Ratio of von Willebrand Factor Propeptide to ADAMTS13 Is Associated with Severity of Sepsis

Hidetada Fukushima, M.D.; Kenji Nishio, M.D., Ph.D.; Hideki Asai, M.D.; Tomoo Watanabe, M.D.; Tadahiko Seki, M.D.; Hideto Matsui, M.D., Ph.D.; Mitsuhiko Sugimoto, M.D., Ph.D.; Masanori Matsumoto, M.D., Ph.D.; Yoshihiro Fujimura, M.D., Ph.D.; and Kazuo Okuchi, M.D., Ph.D.

Department of Emergency and Critical Care Medicine (HF, KN, HA, TW, TS, KO), General Medicine (KN), Regulatory Medicine for Thrombosis (HM, MS), and Blood Transfusion Medicine (MM, YF), Nara Medical University, Kashihara, Nara, Japan.

Correspondence: Kenji Nishio, M.D., Ph.D.,
840 Shijo-Cho, Kashihara City, Nara 634-8522, Japan
TEL +81-744-29-8905
FAX +81-744-24-5739
e-mail: knishio@naramed-u.ac.jp

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).

Running head: VWF propeptide/ ADAMTS13 ratio in sepsis

Conflicts of Interest: MM and YF are members of the advisory board of Alexion Pharma. YF is a member of the advisory board of Baxter BioScience. The other authors have no conflict of interest to declare.

Statement of Authorship: HF performed most of the experiments, data analysis, and manuscript preparation. HF, KN, HA, TW, TS, HM, and MM participated to the acquisition of blood samples and measurement of several parameters. HF, KN, MS, YF, and KO participated in analysis and interpretation of data. KN and KO 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.

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

ABSTRACT

Von Willebrand factor (VWF)-cleaving protease (ADAMTS13) cleaves ultralarge VWF secreted from endothelium and by which is regulating its physiologic function. An imbalance between ultralarge VWF 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 < 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 > 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 (APACHE II) score on days 1 and 5; Sepsis-related Organ Failure Assessment (SOFA) score on days 1, 3, and 5; maximum SOFA 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 > 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 ultralarge VWF secretion and ADAMTS13 level.

KEY WORDS: 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).

VWF is a multimeric glycoprotein that circulates in plasma and functions as a bridge between the subendothelial matrix and platelets. The subunit precursor proVWF (350 kDa) 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, over 20 000 kD). ULVWF condenses into tubules and forms Weibel-Palade bodies. ULVWF 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

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. Since 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).

Since 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 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 < 10% normal is enough to prevent the clinical manifestation of primary thrombotic microangiopathy (TMA) in patients with congenital ADAMTS13 deficiency (16). This suggests that patients with sepsis have a high enough ADAMTS13 level to prevent TMA, 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 < 120 000/μL. Exclusion criteria were: (i) patients aged < 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 non-survivors 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 maximum SOFA score during the clinical course at any time ≤ day 28. 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 1500 x g for 10 minutes 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 (9 men and 6 women; age: range, 23 to 55 y [mean, 40 y]), 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 interleukin 6 (IL-6) (R&D Systems Inc., Minneapolis, MN), TNF-α (R&D Systems Inc., Minneapolis, MN), and VWF (Dako, Glostrup, Denmark) were measured.

Data analysis

Data analysis was performed with statistical software (SPSS, Inc., Armonk, NY and GraphPad, San Diego, CA). 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 test, and the relation between 2 variables was evaluated with Spearman rank correlation. Statistical significance was defined by $P \leq .05$ for 2-sided tests.

RESULTS

Most patients were men and the most common diagnosis was intra-abdominal infection (Table 1). Most patients (20 patients out of 27 patients) were survivors; 1 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. There were no differences between survivors and non-survivors 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 non-survivors (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 non-survivors (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 day 5 and day 7) (Table 2). The mean level of ADAMTS13 was significantly higher in survivors than in non-survivors 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 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 > stage 1

within 48 hours 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%; $P \leq .02$) and VWF-pp/ADAMTS13 ratio (AKI, 15% ± 7%; no AKI, 7% ± 6%; $P \leq .001$) 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 non-survivors 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 to 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 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 of VWF-pp level on admission significantly decreased by day 3, but remained > 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 pro-inflammatory 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 E. 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.

ACKNOWLEDGEMENTS

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

REFERENCES

Reference

1. Dellinger RP. Inflammation and coagulation: implications for the septic patient. *Clin Infect Dis* 36(10):1259-65, 2003.
2. Vallet B. Bench-to-bedside review: endothelial cell dysfunction in severe sepsis: a role in organ dysfunction? *Crit Care* 7(2):130-8, 2003.
3. Sadler JE. Biochemistry and genetics of von Willebrand factor. *Annu Rev Biochem* 67:395-424, 1998.
4. van Mourik JA, Boertjes R, Huisveld IA, Fijnvandraat K, Pajkrt D, van Genderen PJ and Fijnheer R. von Willebrand factor propeptide in vascular disorders: A tool to distinguish between acute and chronic endothelial cell perturbation. *Blood* 94(1):179-85, 1999.
5. Borchiellini A, Fijnvandraat K, ten Cate JW, Pajkrt D, van Deventer SJ, Pasterkamp G, Meijer-Huizinga F, Zwart-Huinink L, Voorberg J and van Mourik JA. Quantitative analysis of von Willebrand factor propeptide release in vivo: effect of experimental endotoxemia and administration of 1-deamino-8-D-arginine vasopressin in humans. *Blood* 88(8):2951-8, 1996.
6. Salat C, Boekstegers P, Holler E, Werdan K, Reinhardt B, Fateh-Moghadam S, Pihusch R, Kaul M, Beinert T and Hiller E. Hemostatic parameters in sepsis patients treated with anti-TNF alpha-monoclonal antibodies. *Shock* 6(4):233-7, 1996.
7. Dong JF, Moake JL, Nolasco L, Bernardo A, Arceneaux W, Shrimpton CN, Schade AJ, McIntire LV, Fujikawa K and Lopez JA. ADAMTS-13 rapidly cleaves newly secreted ultralarge von Willebrand factor multimers on the endothelial surface under flowing conditions. *Blood* 100(12):4033-9, 2002.
8. Chauhan AK, Motto DG, Lamb CB, Bergmeier W, Dockal M, Plaimauer B, Scheiflinger F, Ginsburg D and Wagner DD. Systemic antithrombotic effects of ADAMTS13. *J Exp Med* 203(3):767-76, 2006.
9. Bernardo A, Ball C, Nolasco L, Choi H, Moake JL and Dong JF. Platelets adhered to endothelial cell-bound ultra-large von Willebrand factor strings support leukocyte tethering and rolling under high shear stress. *J Thromb Haemost* 3(3):562-70, 2005.
10. Moake JL. von Willebrand factor, ADAMTS-13, and thrombotic thrombocytopenic purpura. *Semin Hematol* 41(1):4-14, 2004.

11. Ono T, Mimuro J, Madoiwa S, Soejima K, Kashiwakura Y, Ishiwata A, Takano K, Ohmori T and Sakata Y. Severe secondary deficiency of von Willebrand factor-cleaving protease (ADAMTS13) in patients with sepsis-induced disseminated intravascular coagulation: its correlation with development of renal failure. *Blood* 107(2):528-34, 2006.
12. Moake JL and Chow TW. Thrombotic thrombocytopenic purpura: understanding a disease no longer rare. *Am J Med Sci* 316(2):105-19, 1998.
13. Martin K, Borgel D, Lerolle N, Feys HB, Trinquart L, Vanhoorelbeke K, Deckmyn H, Legendre P, Diehl JL and Baruch D. Decreased ADAMTS-13 (A disintegrin-like and metalloprotease with thrombospondin type 1 repeats) is associated with a poor prognosis in sepsis-induced organ failure. *Crit Care Med* 35(10):2375-82, 2007.
14. Bockmeyer CL, Claus RA, Budde U, Kentouche K, Schneppenheim R, Losche W, Reinhart K and Brunkhorst FM. Inflammation-associated ADAMTS13 deficiency promotes formation of ultra-large von Willebrand factor. *Haematologica* 93(1):137-40, 2008.
15. Claus RA, Bockmeyer CL, Budde U, Kentouche K, Sossdorf M, Hilberg T, Schneppenheim R, Reinhart K, Bauer M, Brunkhorst FM and Losche W. Variations in the ratio between von Willebrand factor and its cleaving protease during systemic inflammation and association with severity and prognosis of organ failure. *Thromb Haemost* 101(2):239-47, 2009.
16. Rieger M, Ferrari S, Kremer Hovinga JA, Konetschny C, Herzog A, Koller L, Weber A, Remuzzi G, Dockal M, Plaimauer B and Scheiflinger F. Relation between ADAMTS13 activity and ADAMTS13 antigen levels in healthy donors and patients with thrombotic microangiopathies (TMA). *Thromb Haemost* 95(2):212-20, 2006.
17. Nguyen TC, Han YY, Kiss JE, Hall MW, Hassett AC, Jaffe R, Orr RA, Janosky J and Carcillo JA. Intensive plasma exchange increases a disintegrin and metalloprotease with thrombospondin motifs-13 activity and reverses organ dysfunction in children with thrombocytopenia-associated multiple organ failure. *Crit Care Med* 36(10):2878-87, 2008.
18. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM and Sibbald WJ. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 101(6):1644-55, 1992.
19. Johnson CA, Levey AS, Coresh J, Levin A, Lau J and Eknoyan G. Clinical practice guidelines for chronic kidney disease in adults: Part II. Glomerular filtration rate,

proteinuria, and other markers. *Am Fam Physician* 70(6):1091-7, 2004.

20. Durand F and Valla D. Assessment of the prognosis of cirrhosis: Child-Pugh versus MELD. *J Hepatol* 42 Suppl(1):S100-7, 2005.
21. Knaus WA, Draper EA, Wagner DP and Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* 13(10):818-29, 1985.
22. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, Reinhart CK, Suter PM and Thijs LG. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* 22(7):707-10, 1996.
23. Molitoris BA, Levin A, Warnock DG, Joannidis M, Mehta RL, Kellum JA, Ronco C, Shah SV and Acute Kidney Injury Network working g. Improving outcomes of acute kidney injury: report of an initiative. *Nat Clin Pract Nephrol* 3(8):439-42, 2007.
24. Kremer Hovinga JA, Zeerleder S, Kessler P, Romani de Wit T, van Mourik JA, Hack CE, ten Cate H, Reitsma PH, Wuillemin WA and Lammle B. ADAMTS-13, von Willebrand factor and related parameters in severe sepsis and septic shock. *J Thromb Haemost* 5(11):2284-90, 2007.
25. Crawley JT, Lam JK, Rance JB, Mollica LR, O'Donnell JS and Lane DA. Proteolytic inactivation of ADAMTS13 by thrombin and plasmin. *Blood* 105(3):1085-93, 2005.
26. Reiter RA, Knobl P, Varadi K and Turecek PL. Changes in von Willebrand factor-cleaving protease (ADAMTS13) activity after infusion of desmopressin. *Blood* 101(3):946-8, 2003.
27. Reiter RA, Varadi K, Turecek PL, Jilma B and Knobl P. Changes in ADAMTS13 (von-Willebrand-factor-cleaving protease) activity after induced release of von Willebrand factor during acute systemic inflammation. *Thromb Haemost* 93(3):554-8, 2005.
28. Metcalf DJ, Nightingale TD, Zenner HL, Lui-Roberts WW and Cutler DF. Formation and function of Weibel-Palade bodies. *J Cell Sci* 121(Pt 1):19-27, 2008.
29. Bockmeyer CL, Reuken PA, Simon TP, Budde U, Losche W, Bauer M, Birschmann I, Becker JU, Marx G and Claus RA. ADAMTS13 activity is decreased in a septic porcine model. Significance for glomerular thrombus deposition. *Thromb Haemost* 105(1):145-53, 2011.
30. Kayal S, Jais JP, Aguini N, Chaudiere J and Labrousse J. Elevated circulating E-selectin, intercellular adhesion molecule 1, and von Willebrand factor in patients with severe

infection. *Am J Respir Crit Care Med* 157(3 Pt 1):776-84, 1998.

31. van Mourik JA and Romani de Wit T. Von Willebrand factor propeptide in vascular disorders. *Thromb Haemost* 86(1):164-71, 2001.
32. Shida Y, Nishio K, Sugimoto M, Mizuno T, Hamada M, Kato S, Matsumoto M, Okuchi K, Fujimura Y and Yoshioka A. Functional imaging of shear-dependent activity of ADAMTS13 in regulating mural thrombus growth under whole blood flow conditions. *Blood* 111(3):1295-8, 2008.
33. Doi M, Matsui H, Takeda Y, Saito Y, Takeda M, Matsunari Y, Nishio K, Shima M, Banno F, Akiyama M, Kokame K, Miyata T and Sugimoto M. ADAMTS13 safeguards the myocardium in a mouse model of acute myocardial infarction. *Thromb Haemost* 108(6), 2012.
34. Fujioka M, Hayakawa K, Mishima K, Kunizawa A, Irie K, Higuchi S, Nakano T, Muroi C, Fukushima H, Sugimoto M, Banno F, Kokame K, Miyata T, Fujiwara M, Okuchi K and Nishio K. ADAMTS13 gene deletion aggravates ischemic brain damage: a possible neuroprotective role of ADAMTS13 by ameliorating postischemic hypoperfusion. *Blood* 115(8):1650-3, 2010.
35. Fujioka M, Nakano T, Hayakawa K, Irie K, Akitake Y, Sakamoto Y, Mishima K, Muroi C, Yonekawa Y, Banno F, Kokame K, Miyata T, Nishio K, Okuchi K, Iwasaki K, Fujiwara M and Siesjo BK. ADAMTS13 gene deletion enhances plasma high-mobility group box1 elevation and neuroinflammation in brain ischemia-reperfusion injury. *Neurol Sci* 33(5):1107-15, 2012.

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

* N = 27 patients. Data reported as mean ± SD; number (%)

† Acute Physiology and Chronic Health Evaluation II

‡ Sequential Organ Failure Assessment

§ Systemic inflammatory response syndrome score >3

ADAMTS13, von Willebrand factor propeptide, and von Willebrand factor are expressed as a percentage of normal controls.

Table 2. Levels of VWF, VWF-pp, ADAMTS13, and Inflammatory Markers in Patients with Severe Sepsis or Septic Shock *

Variables	Control subjects	Patients with severe sepsis or septic shock			
		Day			
		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
Non-survivors	NA	19 ± 5.4	27 ± 8.2	25 ± 10	24 ± 9.4
P	NA	.03§	NS	.03§	.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
Interleukin 6 (pg/mL) ††	<2.4	1220 (362 to 3610)	206 (58 to 1050)	115 (29 to 338)	75 (20 to 446)
TNF α (pg/mL) ‡‡	<1.8	5.8 (3.3 to 21.3)	3.4 (2.4 to 5.8)	2.5 (1.2 to 4.0)	2.0 (1.4 to 3.3)

* N = 27 patients (day 1) or 26 patients (days 3, 5, and 7) with sepsis or septic shock; 15 normal control subjects. Data reported as mean ± SD or median (interquatile range). VWF, VWF-pp , and ADAMTS13are expressed as a percentage of normal controls.

† VWF-pp: difference between day 1 and day 3, $P \leq .05$

‡ ADAMTS13: difference between normal controls and patients on day 1, $P \leq .001$; difference between day 1 and day 3, $P \leq .01$; difference between day 3 and day 5, $P \leq .05$.

§ ADAMTS13: Difference between survivors and non-survivors, $P \leq .05$; NS, not significant ($P > .05$)

‖ VWF-pp/ADAMTS13 ratio: difference between normal controls and patients on day 1, $P \leq .001$; difference between day 1 and day 3, $P \leq .01$

†† Upper limit of normal, 2.41 pg/mL

‡‡ Upper limit of normal, 1.79 pg/mL

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 †	$P \leq$ ‡	r†	$P \leq$ ‡	r†	$P \leq$ ‡	r†	$P \leq$ ‡
VWF-pp								
APACHE II	0.32	NS	0.16	NS	0.45	.05	0.07	NS
SOFA	0.51	.007	0.47	.02	0.55	.01	-0.62	NS
Max SOFA	0.25	NS	0.38	NS	0.44	.03	0.59	.003
TNF-α	0.47	.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	.001	0.34	NS	0.32	NS	0.18	NS
ADAMTS-13								
APACHE II	-0.54	.004	-0.30	NS	-0.68	.001	-0.34	NS
SOFA	-0.32	NS	-0.30	NS	-0.57	.007	-0.13	NS
Max SOFA	-0.53	.005	-0.42	.03	-0.47	.02	-0.29	NS
TNF-α	-0.07	NS	-0.37	NS	-0.40	.05	-0.43	NS
IL-6	-0.04	NS	-0.36	NS	-0.39	NS	-0.45	.05
Creatinine	-0.33	NS	-0.22	NS	-0.35	NS	-0.47	.02
VWF-pp/ADAMTS-13 ratio								
APACHE II	0.45	.03	0.15	NS	0.69	.001	0.19	NS
SOFA	0.65	.001	0.41	.04	0.68	.001	-0.61	NS
Max SOFA	0.45	.03	0.41	.04	0.52	.007	0.63	.001
TNF-α	0.44	.03	0.57	.002	0.44	.03	0.59	.007
IL-6	0.12	NS	0.04	NS	0.48	.02	0.70	.001
Creatinine	0.76	.001	0.29	NS	0.49	.02	0.48	.02

* Abbreviations: ADAMTS13, a disintegrin-like and metalloprotease with thrombospondin type 1 motif, member 13 (also known as von Willebrand Factor cleavage protease); APACHE II, Acute Physiology and Chronic Health Evaluation II; IL-6, interleukin 6; Max SOFA, maximum Sepsis-related Organ Failure Assessment score during the clinical course at any time ≤ day 28; SOFA, Sepsis-related Organ Failure Assessment score; TNF-α, tumor necrosis factor α; VWF, von Willebrand Factor; VWF-pp, von Willebrand Factor propeptide

† Spearman rank correlation (ρ)

‡ NS, not significant ($P > .05$)