72.0	129.0	52.0	65.8	75.8	89.0	196.0	86.0
ADAMTS 13													
Activity													
VWFM	(%)		<3.0	<3.0	<3.0								
Chr-ELISA	(%)	70-120	<0.5	<0.5	<0.5	39	49	<0.5	<0.5	47	69	<0.5	<0.5
Inhibitor													
VWFM	(BU/mL)		1.8	1.6	1.3								
Chr-ELISA	(BU/mL)	<0.5	1.6	2.1	2.2	ND	ND	1.4	1.1	ND	ND	2.0	2.2

Among the 11 patients with TTP, 5 had no underlying diseases and were termed acquired idiopathic TTP (ai-TTP) and 5 had TTP that could be associated with connective tissue diseases. Of this second group, 2 patients had systemic lupus erythematosus (SLE), 2 patients had systemic sclerosis (SSc), and 1 patient had overlapping disease (OS: SLE + SSc). The remaining non-idiopathic patient had non-Hodgkin lymphoma (NHL).

Intern Med 52: 1883-1891, 2013 DOI: 10.2169/internalmedicine.52.8253

Table 2. Treatment and Therapeutic Outcomes on Eleven Patients with Acquired TTP

Case	1	2	3	4	5	6	7	8	9	10	11
Initial Therapy											
Intubation Extubation	HD 1 HD 13	HD 1 HD 10	HD 1 HD 8	- -	HD 3 HD 10	HD 3 HD 5	HD 1 HD 16	HD 7 HD 13	HD 1 HD 3	HD 1 HD 10	HD 1 HD 7
Plasma Exchange (PE)	30 times	19 times	12 times	9 times	14 times	6 times	10 times	8 times	2 times	13 times	14 times
Methyl prednisolone pulse	HD 1-3 and HD 14-16	HD 1-3	HD 1-3 and HD 11-13	HD 7-9	HD 3-5	HD 3-5	HD 1-3	HD 7-9	HD 1- 2	HD 1-3	HD 1-3
Prednisolone (1mg/kg)				HD 1-6	HD 1-3			HD 1-6			
Additional Treatment											
Intravenous γ-globulin (a dose of 400 mg/kg)	HD 49-53	HD 21-25	HD 15-19								
Cyclosporine (4-5 kg/m^2)							HD 15 - 28			HD 20 - the present time	
Vincristine (a dose of 1mg/m^2)	HD 21,28, 35 and 42			HD 15, 22, 29 and 36							
Cyclophosphamide pulse (500 mg/m^2)					HD 15			HD 14			HD 20
Rituximab (a dose of 375 mg/m^2)				HD 29, 36, 42 and 49		HD 13, 20, 27 and 34		HD 21,28,35 and 42			
Maintenance Treatment											
Prednisolone Cyclosporine	5mg	5mg	5mg	-	-	5mg	-	-	-	5mg 50mg	5mg
Response	CR	CR	CR	no response	PR	CR	PR	PR	no response	CR	CR
Outcome	DFS	DFS	DFS	dead (HD 76)	dead (HD102)	DFS	dead (HD 28)	dead (HD 56)	dead (HD 3)	DFS	DFS

(HD : hospital days, CR : complete remission, PR : partial remission, DFS : disease-free survival)

All 11 patients were treated with this combination regimen of PE and high-dose PSL therapy. Some of the patients were also treated with the following adjunctive therapies: high-dose IVIG, cyclosporine, vincristine, cyclophosphamide, and rituximab.

13 inhibitor (1.8 BU/mL) were present on admission, confirming the diagnosis of ai-TTP with a severe deficiency of ADAMTS13 activity. Because thrombocytopenia persisted (10×10^9/L), 4 cycles of vincristine at a dose of 1.0 mg/m^2 per week were initiated starting on hospital day 21. Moreover, an intravenous infusion of gamma globulin (IVIG) was administered at a dose of 400 mg/kg for 5 consecutive days starting on hospital day 49 because of persistent thrombocytopenia (25×10^9/L). After these treatments, combined with 30 rounds of PE therapy, a CR was achieved on hospital day 63. To date, the patient maintains disease-free survival (DFS) with an oral intake of 5 mg PSL/day.

Case 2: In 2005, a 50-year-old woman developed petechiae and a fever, and thereafter fell into a coma prior to her admission to our hospital. Based on the clinical diagnosis of TTP, PE therapy was immediately initiated with high-dose mPSL pulse therapy under sedation and with intubation. The patient was extubated on hospital day 10 because her platelet count increased to 100×10^9/L. On hospital day 12, the laboratory findings revealed that a low plasma level of ADAMTS13 activity (<3%) and positivity for an ADAMTS 13 inhibitor (1.6 BU/mL) were present at admission, confirming the diagnosis of ai-TTP with a severe deficiency of ADAMTS13 activity. Because clinical aggravations were observed while tapering off the administration of PE and PSL, a high-dose IVIG treatment was administered for 5 consecutive days starting on hospital day 21. A CR was achieved on hospital day 40 after 19 rounds of PE therapy. To date, the patient maintains DFS with an oral intake of 5 mg PSL/day.

Case 3: In 2007, a 48-year-old man developed a fever with subsequent eye deviation to the right and right hemiplegia prior to his admission to our hospital. Based on the clinical diagnosis of TTP, PE therapy was immediately initiated with high-dose mPSL pulse therapy under sedation and with intubation. The patient was extubated on hospital day 8. On hospital day 11, laboratory findings revealed that a low plasma level of ADAMTS13 activity (<3%) and positivity for an ADAMTS13 inhibitor (1.3 BU/mL) were present on admission, confirming the diagnosis of ai-TTP with a severe deficiency of ADAMTS13 activity. Because the clinical and laboratory findings were exacerbated when the PE therapy was tapered off, a second course of high-dose mPSL pulse therapy was initiated starting on hospital day 11. In addition, a high-dose IVIG treatment was administered for 5 consecutive days starting on hospital day 15, which remarkably improved the neurological and hematological findings. A CR was achieved on hospital day 42 after 12 rounds of PE therapy. Relapse has not been observed to date with an oral intake of 5 mg PSL/day. However, the patient did develop osteonecrosis of the right femur requiring an anterior rotation osteotomy in April 2011 which may have been associated with an adverse reaction to the two courses of high-dose mPSL pulse therapy.

Case 4: In 2007, a 56-year-old man developed petechiae without a fever and subsequently developed anemia and thrombocytopenia prior to his admission to our hospital. The clinical and laboratory findings upon admission suggested a diagnosis of TTP, but this patient also had a history of non-Hodgkin lymphoma (NHL) with CS IIIA at the age of 54. He achieved a CR from the NHL after 8 courses of treatment with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) therapy, but relapsed at the age of 55. However, he achieved a second CR after being treated in December 2006 with salvage therapy and

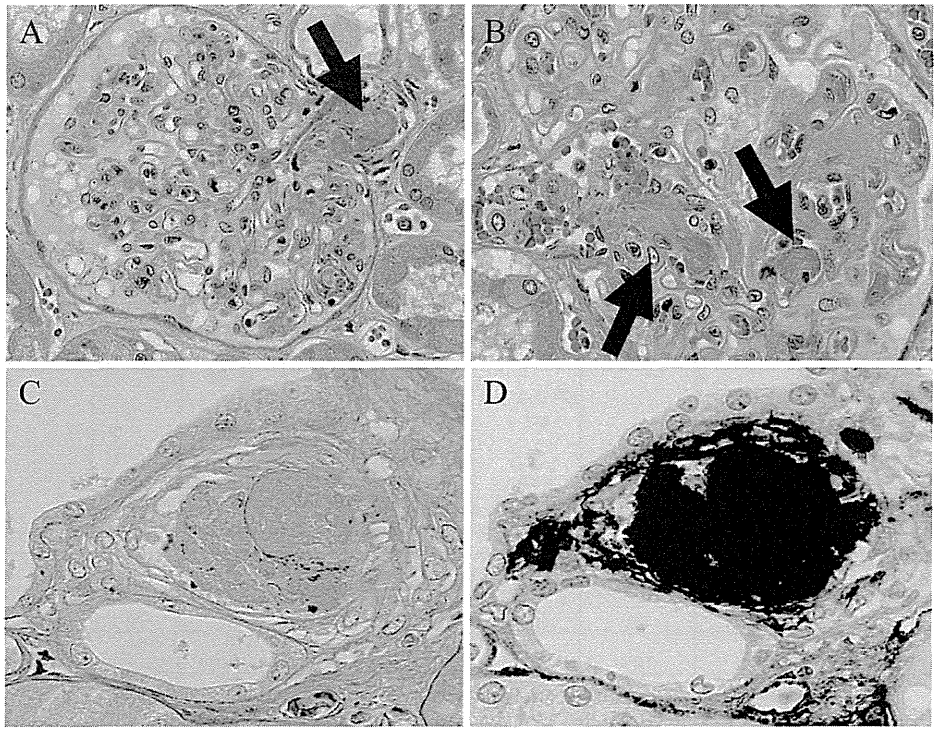

Figure. A post-mortem histological examination of a renal specimen from a patient with non-ADAMTS13 activity deficient TTP (See Case 5). Both Figures A and B show Hematoxylin and Eosin staining, and the arrows indicate platelet thrombi. Figure C is the immuno-staining with the anti-fibrinogen polyclonal antibody (DAKO, Glostrup, Denmark), and Figure D is the immune-staining with the anti-von Willebrand factor (VWF) polyclonal antibody (DAKO, Glostrup, Denmark). Note that the VWF-rich platelet thrombi in this patient show a sharp contrast to those in patients with disseminated intravascular coagulation (DIC) (17).

high-dose chemotherapy followed by an autologous peripheral blood stem cell transplantation (PBSCT). Because of his clinical background, he was initially treated with oral PSL (1 mg/kg) in 2007, but his condition soon worsened and he fell into a coma on hospital day 7. Under a clinical diagnosis of PBSCT-associated TTP, he received PE therapy with high-dose mPSL therapy starting on hospital day 7. On that day, the laboratory findings revealed that a slightly decreased plasma level of ADAMTS13 activity (39%) and negativity for an ADAMTS13 inhibitor were present on admission. Despite treatments with PE and mPSL therapy, his platelet count did not increase. For this reason, 4 cycles of vincristine administered at a dose of 1.0 mg/m^2 per week were initiated starting on hospital day 15, but the patient failed to improve. Four additional cycles of rituximab administered at a dose of 375 mg/m^2 per week were initiated starting on hospital day 29, but did not improve his clinical condition. He died of sepsis due to methicillin-resistant Staphylococcus aureus (MRSA) on hospital day 76 after receiving PE therapy 6 times.

Case 5: In 2007, a 50-year-old woman developed a fever, general fatigue, and dermatitis. She was admitted to our hospital after her family doctor found evidence of anemia and thrombocytopenia. Based on her low serum levels of complement, the presence of a skin rash, and the presence of anti-nuclear antibodies (ANA), the anti-ds DNA antibody, the anti-Sm antibody, and the anti-Scl 70 antibody, she was diagnosed with OS (SLE and SSc) and was treated with oral PSL (1 mg/kg). However, she developed the neurological symptoms of coma and tonic-clonic convulsions on hospital day 3. Taken together, these findings indicated a diagnosis of OS-associated TTP. PE therapy was initiated with high-dose mPSL therapy in parallel with continuous hemodialysis under sedation and with intubation from hospital day 3. The patient was extubated on hospital day 10. On that same day, laboratory findings revealed that a slightly decreased plasma level of ADAMTS13 activity (49%) and negativity for an ADAMTS13 inhibitor were present on admission. The exacerbation of the neurological symptoms and thrombocytopenia were observed while tapering off the administration of PE and PSL. Thus, cyclophosphamide (500 mg/m^2) was administered starting on hospital day 15 which resulted in a transient rise in her platelet count. A partial remission (PR) was achieved on hospital day 44 after 14 rounds of PE therapy. However, the patient died of an uncontrolled progression of aspergillus pneumonia on hospital day 102 despite the administration of liposomal amphotericin B. During the autopsy, platelet thrombi stained with the anti-VWF polyclonal antibody were detected in her kidney, heart, brain, and intestine (Figure). Since platelet thrombi in the microvasculatures that are rich in VWF have been hallmarks of ADAMTS13 activity deficient TTP (17), it was interesting

to note that the same pathological change was observed in this instance of non-ADAMTS13 activity deficient TTP. This finding indicates that both the severe deficiency of ADAMTS13 activity and a moderate deficiency with an extremely low ratio of ADAMTS13:Unusually Large VWF Multimers (UL-VWFM) lead to the same pathological results.

Case 6: In 2008, a 17-year-old female patient developed a fever, general fatigue, headache, vomiting, and arthralgia. She was admitted to our hospital after her family doctor found evidence of anemia and thrombocytopenia. She also had Raynaud's phenomenon, low serum levels of complement, and was positive for ANA, the anti-ds DNA antibody, and the anti-Sm antibody. These findings indicated a diagnosis of SLE and she was treated with PSL (1 mg/kg). She developed the neurological symptom of tonic-clonic convulsions on hospital day 3. By this point, she had been diagnosed with SLE-associated TTP, and PE therapy with high-dose mPSL pulse therapy under sedation and with intubation was instituted starting on hospital day 3. The patient was extubated on hospital day 5 after her platelet count increased (70×10^9/L). On hospital day 7, the laboratory findings revealed that a low plasma level of ADAMTS13 activity (<0.5%) and positivity for an ADAMTS13 inhibitor (1.4 BU/mL) were present on admission, confirming a diagnosis of SLE-associated acquired TTP with a severe deficiency of ADAMTS13 activity. Her neurological symptoms worsened on hospital day 12 despite intensive PE and mPSL therapy. As a result, an off-label treatment with rituximab was initiated starting on hospital day 13 which remarkably improved the abnormal clinical and laboratory findings. A CR was achieved on hospital day 45 after 6 rounds of PE therapy. To date, the patient maintains DFS with an oral intake of 5 mg PSL/day.

Case 7: In 2008, a 71-year-old woman developed a fever and chest pain and soon fell into a coma prior to being transferred to our hospital. The findings of abnormal electrocardiogram, elevated cardiac enzymes, and the asynergy of the antero-septal wall by ultrasound cardiogram confirmed a cardiac failure due to acute myocardial infarction (AMI). Due to her cardiac condition, PE therapy with a reduced amount of FFP (30 mL/kg) was initiated with high-dose mPSL therapy under sedation and with intubation starting on hospital day 1 in parallel with continuous hemodialysis. The laboratory findings on hospital day 9 revealed that a low plasma level of ADAMTS13 activity (<0.5%) and positivity for an ADAMTS13 inhibitor (1.1 BU/mL) were present on admission, thus confirming a diagnosis of ai-TTP with a severe deficiency of ADAMTS13 activity. Worsening clinical signs of acute cardiac failure and pulmonary edema necessitated the cessation of PE therapy after 10 rounds. Instead, cyclosporine therapy at a dose of 4 mg/day was started on hospital day 15. Although these treatments resulted in a partial remission (PR) for TTP, the patient died of cardiac shock associated with her AMI on hospital day 28.

Case 8: In 2008, a 68-year-old woman with dermatitis and chronic heart failure was diagnosed with anemia and thrombocytopenia by her family doctor and admitted to our hospital. Due to the presence of a skin rash, a positive ANA result, and the presence of the anti-Scl 70 antibody, she was diagnosed with SSc and was treated with PSL (1 mg/kg). During the treatment period, she went into a coma and experienced a pulmonary hemorrhage on hospital day 7. She was subsequently diagnosed with SSc-associated TTP. PE therapy at a reduced volume of FFP (30 mL/kg) with high-dose mPSL therapy was initiated in parallel with continuous hemodialysis with intubation starting on hospital day 7. The patient was extubated on hospital day 13. On that same day, the laboratory findings revealed that a slightly decreased plasma level of ADAMTS13 activity (47%) and negativity for an ADAMTS13 inhibitor were present on admission. The neurological symptoms and pulmonary hemorrhage were exacerbated when the PE and PSL administration were tapered off. As a result, we added cyclophosphamide (500 mg/m^2) pulse therapy on hospital day 14, but the patient failed to improve. We subsequently initiated off-label therapy with rituximab starting on hospital day 21. This treatment transiently improved the clinical signs and a PR was achieved on hospital day 42 after 8 rounds of PE therapy. However, the progression of a nosocomial aspergillus pneumonia could not be controlled despite the use of amphotericin B, and the patient died on hospital day 56.

Case 9: In 2008, a 56-year-old woman visited a local physician who identified dermatitis of Gottron's sign, arthritis, and the presence of ANA and the anti-SCL70 antibody. She was diagnosed with SSc and treated with PSL (1 mg/kg). Soon after, she developed anemia and thrombocytopenia and fell into a coma with cerebral hemorrhage. Simultaneously, she had lung lesions indicative of interstitial pneumonia with pulmonary hemorrhage. Due to these serious clinical conditions, she received a platelet transfusion with a dose of 10 units (2×10^{11} platelets) after which her clinical signs worsened and necessitated her transfer to our hospital. Clinical and laboratory findings upon admission indicated a clinical diagnosis of SSc-related TTP. The patient immediately received PE therapy for 2 consecutive days concurrently with high-dose mPSL pulse therapy. However, she died from the progression of a cerebral hemorrhage on hospital day 3 without appreciable clinical improvements. After her death, laboratory findings revealed that a normal plasma level of ADAMTS13 activity (69%) and negativity for an ADAMTS13 inhibitor were present on admission.

Case 10: In 2009, a 59-year-old man developed a fever, diarrhea, and gastrointestinal hemorrhage. The patient subsequently fell into a coma with left hemiplegia and was admitted to our hospital. PE therapy was initiated with high-dose mPSL pulse therapy after sedation and with intubation on hospital day 1. The patient was extubated on hospital day 10 because his clinical signs had improved. On hospital day 10, laboratory findings revealed that a deficient plasma ADAMTS13 activity (<0.5%) with positivity for an

ADAMTS13 inhibitor (2.0 BU/mL) were present upon admission, thus confirming a diagnosis of ai-TTP with severe deficiency of ADAMTS13 activity. However, exacerbation of the thrombocytopenia was observed when PE and PSL administrations were tapered off. Thus, cyclosporine (5 mg/kg) was administered starting on hospital day 20 which resulted in an increased platelet count. A CR was achieved on hospital day 35 after 13 rounds of PE therapy. To date, the patient maintains DFS with an oral intake of PSL 5 mg/day.

Case 11: In 2009, a 51-year-old woman developed a fever and sore throat and subsequently fell into a coma prior to her transfer to our hospital. In addition, she had a skin rash, low serum levels of complement, and was positive for ANA, the anti-ds DNA antibody, and the anti-Sm antibody. On the basis of these additional findings, she was diagnosed with SLE-associated TTP. PE therapy was initiated immediately with high-dose mPSL therapy under sedation and with intubation starting on hospital day 1. These treatments rapidly improved her laboratory and clinical findings, and she was extubated on hospital day 7. On hospital day 9, the laboratory findings revealed a low plasma level of ADAMTS13 activity (<0.5%) and positivity for an ADAMTS13 inhibitor (2.2 BU/mL), confirming the diagnosis of SLE-associated TTP with a severe ADAMTS13 deficiency. The neurological symptoms and thrombocytopenia were exacerbated when the PE and PSL administrations were tapered off. Thus, cyclophosphamide (500 mg/m^2) pulse therapy was added starting on hospital day 20, which gradually improved thrombocytopenia. A CR was achieved on hospital day 32 after 14 rounds of PE therapy. The patient maintains DFS with an oral intake of 5 mg PSL/day.

Case series summary: Of our 11 patients, 7 had ADAMTS13 activity-deficient TTP and 4 had non-ADAMTS13 activity-deficient TTP. Although idiopathic TTP develops with a fever in the absence of any known etiology, TTP can also be associated with various underlying diseases. Of the 11 patients included in our study, 5 were idiopathic and 6 had cases that were associated with underlying causes such as non-Hodgkin lymphoma (n=1) and connective tissue diseases (n=5) including SLE, SSc, and OS.

Regarding the clinical pentad in TTP, all of our 11 patients had a fever, hemolytic anemia, thrombocytopenia, and neurological signs upon admission, but 5 lacked renal dysfunction (Table 1). The laboratory findings indicated that the platelet counts and serum LDH levels were highly variable, but the serum haptoglobin levels were uniformly very low (<10 mg/dL). Moreover, non-ADAMTS13 activity-deficient TTP patients had tendency to present with high values of LDH and low values of bilirubin. With regard to ADAMTS 13, 7 of our patients had ADAMTS13 activity-deficient TTP with a low titer of ADAMTS13 inhibitors (1.1-2.2 BU/mL), whereas the remaining 4 had a slightly reduced or almost normal ADAMTS13 activity levels without detectable inhibitors.

Of the 11 patients treated for TTP, 6 are alive with a mean survival time of 1,014.6±1,101.0 days (mean ± SD). The survival rates of ADAMTS13 activity-deficient TTP patients and non-ADAMTS13 activity-deficient TTP patients were 85.7% and 0%, respectively, and the respective mean survival times were 1,816.0±853.3 days and 53.0±38.9 days.

The causes of death identified during this study included cardiogenic shock due to heart failure (patient 5), aspellugillus pneumonia (patients 8 and 10), MRSA sepsis (patient 9) and cerebral hemorrahage (patient 11). Patients with idiopathic, ADAMTS13 activity-deficient TTP did not develop any other diseases such as collagen diseases during the follow-up periods.

Discussion

The detailed clinical and laboratory findings were well preserved in this study because all 11 patients with TTP were treated in a single institution at Miyazaki during the past 12 years. Of these 11 patients, 5 had idiopathic TTP and 6 had cases of TTP that were associated with underlying issues such as non-Hodgkin lymphoma or connective tissue diseases. Regarding the clinical pentad of TTP, all 11 of the patients had a fever, hemolytic anemia, thrombocytopenia, and neurological signs upon admission, but 5 lacked renal dysfunction (Table 1). Although the platelet counts and serum LDH levels were highly variable between patients, their serum haptoglobin levels were uniformly very low (<10 mg/dL). Of these 11 patients, 7 had ADAMTS13 activity deficient TTP with low-titer ADAMTS13 inhibitors (1.1-2.2 BU/mL), whereas the remaining 4 had a slightly reduced or almost normal ADAMTS13 activity without any detectable inhibitors. The non-ADAMTS13 activity deficient TTP patients tended to display high LDH values and low bilirubin values. Notably, the plasma levels of fibrin degradation products (FDP)-P and D-dimer were slightly elevated in 3 out of the 4 non-ADAMTS13 activity-deficient TTP patients. These findings suggested that non-ADAMTS13 activity deficient TTP patients who suffer from an underlying disease are prone to suffer from a fibrinogen consumption commonly known as disseminated intravascular coagulation (DIC). Habe et al. (18) recently reported that ADAMTS13/VWF profiles may have important roles in the pathogenesis of DIC, and that the plasma levels of both ADAMTS13 and VWF propeptide are useful indicators for the diagnosis and prognosis of DIC. In contrast, the marker for immune thrombocytopenia (ITP), platelet-associated (PA) IgG, was present in significantly increased levels in all of our TTP patients, thereby indicating that PAIgG is not useful for differentiating between ITP and TTP.

Of our 11 patients with TTP, 6 are still alive. The survival rates of ADAMTS13 activity-deficient TTP patients and non-ADAMTS13 activity-deficient TTP patients were 85.7% and 0%, respectively, and the respective mean survival times were 1816.0±853.3 days and 53.0±38.9 days. Statistically significant differences between these two groups were observed in the incidence of renal dysfunction (p=0.02) and in

the total bilirubin counts (p=0.002).

Since 1991, PE therapy with or without an adjunctive regular or high-dose (pulse) steroid therapy has been used as the first-line treatment of TTP (6, 19-25). The discovery of ADAMTS13 allowed the efficacy of PE therapy in TTP patients to be quantifiably analyzed. Normal hemostasis can now be achieved by the replenishment of ADAMTS13 and regular-sized VWFM in addition to the removal of hazardous materials such as UL-VWFM, anti-ADAMTS13 autoantibodies, and the elevated inflammatory cytokines that upregulate UL-VWFM release from vascular endothelia cells. All 11 of our patients were treated with the combination regimen of PE therapy with high-dose mPSL pulse therapy because ADAMTS13 data were not readily available upon admission. The patient group received PE therapy between 6 and 30 times, and none of the patients showed a rebound or increase in their inhibitor titers during their PE therapy and subsequent clinical courses. The high-dose mPSL pulse therapy administered in tandem aimed to sedate the severe, fluctuating neurological signs associated with TTP as well as to suppress the anti-ADAMTS13 antibody production in patients with ADAMTS13 activity-deficient TTP. Ito-Habe et al. (26) report that a high-dose mPSL pulse therapy (1 g/day) is now administered in the majority of Japanese institutions rather than standard PSL therapy (1 mg/kg). According to their response to the combination regimen of PE therapy and mPSL pulse therapy, some of our patients were treated with the following adjunctive therapies: high-dose IVIG, cyclosporine, vincristine, cyclophosphamide, or rituximab. A few of these adjunctive drugs now have well-documented efficacies based on pharmacokinetics. The adjunctive therapies were administered based on the underlying disease, the unrecovered clinical symptons, the unresolved laboratory data, and the degree of organ dysfunction. We tended to administer rituximab for the refractory setting of TTP after the drug became available for off-label use in 2007. Due to our therapeutic regime, 6 out of the 7 patients with ADAMTS13 activity-deficient TTP accomplished CR with the exception from this group being the case that died from a complication of her AMI. In contrast, all 4 of the non-ADAMTS13 activity deficient TTP patients died.

While a few studies documented the efficacy of high-dose IVIG therapy in TTP patients prior to the discovery of ADAMTS13, subsequent studies could not confirm this finding (27, 28). Between 2003 and 2007, we used high-dose IVIG therapy at a dosage of 400 mg/kg IVIG for 5 consecutive days in 3 patients with ADAMTS13 activity-deficient TTP (cases 1-3) as an adjunctive therapy when the clinical aggravation of thrombocytopenia and persistent thrombocytopenia were observed during the tapering off period of the PE and PSL therapies. Interestingly, all the patients showed a good response to high-dose IVIG therapy. However, as described below, rituximab is now preferred over high-dose IVIG therapy for the treatment of TTP patients with relapsing and intractable clinical courses. If the efficacy of high-dose IVIG is proven on a scientific basis in the future, this therapy might be revived.

Among the adjunctive therapies, rituximab treatment may be most reasonable and powerful therapeutic modality for depleting the B cells that produce the autoantibodies for ADAMTS 13. Several reports in the literature have clearly shown that rituximab, an anti-CD20 chimeric monoclonal antibody, is highly efficient as an adjunctive therapy for TTP patients who do not respond adequately to a standard combination therapy of PE and steroids (29-31). Globally, the on-label therapeutic use of rituximab is for CD20-positive malignant B-cell lymphoma. Because the B-lymphocyte is an IgG antibody-producing cell, a broad spectrum of autoimmune diseases and associated symptoms can be targeted by rituximab, including the ADAMTS13 deficiency in TTP patients due to the presence of autoantibodies. The most recent study indicates that rituximab administration at a dosage of 375 mg/m^2 weekly for 4 cycles almost completely depletes the circulating B-lymphocytes from 16 hospital days to 3 months (32). Three of our TTP patients (cases 4, 6, and 8) were treated with rituximab between 2007 and 2008. The patient with ADAMTS13 activity deficient TTP (Case 6) due to a low titer of ADAMTS13 inhibitor (1.4 BU/mL), responded well to this treatment and has maintained DFS to date. However, the two patients with non-ADAMTS13 activity deficient TTP (Cases 4 and 8) died on hospital days 76 and 56, respectively. We therefore did not find any benefit in rituximab therapy for patients with non-ADAMTS13 activity-deficient TTP. However, of our 7 patients with ADAMTS13 activity-deficient TTP due to its inhibitors (Cases 1, 2, 3, 6, 7, 10, and 11), Case 6 alone was treated with rituximab and had a favorable outcome. During her course of treatment, Case 6 received less PE therapy than the other cases (6 rounds in Case 6 versus 9 to 30 times in our other cases), suggesting that the adjunctive use of rituximab has a plasma-sparing effect that must be confirmed in future studies. Scully et al. (32) reported in a Phase 2 study that rituximab treatment with PE was a safe and effective treatment for ai-TTP patients, thus validating our observations. Kameda et al. (33) have recently reported that rituximab treatment may be effective for non-ADAMTS 13 activity deficient TTP patients, but we did not confirm this to be true for our study. Their results may be explained by the reduction of excessive cytokine production through the rituximab-driven B cell depletion in non-ADAMTS13 activity deficient TTP patients, but that hypothesis needs to be carefully evaluated in future studies. We are presently unable to discuss the efficacies of the additional adjunct therapies, including including vincristine, high-dose cyclophosphamide, and cyclosporine, due to their low administration frequency to patients in this study.

In conclusion, we believe that this case-oriented study will be highly useful to the physicians who directly care for TTP patients.

The authors state that they have no Conflict of Interest (COI).

Acknowledgement

This work was supported in part by research grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan, from the Ministry of Health, Labor, and Welfare of Japan, and from the Takeda Science Foundation.

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