(Diagnostica Stago, respectively). The plasma PC antigen concentration was measured based on a latex agglutination test using a LPIA-ACE PC kit (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). The plasma AT activity was measured according to a synthetic substrate assay using a Chromorate ATIII (C) kit (Mitsubishi Chemical Medience Corporation). The dilute Russell's viper venom time (DRVVT) was measured with the clotting time method using a Gradipore LA test (Gradipore, Sydney, Australia). The titers of anti-cardiolipin-β2 glycoprotein I (ACL-β2GPI) antibodies were measured with an ELISA kit (Yamasa Co, Tokyo, Japan) [17].

Gene analysis of AT, PC and PS

Genomic DNA was prepared from peripheral blood leukocytes using a QIAamp DNA Blood Mini Kit (QIAGEN) according to the manufacturer's instructions. Each exon and exon/intron boundary of the gene was amplified from genomic DNA using polymerase chain reaction (PCR), as previously described. The PCR products were directly sequenced using a Big-Dye Terminator Cycle Sequencing Kit and Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) [17]. Gene analyses were carried out in cases with AT, PC or PS levels less than 70 %.

Statistical analysis

The data are expressed as the median (25th–75th percentile). Differences between the groups were examined for statistical significance using the Mann–Whitney *U* test. A p value of <0.05 denoted the presence of a statistically significant difference.

Results

There were 17 patients with pregnancy-related VTE, including one woman with congenital AT deficiency and her mother. There were five families with congenital AT deficiency and two families with congenital PC deficiency. One woman with PC deficiency showed PS Tokushima (Table 1). These patients were diagnosed as having thrombophilia based on a genetic analysis after developing VTE. The age of onset of thrombosis was 30.0 years (28.8–34.3 years), and thrombosis appeared during first trimester in eight cases, during second trimester in three cases, during third trimester in five cases and after delivery in two cases. Fourteen of the eighteen women demonstrated thrombosis at the first pregnancy. There were 14 cases of DVT, three cases of CVST, one case of DIC and one case of TIA. The cause of thrombosis was considered to be AT

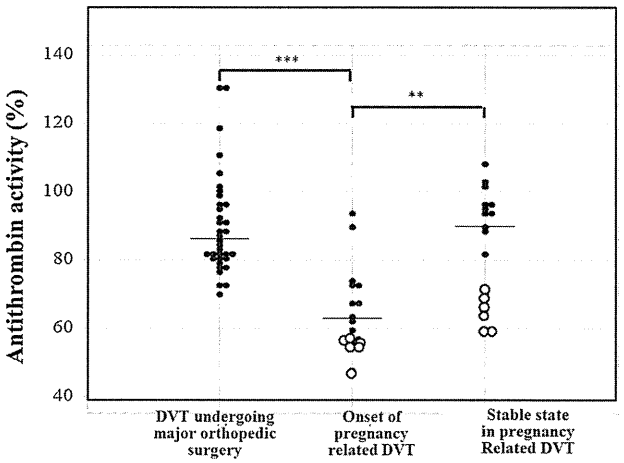

Fig. 1 AT activity at onset and in the stable state of pregnancy-related VTE and in the DVT patients undergoing major orthopedic surgery. $^{**}p < 0.01$, $^{***}p < 0.005$. *Open circle* congenital AT deficiency. *DVT* deep vein thrombosis, *VTE* venous thromboembolism

deficiency in eight cases, APS in two cases, PC deficiency in one case, PC and PS deficiency in one case, bed rest in one case, pregnancy-induced hypertension in one case, dehydration in one case and unknown in three cases. An abortion occurred in only two cases.

The AT activity levels were significantly lower at the onset of thrombosis (62.0 %: 56.8–72.5 %) than after delivery and anticoagulant therapy (89.2 %: 67.7–95.7 %, $p < 0.005$) and in the DVT patients undergoing major orthopedic surgery (86.2 %; 80.7–96.4 %, $p < 0.001$, Fig. 1). The PS activity and antigen levels were also significantly lower at the onset of thrombosis (50.0 %: 40.2–60.4 % and 60.0 %: 49.4–81.4 %) than after delivery and without warfarin therapy (83.2 %: 69.8–95.3 % and 90.9 %: 73.6–114.0 %, $p < 0.01$ and $p < 0.05$). There were no significant differences in the PC activity or antigen levels between the onset of thrombosis and the stable state after delivery and without warfarin therapy (Table 2). In cases 1–5, the AT activity was significantly low in the stable state and became lower at the onset of thrombosis. Although the AT levels were normal before pregnancy, they subsequently decreased and the patients required the administration of AT after pregnancy in Cases 6 and 11. Table 3 shows the veins in which thrombosis related to pregnancy occurred. The frequency of DVT was relatively higher in the left vein than in the right vein. In the DVT patients undergoing major orthopedic surgery, DVT occurred in the form of soleus vein thrombosis.

In the gene analyses (Table 4), AT Budapest [18] was noted in case 1, AT Toyama [19] was noted in case 2 and AT Glasgow [20] was noted in cases 3-a and 3-b, 4 and 5. Although the patients in cases 3-a and 3-b were from the same family, the patients in cases 3–5 were from different

Table 2 AT, PC and PS levels at the onset of thrombosis

	AT activity (%)		PC activity (%)		PC antigen (%)		PS activity (%)		PS antigen (%)	
	Onset	Stable	Onset	Stable	Onset	Stable	Onset	Stable	Onset	Stable
1	55.5	63.6	–	107	–	108	–	127	–	120
2	56.0	70.0	–	–	98	–	–	–	60	–
3-a	–	59.1	–	147	–	135	–	–	97	114
3-b	59.0	59.4	113	–	–	–	24	–	–	–
4	57.4	71.4	–	89.0	–	–	–	66	–	73
5	47.5	65.4	–	126	–	116	61	89	51	79
6	56.8	108	105	100	91	96	56	77	–	–
7	56.9	89.8	61	60	56	58	61	–	–	–
8	94.2	102	105	102	–	–	49	98	91	102
9	–	93.0	–	98.0	–	–	–	49 ([a])	–	–
10	63.9	82.0	138	–	134	–	50	73	36	63
11	72.0	96.8	104	–	95	–	57	–	58	–
12	62.0	92.9	97	–	90	–	39	–	44	–
13	67.9	–	55	–	39	–	43	–	62	–
14	72.6	96.8	–	–	–	–	–	–	–	–
15	73.3	88.6	109	–	95	–	39	–	–	–
16	89.7	94.6	109	103	100	104	66	92	78	–
17	66.8	103	134		128		27		61	

AT antithrombin, *PC* protein C, *PS* protein S

[a] Pregnant state

Table 3 Thrombosis

Name	Vein
1	DVT (left femoral and soleus vein), CVST (straight sinus)
2	DVT (left femoral and soleus vein)
3-a	DVT (right iliac and femoral vein)
3-b	DVT (left femoral vein)
4	DVT (left iliac, femoral and soleus vein)
5	DVT (left iliac, femoral and soleus vein)
6	DVT (left external iliac and femoral vein), DVT (left and right femoral and soleus vein)
7	DVT (inferior vena cava, left iliac, femoral and soleus vein)
8	CVST (right transverse sinus)
9	Left DVT ([a])
10	CVST (right transverse sinus)
11	DVT (inferior vena cava, left iliac, femoral, superficial femoral and popliteal vein)
12	DVT (right common iliac, external iliac and common femoral vein)
13	DVT (right external iliac vein)
14	No findings in MRI
15	DIC
16	DVT (right soleus vein)
17	DVT (left external iliac and femoral vein)

DVT deep vein thrombosis, *CVST* cerebral venous sinus thrombosis, *MRI* magnetic resonance imaging

[a] Data were not available

families and were not relatives. Protein C Tochigi [21] was observed in case 7 and combined heterozygous PC deficiency [22] and PS Tokushima [23] was observed in case 13. There were no cases in which the AT, PC or PS levels were less than 70 % of among the DVT patients undergoing major orthopedic surgery.

Table 4 Gene analysis

Name			cDNA change	Amino acid change
1	AT Budapest [18]	Type II	c.1382C>T	p.Pro461Leu
2	AT Toyama [19]	Type II	c.235C>T	p.Arg79Cys
3-a	AT Glasgow [20]	Type II	c.1274G>A	p.Arg425His
3-b	AT Glasgow [20]	Type II	c.1274G>A	p.Arg425His
4	AT Glasgow [20]	Type II	c.1274G>A	p.Arg425His
5	AT Glasgow [20]	Type II	c.1274G>A	p.Arg425His
7	Protein C Tochigi, Protein C Osaka-1 [21]	Type I	c.631C>T	p. Arg211Trp
13	Protein C [22]	Type I	c.400G>T	p.Glu134X
	Protein S Tokushima [23]	Type II	c.586A>G	p.Lys196Glu

AT antithrombin, *PC* protein C, *PS* protein S

Discussion

Maternal factors are important for the onset of pregnancy-related VTE [14]. In the current study, the median age of pregnancy-related VTE was 30.0 years, suggesting that late child bearing is not a main cause of pregnancy-related VTE.

Several previous reports [24, 25] have suggested that the onset of pregnancy-related VTE in cases of thrombophilia occurs in early pregnancy. In our study, pregnancy-related VTE tended to occur during the first and second trimesters, and most case of pregnancy-related VTE appeared during the patient's first pregnancy, suggesting that thrombophilia is an important factor for the onset of thrombosis during pregnancy. VTE, such as DVT and CVST, was observed in this study. Although DVT is the most frequent type of VTE, CVST [26, 27] is rare among cases of VTE. The diagnosis of CVST provides an important clue to suspect CVST. Most lesions of pregnancy-related VTE involved proximal DVT in this study; however, DVT was distal in the patients undergoing major orthopedic surgery, as patients undergoing major orthopedic surgery are treated with anticoagulants [28]. Although patients with pregnancy-related VTE have a risk of developing fatal PE, pregnant women are usually not treated with anticoagulants such as warfarin. Therefore, these patients require intravenous heparin administration.

In terms of the causes of thrombosis, hemostatic abnormalities were noted in 12 cases (approximately 66 %). In particular, congenital or acquired AT deficiencies were observed in eight cases (approximately 44 %). The AT activity was significantly low in the stable state of congenital AT deficiency and decreased further at the onset of thrombosis. Although the AT levels were normal before pregnancy in the cases of acquired AT deficiency, they subsequently decreased and the patients required the administration of AT during pregnancy and after delivery. AT deficiency has been reported to be an independent risk factor for pregnancy-related VTE [10]. AT deficiency is also observed in patients with pregnancy-induced hypertension [29]. Congenital or acquired AT deficiency may be important for the onset of pregnancy-related VTE. However, AT deficiency was not observed in the DVT patients undergoing major orthopedic surgery in this study, and the PS activity and antigen levels were also significantly low at the onset of pregnancy-related VTE in comparison to that observed in the stable state or in the DVT patients undergoing major orthopedic surgery. Although the decreased PS levels noted in pregnant women have been reported to be caused by estrogen [30], the relationship between decreased PS levels and pregnancy-related VTE remains unclear. APS is also important for pregnancy-related VTE, as reported in Case 15 [31]. While there were two cases of congenital PC deficiency, in the current study, there were no significant differences in the PC levels between the onset of VTE and in the stable state, suggesting that pregnancy may not decrease the PC levels.

In the gene analyses, six pregnant women with VTE were diagnosed as having congenital AT deficiency, indicating that the rate of congenital AT deficiency is markedly high in cases of pregnancy-related VTE. A previous study [10] reported that AT deficiency is a risk factor for pregnancy-related VTE. Notably, AT Budapest [18], AT Toyama [19] and AT Glasgow [20] were noted in this study. Although the patients in Cases 3–5 from different families were not relatives, the far ancestors of these families may be the same. There were two cases of congenital PC deficiency in this report; however, the relationship between PC deficiency and pregnancy-related VTE was not clarified.

In conclusion, a deficiency of natural anticoagulants, especially AT, is frequently observed in patients with pregnancy-related VTE and is an important cause of pregnancy-related VTE.

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Compliance with ethical standards

Conflict of interest There are no conflicts of interest for any of the authors in association with this study.

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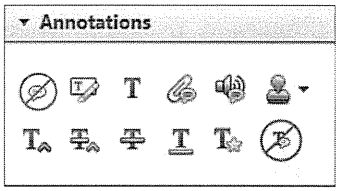

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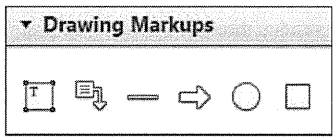

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Elevated Soluble Platelet Glycoprotein VI Levels in Patients After Living Donor Liver Transplantation

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Abstract

Plasma-soluble platelet glycoprotein VI (sGPVI) levels were examined in patients undergoing living donor liver transplantation (LDLT), and the relationship between platelet activation and thrombocytopenia was evaluated to understand the mechanism of thrombocytopenia in LDLT. Platelet counts were significantly higher in the donors compared to the recipient, and the plasma sGPVI levels increased in both groups after the operation. Regarding the relationship between the platelet counts and the sGPVI levels, the slope varied on different days, and it became negative on day 3, suggesting that the plasma sGPVI levels are related to platelet activation in LDLT. The frequency of complications was high in the nonsurvivors. The platelet counts were higher in the survivors than in the nonsurvivors on days 14 and 28. Although the plasma levels of sGPVI in the survivors increased after the operation, those in the nonsurvivors were high only on day 3. Although the ADAMTS13 levels were markedly reduced, von Willebrand factor (VWF) and VWF propeptide (VWFpp) were markedly elevated during LDLT. The antithrombin activity was significantly lower (day 14) and VWFpp (day 28) was significantly higher in the nonsurvivors than in the survivors. These findings suggest that platelet activation first occurs after LDLT, and it is high in the nonsurvivors on day 3. Thereafter, the hemostatic abnormality and vascular endothelial cell injuries may appear on days 14 and 28.

Keywords

sGPVI, living donor liver transplantation, thrombocytopenia, mortality

Introduction

Living donor liver transplantation (LDLT) was first performed in Japan in 1989,[1] and unique technical, physiological, and logistical innovations in LDLT[2,3] have developed since then. Technical improvements in living donor surgery have led to the generalization of pediatric LDLT with excellent patient and graft survival outcomes. Despite the application of preoperative plasma exchange, splenectomy, and enhanced immunosuppression, the 5-year graft survival rate is less than 70% in ABO-incompatible LDLT in children.[4,5] Specific diseases and preoperative patient conditions are associated with the transplantation outcomes.[6-8] In the registry of the Japanese Liver Transplantation Society between November 1989 and December 2010, the 1-, 5-, 10-, and 20-year patient survival rates were 88.3%, 85.4%, 82.8%, and 79.6%, respectively.[9] Nationwide surveys of acute liver failure (ALF) are conducted annually in Japan, and 20% of patients with ALF undergo liver transplantation (LT).[10] In LDLT for patients with ALF, the cumulative patient survival rate at 1 year after LT was 79%.[10]

Transient thrombocytopenia is a common phenomenon after LT, and the recovery of platelet counts is clinically significant. In 1992, McCaughan et al[11] revealed the nadir platelet counts after LT to predict allograft dysfunction. Following this report, 3 additional studies[12-14] confirmed that severe thrombocytopenia after LT was associated with graft and patient survival. However, the precise mechanisms of posttransplant thrombocytopenia and its relationship with graft dysfunction still remain unclear. The mechanisms contributing to graft dysfunction are multifactorial and include ischemic reperfusion injury,

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sinusoidal endothelial cells injury, platelet aggregation, immunological reactions, and inflammatory responses.[15,16]

Platelet glycoprotein VI (GPVI), a type I transmembrane glycoprotein of the immunoreceptor family, is constitutively associated and expressed with the Fc receptor γ-chain, an immunoreceptor tyrosine-based activation motif-bearing receptor.[17,18] Upon platelet activation, the platelet surface GPVI is cleaved off by proteases, such as ADAM10, releasing the soluble form of GPVI (sGPVI).[19,20] The soluble form of GPVI has recently received much attention as a platelet activation marker, as described subsequently. Several groups have reported that sGPVI is a useful biomarker of diseases caused by platelet activation, such as acute coronary syndrome and stroke.[21,22] C-type, lectin-like receptor 2 is a transmembrane glycoprotein similar to GPVI that has been reported to be a potential thrombotic marker.[23]

A disintegrin and metalloprotease with thrombospondin type I domain, member 13 (ADAMTS13) is a metalloprotease that specifically cleaves the multimeric von Willebrand factor (VWF).[24] The pre–pro-VWF is synthesized in endothelial cells and megakaryocytes, and the VWF propeptide (VWFpp) is cleaved but remains stored together with mature VWF in α-granules (megakaryocytes) and Weibel-Palade bodies (endothelial cells). After the secretion of VWFpp and VWF into the plasma from endothelial cells induced by physiological or pathological stimuli, VWFpp dissociates from VWF.[25] The behaviors of ADAMTS13, VWF, and VWFpp have been reported previously in LDLT patients with thrombotic microangiopathy (TMA),[26,27]

In this study, the sGPVI levels were measured in 79 patients undergoing LDLT, and the relationship between the sGPVI levels and thrombocytopenia was examined to evaluate the mechanism of thrombocytopenia in LDLT patients with a poor outcome.

Materials and Methods

The plasma levels of sGPVI and platelet counts were determined in 79 recipients (34 females and 45 males) and 12 donors during LDLT from January 1, 2002, to March 31, 2014. The patients who provided consent were continuously included in this study, but the patients with unavailable plasma were excluded. The underlying diseases necessitating LDLT were liver cirrhosis (LC) without hepatic cell carcinoma (HCC) in 40 cases, LC with HCC in 23 cases, HCC without LC in 3 cases, and other diseases in 13 cases (ie, 6 cases of biliary atresia, 4 cases of acute hepatitis, 2 cases of primary sclerosing cholangitis, and 1 case of Alagille syndrome). The causes of LC were hepatitis C virus infection in 23 cases, hepatitis B virus infection in 12 cases, non-B and non-C hepatitis in 11 cases, primary biliary cirrhosis in 9 cases, alcoholic disturbances in 4 cases, progressive intrahepatic cholestasis in 3 cases, and secondary biliary cirrhosis in 1 case. The mortality rate was evaluated on day 90 after surgery.[13] The study protocol was approved by the Human Ethics Review Committee of Mie University School of Medicine, and written informed consent was obtained from each participant. The immunosuppression protocol consisted of tacrolimus and low-dose steroids. The target trough level for tacrolimus in whole blood was 10 to 15 ng/mL during the first 2 weeks, approximately 10 ng/mL during the next 2 weeks, and 5 to 10 ng/mL thereafter. Methylprednisolone (10 mg/kg body weight) was administered intravenously immediately before perfusion of the graft portal vein. Methylprednisolone (1 mg/kg) was given intravenously daily on postoperative days (PODs) 1 to 3, and a dose of 0.5 mg/kg was given daily on PODs 4 to 6. On POD 7, the steroid was switched to oral prednisolone (0.5 mg/kg) daily, and at 1 month, the dose was reduced to 0.1 mg/kg. Patients whose liver function was stable were weaned off the steroid approximately 3 to 6 months postoperatively.

The Child-Pugh (CP) score, prothrombin time–international normalized ratio (PT-INR), total bilirubin, albumin, graft-to-recipient weight ratio (GRWR), platelet counts, and plasma sGPVI levels were measured in the patients undergoing LDLT.

The plasma level of sGPVI was quantified using a sandwich enzyme-linked immunosorbent assay, which consisted of 2 mouse anti-GPVI monoclonal antibodies, F1232-7-1 and F1232-10-2 able to recognize the extracellular domain 1 (D1) N-terminal loop and extracellular domain D2 loop of GPVI, respectively.[28-30]

The platelet counts were measured using the fully automated hematology analyzer XE-2100 (Sysmex, Kobe, Japan). The PT-INR was measured using a Thromborel S (Sysmex) by the fully automated hematology analyzer CA-7000 (Sysmex). The ADAMTS13 was measured using FRETS-VWF73, which was chemically synthesized by the Peptide Institute, Inc (Osaka, Japan) according to the method of Kokame et al.[31] The VWF and VWFpp levels were measured with a VWF & Propeptide Assay kit (GTI Diagnostics, Waukesha, Wisconsin).[27] The AT activity was measured by a chromogenic assay, and D-dimer was measured according to the latex agglutination method using Nanopia D-dimer (Sekisui Medical).[32]

The behaviors of biomarkers such as sGPVI and platelet counts in the recipients were first compared with those in the donors to examine the relationship between the change in the biomarkers and the stress of the operation. Markers including VWFpp and ADAMTS13 in the nonsurvivor were subsequently compared with those in the survivors.

Statistical Analysis

The data are expressed as the median and 25th to 75th percentiles. Differences between groups were examined for significance using the Wilcoxon test, and analysis of variance (ANOVA) was also performed. A P value of less than .05 was considered to indicate a significant difference. All statistical analyses were performed using the Stat flex, version 6, software package (Artec Co Ltd, Osaka, Japan).

Results

Twelve patients died within 90 days of transplantation (nonsurvivors) and 67 patients survived over 90 days (survivors; Table 1).

Table 1. Survivors and Nonsurvivors With LDLT.

	Survivors (n = 67)	Nonsurvivors (n = 12)	P
Age	52 (44-60)	54 (48-60)	.424
Sex (male, female)	(39, 28)	(6, 6)	.832
Primary disease			
LC	52	11	.468
HCC	21	5	.616
Preoperative liver function			
CP score	10 (8-11)	12 (10.5-14)	**.006**
PT-INR	1.36 (1.18-1.77)	1.90 (1.29-3.03)	.086
Total bilirubin	3.6 (2.5-3.1)	3.0 (2.4-3.6)	.107
Albumin	2.8 (2.5-3.1)	3.0 (2.4-3.6)	.366
Graft			
Right lobe	38	8	.745
Left lobe	24	3	.691
Others	5	1	1.00
GRWR	0.967 (0.840-1.16)	0.957 (0.837-1.04)	.546
Donor age	37 (28-47)	35 (21.8-56.3)	.994

Abbreviations: CP, Child-Pugh; GRWR, graft-to-recipient weight ratio; HCC, hepatic cell carcinoma; LC, liver cirrhosis; LDLT, living donor liver transplantation; PT-INR, prothrombin time–international normalized ratio.

Table 2. Postoperative Complications.

	Survivors (n = 67)	Nonsurvivors (n = 12)	P
No complication	30 (44.8%)	0 (0%)	.009
Thrombosis	6 (9.0%)	3 (25%)	.264
Bleeding	10 (14.9%)	7 (58.3%)	.003
Acute rejection	13 (19.4%)	1 (8.3%)	.607
Infection	17 (25.4%)	5 (41.7%)	.418
Others	15 (22.4%)	7 (58.3%)	.027

The causes of death were liver failure in 6 cases, sepsis in 2 cases, gastrointestinal bleeding in 1 case, cardiac failure in 2 cases, and cerebral hemorrhage in 1 case. There were no significant differences in age, sex, LC, HCC, PT-INR, total bilirubin, albumin, and GRWR between survivors and nonsurvivors. The CP score was higher in the nonsurvivors than in the survivors ($P < .01$). The incidence of complications, especially bleeding, was significantly higher in the nonsurvivors than in the survivors ($P < .01$; Table 2).

In comparison between donor with mild stress by LDLT and recipient who had marked stress by LDLT, the platelet counts in the donors were significantly decreased on day 3 ($P < .05$), but they were increased on day 14 ($P < .001$). They were significantly lower in the recipients than in the donors on days 1 ($P < .001$), 3 ($P < .001$), 7 ($P < .001$), and 14 ($P < .05$; Figure 1A). The platelet counts in the recipients were significantly decreased on day 3 ($P < .05$) and increased on day 14 ($P < .05$). The plasma levels of sGPVI in the donors were significantly higher on days 1 ($P < .01$), 3 ($P < .001$), 7 ($P < .01$), and 14 ($P < .001$) compared to the levels before the operation, but those in the recipient were significantly higher on days 1, 3, and 14 ($P < .05$, respectively) compared to the values before the operation (Figure 1B). The plasma levels of GPVI were significantly higher in the recipients than in the donors on day 14 ($P < .05$). According to the ANOVA, the F value was 8.83 ($P < .001$) in platelet counts and 4.08 ($P < .01$) in sGPVI. In terms of the relationship between the platelet counts and the sGPVI levels, the slope varied on different days and it became negative on day 3 (Figure 2 and Table 3).

The platelet counts significantly increased in survivors on days 14 and 28 compared to values before the operation ($P < .001$). They were significantly higher in the survivors than in the nonsurvivors on days 14 ($123 \times 10^3/\mu L$: $72.0\text{-}223.5 \times 10^3/\mu L$ vs $56.0 \times 10^3/\mu L$: $43.5\text{-}73.8 \times 10^3/\mu L$; $P < .001$) and 28 ($131.0 \times 10^3/\mu L$: $68.0\text{-}194.0 \times 10^3/\mu L$ vs $60.5 \times 10^3/\mu L$: $41.5\text{-}118.5 \times 10^3/\mu L$; $P < .05$), although there were no differences in the platelet counts between the groups before the operation and on days 1, 3, and 7 (Figure 3A). The plasma levels of sGPVI in the survivors were significantly higher on days 1, 3, 7, 14, and 28 ($P < .001$) compared to values before the operation (14.7 ng/mL: 9.7-24.7 ng/mL), and those in the nonsurvivors were significantly higher ($P < .05$) on day 3 compared to values before the operation. The plasma levels of sGPVI on day 3 were significantly higher ($P < .01$) in the nonsurvivors (38.1 ng/mL: 32.3-44.1 ng/mL) than in the survivors (22.5 ng/mL: 17.3-35.8 ng/mL; Figure 3B). The ratio of sGPVI to platelets of the survivors was significantly higher on days 1 ($P < .01$), 3 ($P < .001$), and 7 ($P < .01$) and significantly lower on day 28 ($P < .05$) compared to values before the operation. The ratio for nonsurvivors was significantly higher ($P < .01$) on day 3 (Figure 3C), and it was significantly higher in the nonsurvivors than in the survivors before the operation ($P < .05$) and on days 3 ($P < .01$) and 28 ($P < .05$). In the ANOVA between the survivor and the nonsurvivors, the F values for the platelet counts, plasma levels of sGPVI, and the ratio of sGPVI to platelets were not significant.

The plasma levels of ADAMTS13 in patients with LDLT were significantly decreased on days 1, 7, 14, and 28 and were significantly lower in the nonsurvivors than in the survivors on days 7 and 28 ($P < .05$; Figure 4A). Although the plasma levels of VWF were high in the patients during LDLT, they were significantly decreased in the survivors on day 1 ($P < .001$; Figure 4B). The plasma levels of VWFpp were markedly higher on day 14 and decreased on day 28 in the survivors ($P < .05$; Figure 4C), and these levels were significantly higher in the nonsurvivors than in the survivors ($P < .01$). There were no significant differences in the PT-INR and D-dimer levels between the survivors and the nonsurvivors on days 7 and 14 (Table 4). Although there was no significant difference in the AT levels between the survivors and the nonsurvivors on day 7, the AT levels were significantly lower in the nonsurvivors than in the survivors on day 14 ($P < .01$).

Discussion

The causes of a poor outcome for LDLT are considered to be graft dysfunction and complications such as infection, thrombosis, bleeding, graft-versus-host disease,[33] TMA,[27,34] disseminated

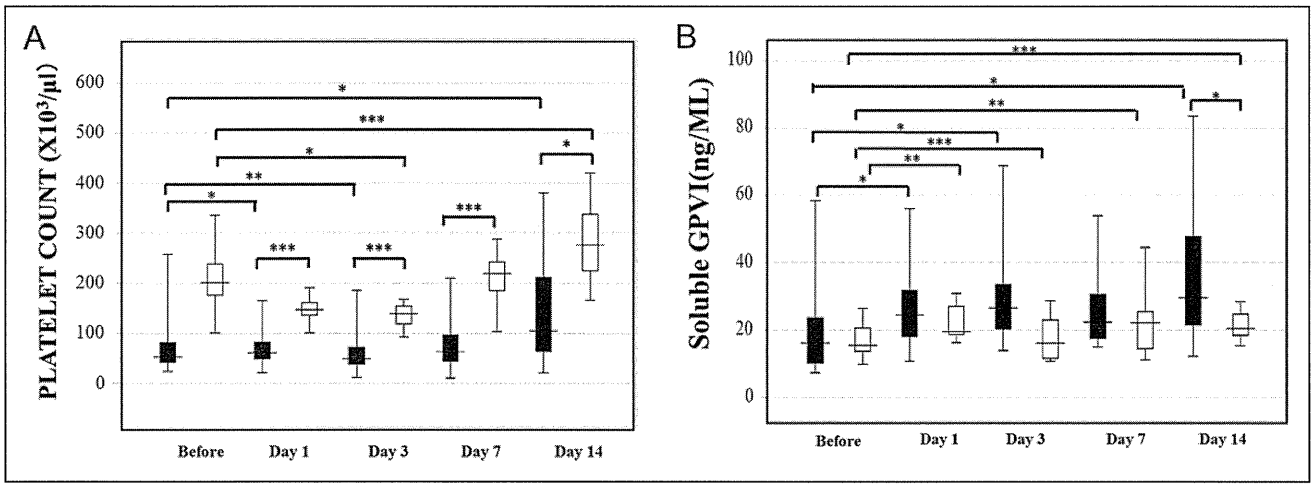

Figure 1. Platelet counts (A) and soluble platelet glycoprotein VI (sGPVI) levels (B) in the donors and recipients for living donor liver transplantation (LDLT). Closed bar: recipient, open bar: donor. *$P < .05$, **$P < .01$, ***$P < .001$.

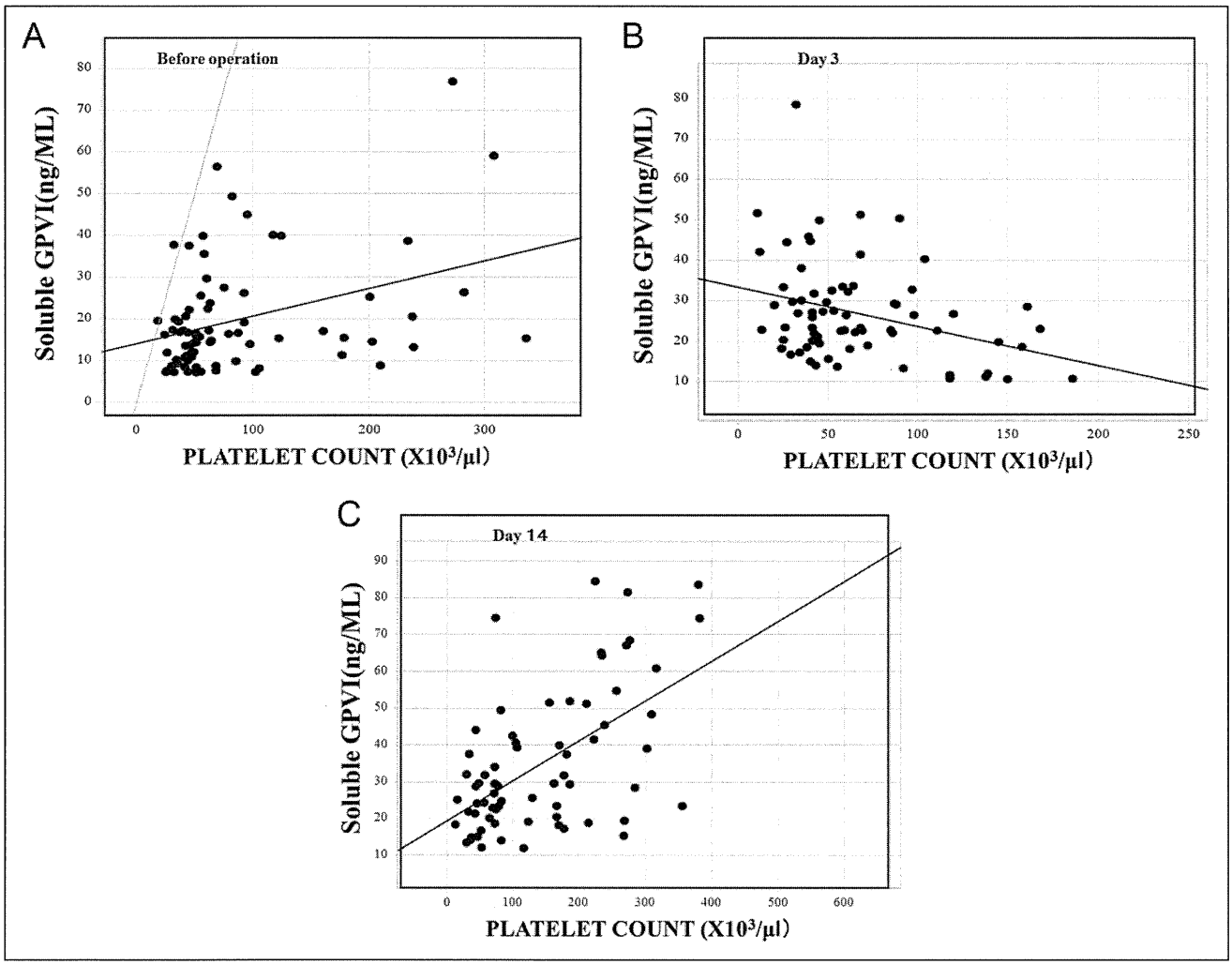

Figure 2. Relationship between the soluble platelet glycoprotein VI (sGPVI) levels and platelet counts in the recipients for living donor liver transplantation (LDLT). Before the operation (A), day 3 (B), and day 14 (C).

Table 3. Relationship Between the Platelet Count and sGPVI Levels.

	All	LDLT (Survivors)	LDLT (Nonsurvivors)
Before operation	$Y = 14.14 + 0.07X$, $R = 0.359$ ($P < .01$)	$Y = 8.73 + 0.15X$, $R = 0.598$ ($P < .001$)	$Y = 17.90 + 0.13X$, $R = 0.268$ ($P < .05$)
Day 1	$Y = 21.24 + 0.05X$, $R = 0.201$ ($P < .05$)	$Y = 16.63 + 0.14X$, $R = 0.461$ ($P < .01$)	$Y = 25.12 - 0.07X$, $R = -0.467$ ($P < .05$)
Day 3	$Y = 33.38 - 0.10X$, $R = -0.331$ ($P < .01$)	$Y = 31.28 - 0.06X$, $R = -0.158$ ($P < .05$)	$Y = 34.29 + 0.08X$, $R = 0.172$ ($P < .05$)
Day 7	$Y = 18.68 + 0.08X$, $R = 0.253$ ($P < .029$)	$Y = 7.19 + 0.27$, $R = 0.492$ ($P < .001$)	$Y = 10.74 + 0.23X$, $R = 0.843$ ($P < .01$)
Day 14	$Y = 19.42 + 0.11X$, $R = 0.566$ ($P < .001$)	$Y = 14.88 + 0.15X$, $R = 0.764$ ($P < .001$)	$Y = 30.93 + 0.001X$, $R = 0.001$ ($P < .05$)
Day 28		$Y = 12.25 + 0.12X$, $R = 0.755$ ($P < .001$)	$Y = 13.52 + 0.15X$, $R = 0.850$ ($P < .05$)

Abbreviations: LDLT, living donor liver transplantation; sGPVI, soluble platelet glycoprotein VI.

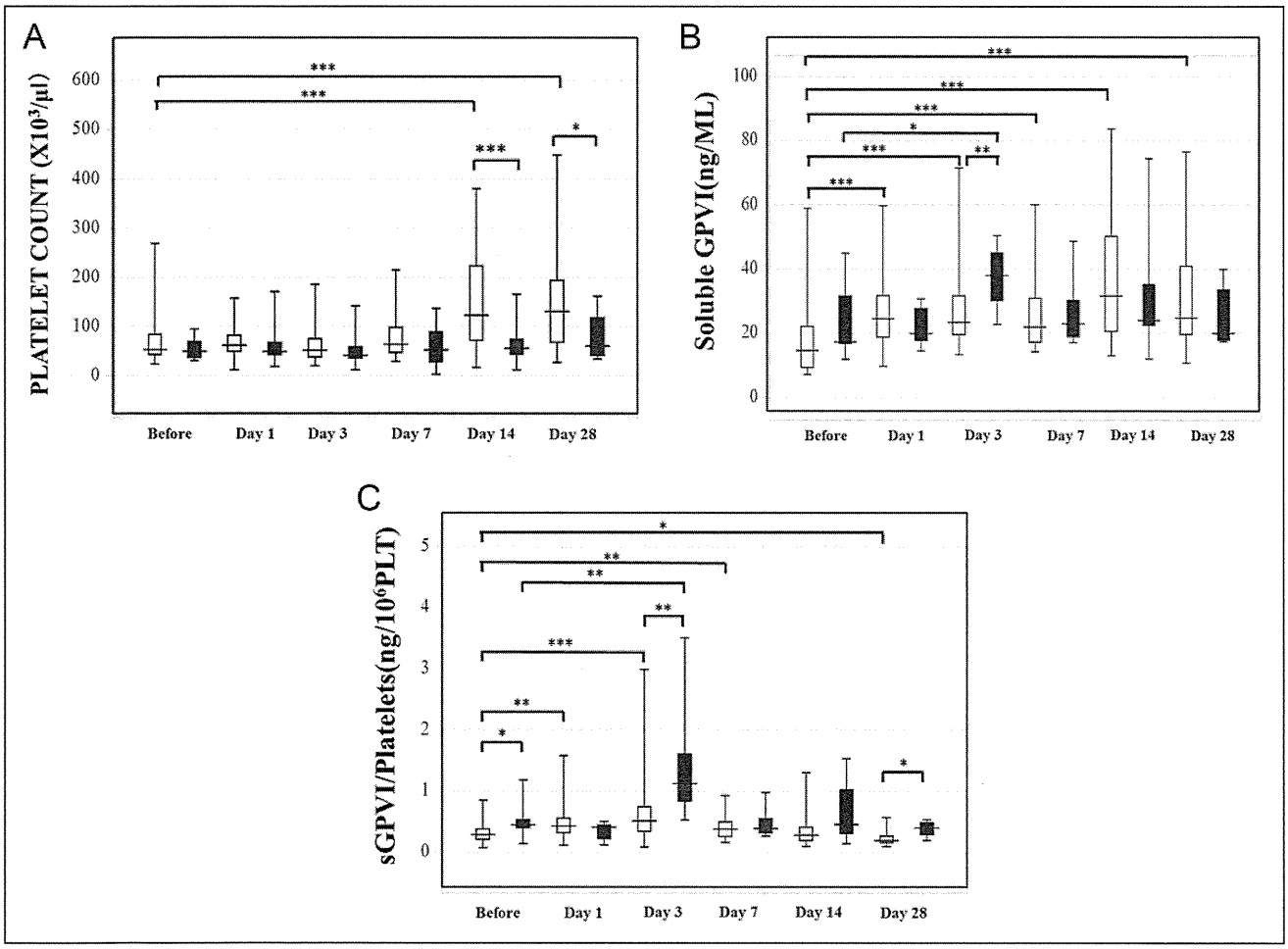

Figure 3. Platelet counts (A), soluble platelet glycoprotein VI (sGPVI) levels (B), and the sGPVI-platelets ratio (C) in the recipients for living donor liver transplantation (LDLT). Open bar: survivors, closed bar: nonsurvivors. *$P < .05$, **$P < .01$, ***$P < .001$.

intravascular coagulation (DIC),[35] and others. Thrombocytopenia is commonly associated with LDLT and many complications of LDLT, suggesting that it is important to know the degree of platelet activation in LDLT patients with thrombocytopenia. The plasma sGPVI levels have been reported to significantly increase in patients with thrombosis during the postoperative period[36] and those with DIC[35] or TMA, thus suggesting that the plasma sGPVI levels increase in a thrombotic state, which thus activates platelets.[30] In a cynomolgus monkey model of lipopolysaccharide-induced thrombocytopenia, the change in sGPVI was more pronounced than the existing platelet activation biomarker, soluble P-selectin.[37]

An ANOVA showed that LDLT had an important effect on the platelet count and the plasma GPVI level in the present

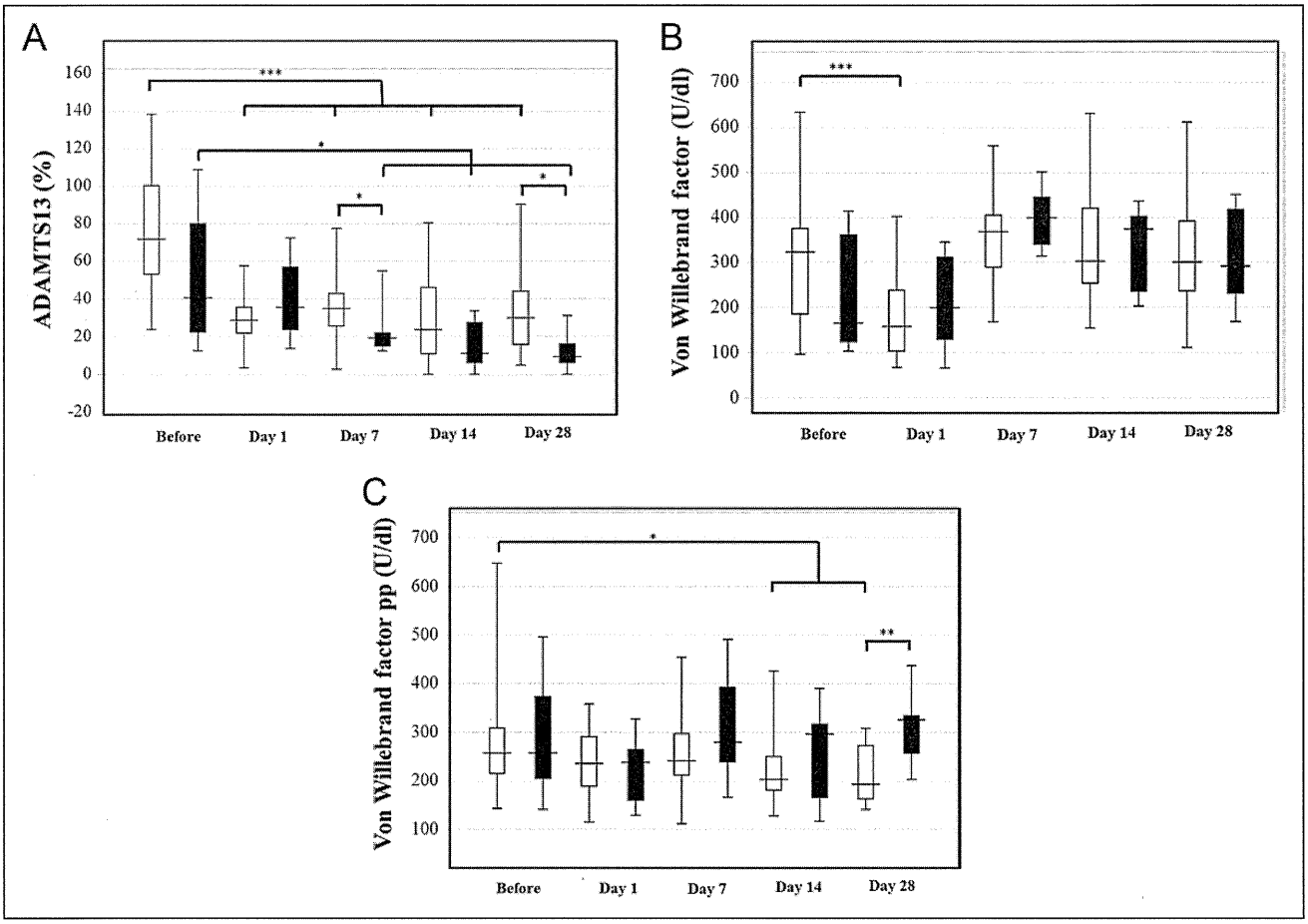

Figure 4. ADAMTS13 (A), von Willebrand factor (VWF) (B), and VWF propeptide (VWFpp) (C) in the recipients for living donor liver transplantation (LDLT). Open bar: survivors, closed bar: nonsurvivors. *$P < .05$, **$P < .01$, ***$P < .001$.

Table 4. Hemostatic Data in the Survivors and Nonsurvivors With LDLT.

	Survivors	Nonsurvivors	Significance
PT-INR			
7 days	1.13 (1.04-1.31)	1.20 (1.06-1.33)	NS
14 days	1.12 (1.01-1.28)	1.25 (1.18-1.46)	NS
D-dimer, μg/mL			
7 days	29.6 (24.7-48.7)	20.0 (8.2-25.2)	NS
14 days	16.8 (10.6-27.1)	18.1 (11.7-29.2)	NS
AT, %			
7 days	86.0 (77.5-101.6)	91.5 (73.6-97.2)	NS
14 days	85.9 (74.0-95.7)	55.7 (34.3-65.0)	$P < .01$

Abbreviations: AT, antithrombin; LDLT, living donor liver transplantation; NS, not significant; PT-INR, prothrombin time–international normalized ratio.

study. These levels were not significantly different among the various underlying diseases. It is therefore typically considered in LDLT that the stress and platelet activation by LDLT are higher in the recipients than in the donors. After LDLT, markedly low platelet counts and relatively high sGPVI levels were observed in the recipients, and slight changes were observed in the donors, suggesting that sGPVI levels and platelet counts will be well affected by LDLT and its complication. The low platelet counts and the high sGPVI levels were observed in the recipients on days 1, 3, and 14. In terms of the relationship between the platelet count and the sGPVI levels, the slope became negative on day 3. These findings indicate that the activation of platelets might occur the highest on day 3. In the nonsurvivors, peak platelet activation might occur earlier. These findings suggest that thrombocytopenia in LDLT may be caused by platelet activation and consumption. The ADAMTS13 levels were markedly low after day 1 and the VWF levels were markedly high during LDLT, which increased the platelet aggregations, thereby leading to thrombosis, organ failure, or TMA. There was no significant difference in the PT-INR between the survivors and the nonsurvivors until day 14, suggesting that the liver function remained stable until day 14. The decreased AT activity in the nonsurvivors on day 14 indicates that hemostatic abnormalities occurred on day 14 following elevated PT-INR on day 28. The elevated VWFpp suggested that vascular endothelial injuries occurred on day 28.

The platelet counts were significantly higher in the survivors than in the nonsurvivors on days 14 and 28, similar to those in previous reports,[12,32] indicating that thrombocytopenia

on day 14 or 28 may be a good marker for a poor outcome in LDLT. ADAMTS13,[38] VWF, and VWFpp[39] were studied previously and reported to be changed on day 14 as biomarkers for complications and poor outcomes for LDLT. Thrombopoietin levels were also reported to be decreased in LDLT patients with poor outcome on day 28.[39] The incidence of complications was high in the nonsurvivors, suggesting that several complications might have contributed to the observed deaths within 90 days of surgery. The plasma VWFpp level was significantly higher in the nonsurvivors than in the survivors. The diagnosis of liver failure on day 14 or 28 may be too late to improve the outcome in the patients with LDLT. The plasma levels of sGPVI in the nonsurvivors were significantly increased on day 3, and those levels were significantly higher in the nonsurvivors than in the survivors on day 3. Although this study is small and the statistical power is low, our findings suggest that elevated plasma sGPVI levels may be an early marker for poor outcome in LDLT. An increased sGPVI to platelets ratio may therefore be a good marker for platelet activation. Decreased ADAMTS13 and elevated VWF, which are related to the platelet aggregations, were observed on day 7, suggesting that these changes may occur after marked platelet activation and aggregation.

We hypothesize that platelet activation occurs after LDLT and it is high in the nonsurvivors on day 3 and thereafter that the hemostatic abnormality and vascular endothelial cell injuries increased on days 14 and 28.

Authors' Note

K.N. contributed to obtaining the measurements of sGPVI but was not involved in interpreting the results.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: K.N. is an employee of Mochida Pharmaceutical Co, Ltd.

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Plasma ADAMTS13, von Willebrand Factor (VWF), and VWF Propeptide Profiles in Patients With Connective Tissue Diseases and Antiphospholipid Syndrome

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Abstract

Thrombotic thrombocytopenic purpura (TTP) frequently develops in patients with connective tissue diseases (CTDs). ADAMTS13 and von Willebrand factor (VWF) are closely related to the onset of TTP. We investigated the roles of ADAMTS13 and VWF in thrombotic events of patients with CTD. ADAMTS13 activity and VWF and VWF propeptide (VWFpp) levels in CTD, primary antiphospholipid antibody syndrome (pAPS), and controls were measured to examine their relationship with thrombosis. ADAMTS13 activity levels were significantly low in the patients with CTD but not in the patients with pAPS. No significant difference in the ADAMTS13 activity levels among the various CTD subgroups was found. The levels of VWF and VWFpp were significantly elevated in the patients with pAPS and CTD compared with that of control groups. Eleven patients with CTD developed TTP, and their ADAMTS13 activity levels were significantly lower than patients having CTD without TTP. However, the ADAMTS13 activity levels showed no difference between the patients having CTD with and without thrombotic events. The VWF antigen levels were significantly high in the patients having CTD with TTP. There were no significant differences in the VWF levels of the patients having CTD with TTP and thrombosis. The VWFpp levels were significantly high in the patients having CTD with TTP and thrombosis. The VWF and VWFpp levels were significantly high in the patients with pAPS. Decreased ADAMTS13 activity and elevated VWF and VWFpp levels were observed in patients with CTD. These abnormalities in patients with CTD may represent the increased risk of thrombosis in CTD.

Keywords

ADAMTS13, von Willebrand factor (VWF), VWF propeptide, connective tissue disease, antiphospholipid syndrome

Introduction

Population-based epidemiological studies have revealed an association between systemic autoimmune diseases and deep venous thrombosis (DVT)/venous thromboembolism (VTE). The etiopathogenesis of the increased risk of VTE in systemic autoimmune diseases is not entirely clear but multiple contributors have been explored.[1] Antiphospholipid antibody syndrome (APS)[2,3] is a well-known systemic thrombotic diathesis that is associated with the presence of antiphospholipid antibodies (aPLs). The mechanisms underlying the development of thrombosis, including cerebral thrombosis[4] and VTE,[5] and obstetric morbidity[6] due to aPLs are poorly understood. Antiphospholipid antibody syndrome consists of the primary APS and secondary APS.[7,8] The underlying diseases of the secondary APS include various connective tissue diseases (CTDs), systemic lupus erythematosus (SLE)[9] and the related

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Table 1. Participants.

		F:M	Age	Total				TTP	THE	APS
CTD	SLE	86:8	57.0 (23.2-82.4)	94	32	SLE	28	4	11	9
						SLE + Sjs	4	0	0	0
	SSc				27	Limited SSc	13	0	3	0
						SSc + Sjs	4	0	1	0
						Diffuse SSc	9	1	0	0
						SSc + Sjs	1	0	1	0
	DM				8			1	0	0
	Primary Sjs				5			0	1	0
	OVS				7	SLE + limited SSc + Sjs	1	0	0	0
						SLE + limited SSc	1	0	0	0
						RA + SLE	1	0	0	0
						Limited SSc + RA	1	0	0	0
						Diffuse SSc + DM	2	1	0	0
						Diffuse SSc + RA	1	0	1	0
	MCTD				7			1	0	0
	RA				8	RA	5	2	1	0
						RA + Sjs	3	1	1	1
Primary APS		8:1	52.0 (43.0-61.0)	9	9			0	9	9
				103				11	29	19

Abbreviations: CTD, connective tissue disease; RA, rheumatoid arthritis; APS, antiphospholipid antibody syndrome; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; DM, dermatomyositis; Sjs, Sjogren syndrome; OVS, overlap syndrome; M, male; F, female; TTP, thrombotic thrombocytopenic purpura; MCTD, mixed CTD.

autoimmune diseases, and idiopathic thrombocytopenic purpura.[10]

Thrombotic thrombocytopenic purpura (TTP)[11] presents specific symptoms, including microangiopathic hemolytic anemia, thrombocytopenia due to platelet consumption and organ dysfunction, and frequently associates with CTD.[12] A disintegrin-like and metalloproteinase with thrombospondin type I motifs 13 (ADAMTS13) is a metalloproteinase that specifically cleaves the multimeric von Willebrand factor (VWF).[13-17] The severe deficiency in ADAMTS13 activity results from either a mutation of the ADAMTS13 gene[14,18] or by the presence of inhibitory antibodies against ADAMTS13.[19] The large VWF multimers (UL-VWFMs) are produced and released by the injured vascular endothelial cells into the plasma of the patients with TTP.[20,21] Pre-pro VWF, synthesized in the endothelial cells and megakaryocytes, requires posttranslational modifications including signal peptide cleavage, C-terminal dimerization, glycosylation, sulfation, and N-terminal multimerization[22] for activation. In the trans-Golgi areas, the VWF propeptides (VWFpp) are processed and stored together with mature VWF in α-granules (megakaryocytes) and Weibel-Palade bodies (endothelial cells). Responding to various physiological and/or pathological stimuli, the endothelial cells discharge VWFpp and VWF into the plasma, and VWFpp dissociates from VWF in plasma.[23]

Elevated plasma levels of VWFpp have been reported in patients with thrombotic microangiopathy (TMA) and disseminated intravascular coagulation (DIC).[24,25]

In the present study, we measured ADAMTS13 activity and VWFpp and VWF antigen levels in plasma samples from 103 patients with CTD or APS and 68 healthy volunteers (HV).

Materials and Methods

Laboratory data were serially investigated in 94 patients with CTD, 9 patients with primary APS, and 68 HV. The patients consulted the Department of Hematology or Department of Dermatology from January 1, 1994, to December 31, 2013. The diseases of the patients with CTD included SLE (n = 32), systemic sclerosis (SSc; n = 27), dermatomyositis (DM; n = 8), primary Sjogren syndrome (n = 5), overlap syndrome (n = 7), rheumatoid arthritis (RA; n = 8), and mixed CTD (MCTD, n = 7; Table 1). Normal plasma specimens were collected from 69 HV from April 1, 2010, to August 31, 2010, and then were stored before performing the assays.

SLE,[26] SSc,[27] and Sjogren's syndrome[28] were diagnosed according to the diagnostic criteria of the American College of Rheumatology. Thrombotic events were diagnosed using echography, venography, computed tomography, magnetic resonance imaging, magnetic resonance venography, or cerebral angiography (CAG). Thrombotic microangiopathy, which results in thrombocytopenia and hemolytic anemia due to the microangiopathy, was identified based on the laboratory data and clinical symptoms such as neurological dysfunction, renal failure, and fever.[13] Thrombotic thrombocytopenic purpura was diagnosed when a patient had TMA and neurological symptom(s) due to TMA.

The human plasma obtained from the whole blood treated with a 1/10 volume of 3.8% sodium citrate by centrifugation at 3000 × *g* at 4°C for 15 minutes. The plasma sample was stored at −80°C until analysis. All assays were performed within 2 years after sampling. Old data were obtained from only patients with TMA, and these assays were performed at the onset of TMA. The ADAMTS13 activity was measured using

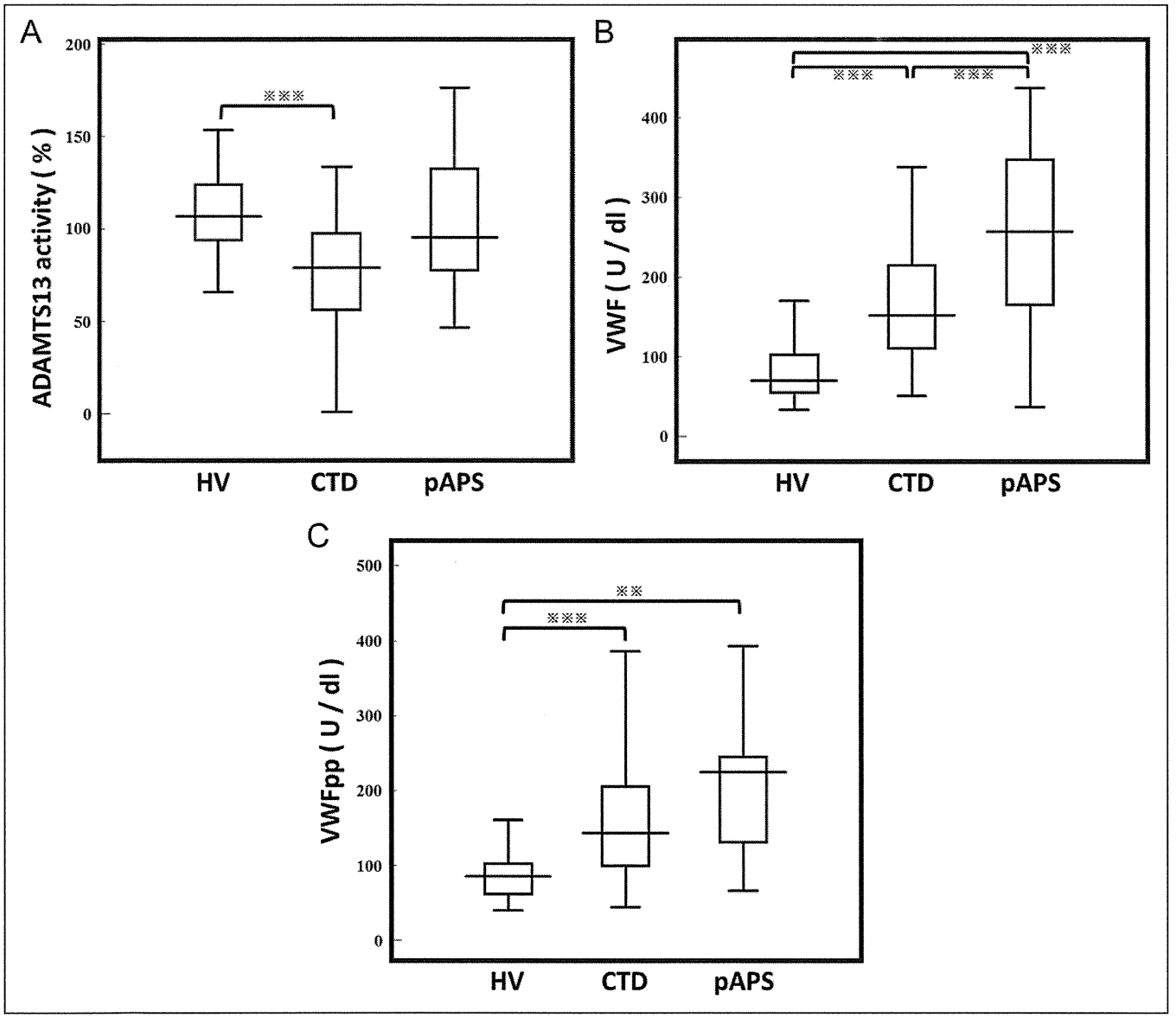

Figure 1. Plasma ADAMTS13 activity (A), VWF (B), and VWF propeptide (C) levels in patients with CTD, pAPS, and HV. CTD indicates connective tissue disease; pAPS, primary antiphospholipid antibody syndrome; HV, healthy volunteers; VWF, von Willebrand factor. ADAMTS13, a disintegrin-like and metalloproteinase with thrombospondin type I motifs 13. ※※※, $P < .001$; ※※, $P < .01$; ※, $P < .05$.

a FRETS-VWF73 peptide, which was chemically synthesized by the Peptide Institute, Inc (Osaka, Japan) according to the methods of Kokame et al.[18] The plasma levels of VWF and VWFpp were measured with a VWF & Propeptide assay kit (GTi DIAGNOSTiCs, Waukesha, Wisconsin).[24] Hemoglobin levels and platelet counts were measured using a fully automated hematology analyzer XE-2100 (Sysmex, Kobe, Japan). The prothrombin time (PT), activated partial prothrombin time (APTT), and lupus anticoagulant (LA) were measured as described previously (25).

The study protocol was approved by the Human Ethics Review Committee of Mie University School of Medicine (approve number 2629), and informed consent was obtained from the patients.

Statistic Analysis

The data are expressed as the medians (25th-75th percentile). Mann-Whitney *U* test was used to examine the statistical significance of difference between the groups. The *P* values of <.05 were considered to indicate a statistically significant difference.

Results

The thrombotic events developed in 11 (34.4%) patients with SLE, 5 (18.5%) patients with SSc, 1 (12.5%) patient with DM, 1 (14.3%) patient with OVS, and 2 (25.0%) patient with RA. The ADAMTS13 activity levels in the patients with CTD

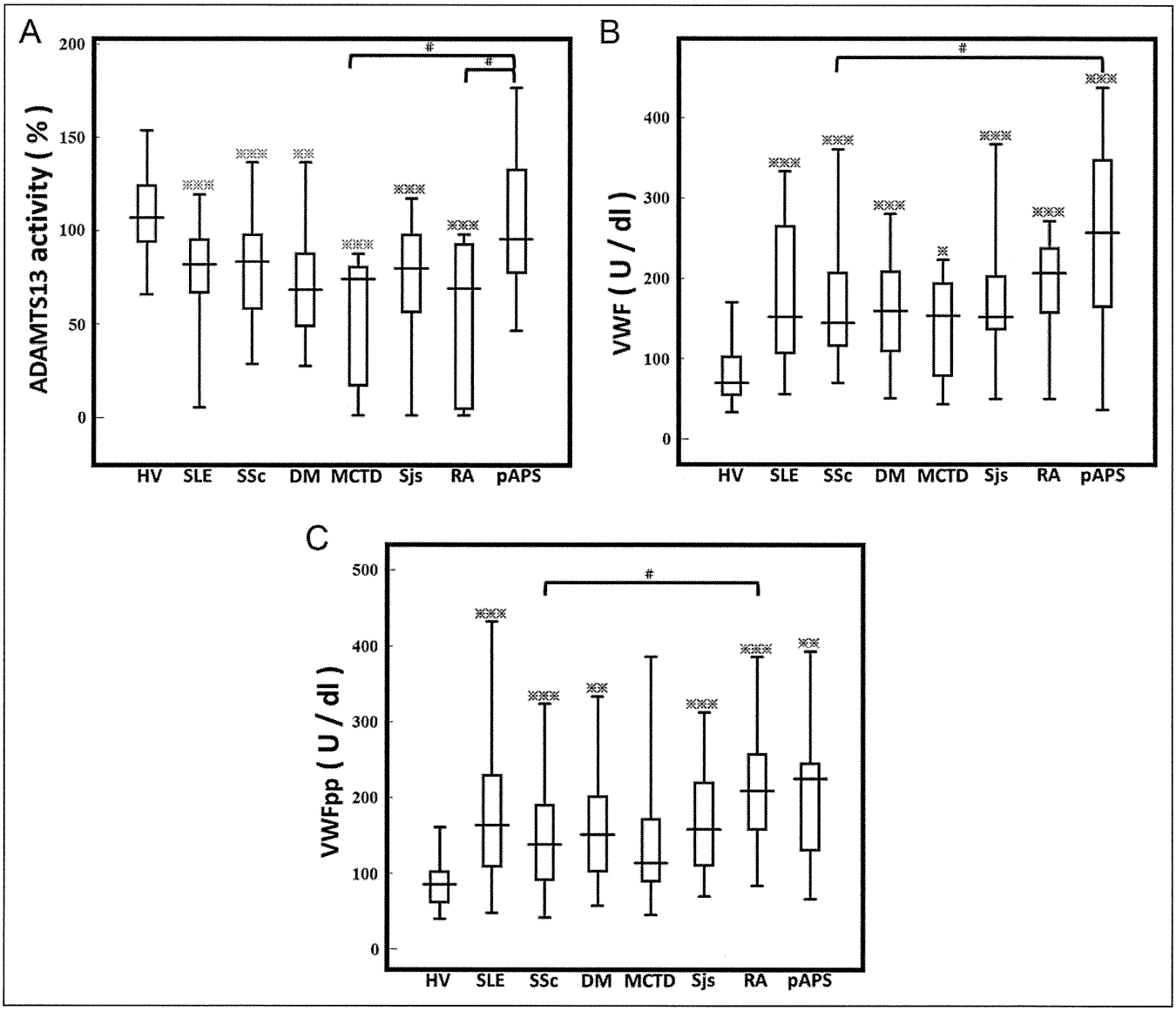

Figure 2. Plasma ADAMTS13 activity (A), VWF (B), and VWF propeptide (C) levels in patients with various CTD and pAPS. CTD, connective tissue disease; pAPS, primary antiphospholipid antibody syndrome; HV, healthy volunteer; VWF, von Willebrand factor; ADAMTS13, a disintegrin-like and metalloproteinase with thrombospondin type I motifs 13. ※※※, $P < .001$; ※※, $P < .01$; ※, $P < .05$ in comparison with HV; #, $P < .05$ between each CTD.

(78.7%: 56.0%-97.2%) were significantly lower than that in HV (107%: 93.7%-124%; $P < .001$). However, the ADAMTS13 activity levels of the primary patients with APS showed no difference to that of the CTD or HV (Figure 1A). The VWF antigen levels in the patients with primary APS (257 U/dL: 165-347 U/dL) were significantly higher than both of the levels in the CTD (152 U/dL: 110-215 U/dL; $P < .001$) and HV (69.5 U/dL: 55.0-102 U/dL, $P < .001$; Figure 1B).

The VWFpp antigen levels in the patients with CTD (143 U/dL: 99.3-205 U/dL) and primary APS (225 U/dL: 131-244 U/dL) were significantly higher than that in HV (85.0 U/dL: 62.0-102 U/dL; $P < .001$ or $P < .01$, respectively; Figure 1C), however, no difference was found between that of the CTD and pAPS.

The ADAMTS13 activity levels in each subgroup of patients with CTD were lower than that of HV, but no significant difference in ADAMTS 13 activity levels was found among the CTD subgroups (Figure 2A). Interestingly, the ADAMTS13 activity levels in MCTD and RA groups showed difference between that of pAPS. The VWF antigen levels in all the CTD subgroups increased compared with HV (Figure 2B). The VWF antigen levels in SSc were lower than that of pAPS. Except MCTD, VWFpp levels were significantly higher in the CTD subgroup than that of HVs (Figure 2C).

Eleven patients with CTD: SLE (n = 4), RA (n = 3), SSc (n = 1), DM (n = 1), OVS (n = 1), and MCTD (n = 1) developed TTP. The ADAMTS13 activity levels in patients having CTD with TTP without thrombotic event (THE)

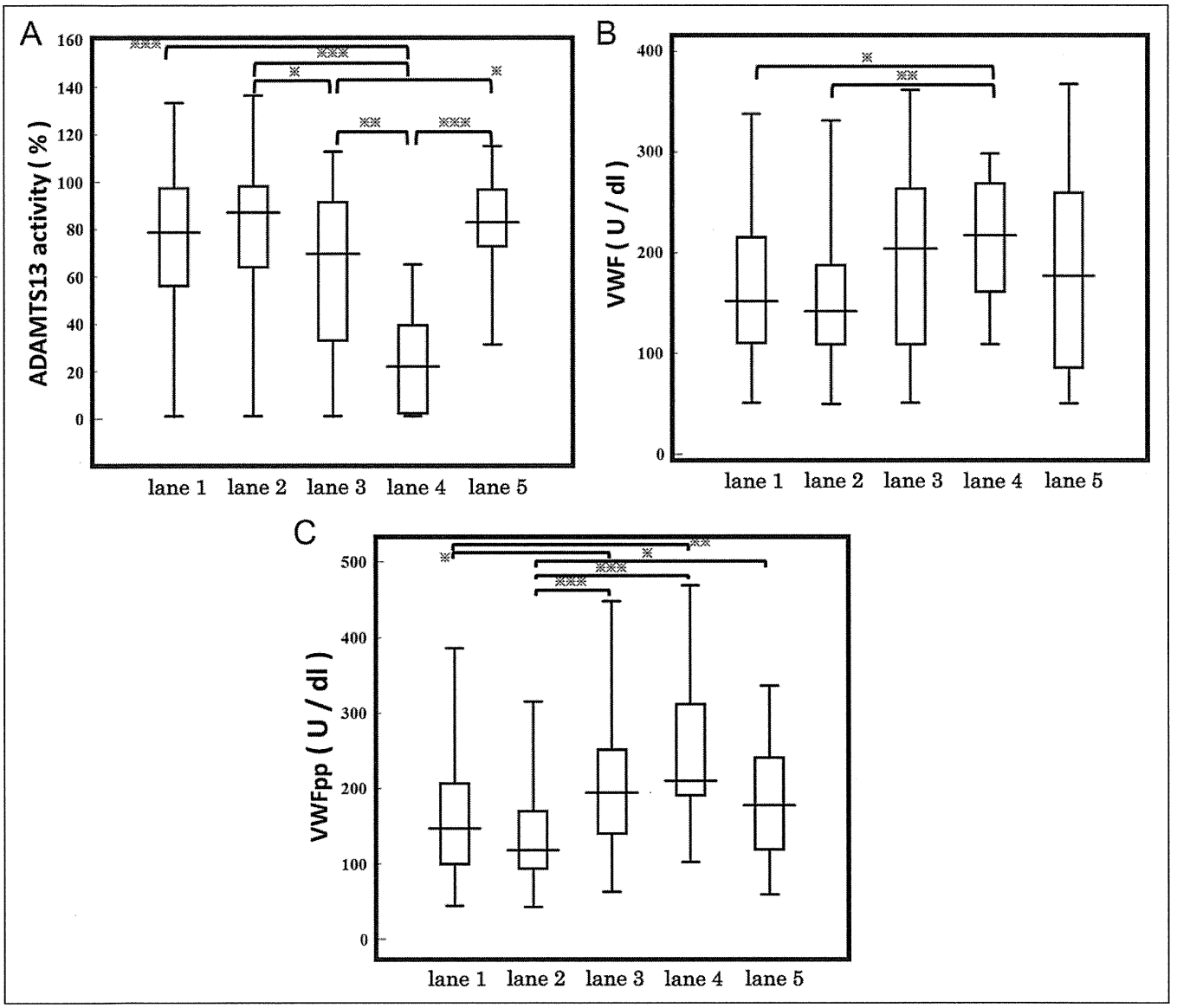

Figure 3. Plasma ADAMTS13 (A), VWF (B), and VWF propeptide (C) levels in patients with CTD and TTP. lane 1, all patients with CTD; lane 2, patients having CTD without THE or TTP; lane 3, patients having CTD with THE or TTP; lane 4, patients having CTD without THE but with TTP; lane 5, patients having CTD without TTP but with THE. ※※※, $P < .001$; ※※, $P < .01$; ※, $P < .05$ in comparison with HV. ADAMTS13 indicates a disintegrin-like and metalloproteinase with thrombospondin type I motifs 13; VWF, von Willebrand factor; CTD, connective tissue disease; TTP, thrombotic thrombocytopenic purpura.

(21.9%: 2.38%-39.4%) were significantly lower than any of whole CTD patient population ($P < .001$), CTD THE−/TTP−, CTD THE+/TTP+, CTD THE−/TTP+, and CDT THE+/TTP−. An ADAMTS13 activity level of less than 10% was observed in 4 patients with TTP. There was no significant difference in the ADAMTS13 activity levels of patients having CTD with and without thrombosis (Figure 3A).

The VWF levels were significantly higher in patients having CTD with TTP (217 U/dL: 161-268 U/dL) than in the whole CTD patient population (152 U/dL: 110-215 U/dL) and in patients having CTD without thrombosis (142 U/dL: 109-187 U/dL). There was no significant difference in the VWF levels of the patients having CTD with TTP and the patients having CTD with thrombosis (Figure 3B). The VWFpp levels were significantly higher in patients having CTD with TTP (210 U/dL: 190-311 U/dL) than in the whole CTD patient population (147 U/dL: 99.0-206 U/dL; $P < .01$) and patients having CTD without thrombosis (118 U/dL: 93.0-169 U/dL; $P < .001$). This level was significantly higher in patients having CTD with thrombosis (177 U/dL: 118-241 U/dL) than in patients having CTD without thrombosis (118 U/dL: 93.0-169 U/dL, $P < .05$; Figure 3C).

There were no significant differences in the ADAMTS13 activity levels among patients having CTD with and without thrombosis, primary APS, and secondary APS (Figure 4A). The VWF levels were significantly higher in the patients with

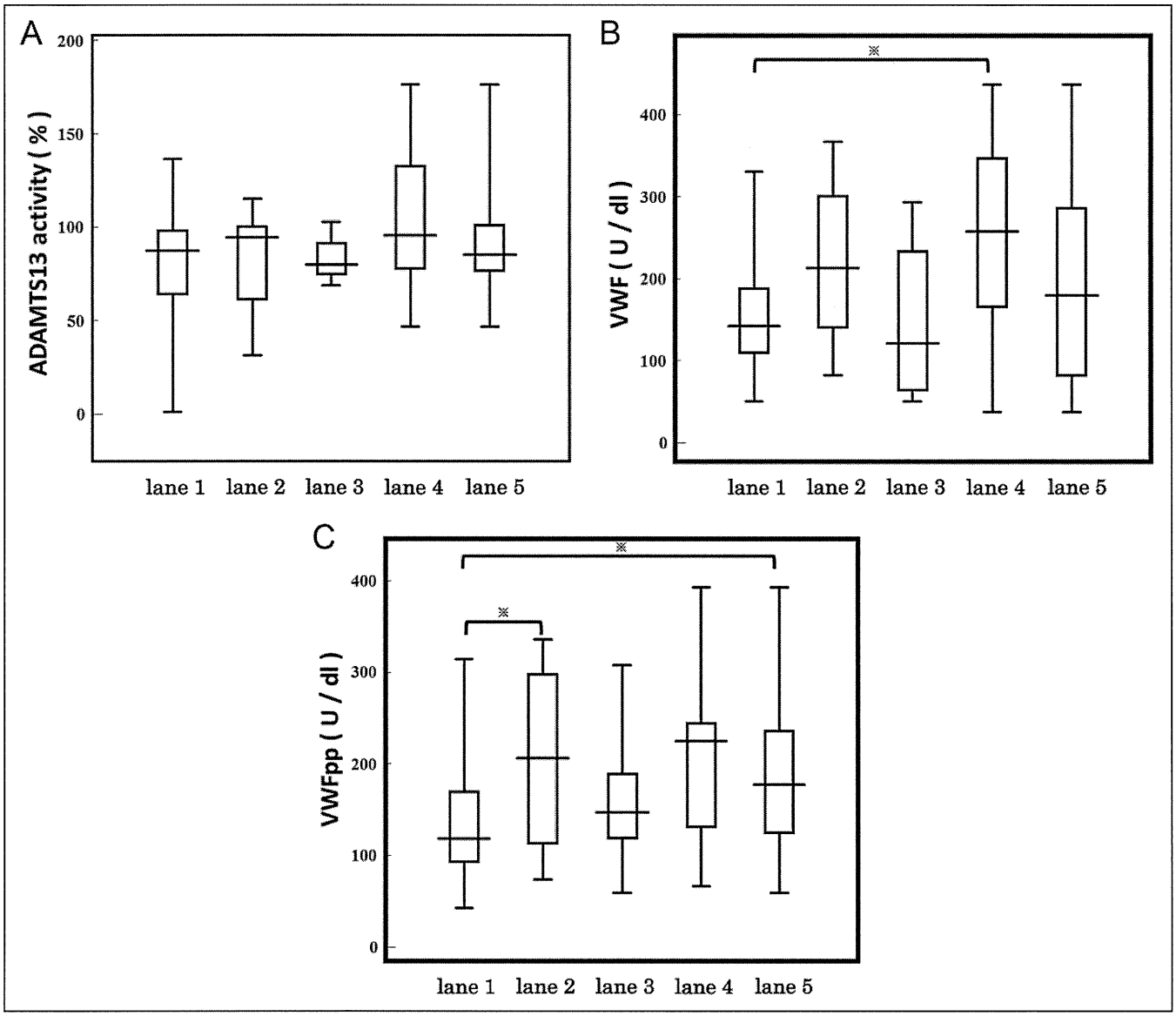

Figure 4. Plasma ADAMTS13 activity (A), VWF (B), and VWF propeptide levels (C) in patients with CTD and APS. lane 1, patients having CTD without THE; lane 2, patients having CTD without APS but with THE; lane 3, patients with secondary APS; lane 4, patients with primary APS; lane 5, patients with secondary APS and patients with primary APS. ADAMTS13 indicates a disintegrin-like and metalloproteinase with thrombospondin type I motifs 13; VWF, von Willebrand factor; CTD, connective tissue disease; APS, antiphospholipid antibody syndrome. ※※※, $P < .001$; ※※, $P < .01$; ※, $P < .05$ ※※※, $P < .001$; ※※, $P < .01$; ※, $P < .05$.

primary APS (257 U/dL: 165-347 U/dL) than in patients having CTD without thrombosis (142 U/dL: 109-187 U/dL; $P < .05$; Figure 4B). The VWFpp levels were significantly higher in patients having CTD with thrombosis (206 U/dL:113-298 U/dL) and patients with APS (177 U/dL: 124-236 U/dL; $P < .05$) than in patients having CTD without thrombosis (118 U/dL:93.0-169 U/dL; Figure 4C). Systemic sclerosis developed several THE cases. Then we divided SSc into 2 groups: the diffuse type SSc and the limited type SSc. However, no significant differences in the ADAMTS13 activity, VWF, and VWFpp levels were found between the limited- and diffuse-type SSc (Table 2).

The frequency of elevated VWF or VWFpp (more than 200 U/dL) and reduced ADAMTS13 activity (less than 50%) was high in patients with RA or those with MCTD and low in those with primary Sjogren syndrome (Figure 5). The levels of lactate dehydrogenase ($P < .001$) and LA ($P < .05$) were significantly higher, and PT ($P < .05$) and APTT were significant longer in patients with reduced ADAMTS13 activity and elevated VWF or VWFpp than in those without (Table 3).

Discussion

ADAMTS13 is an enzyme that cleaves UL-VWFMs to control activation or aggregation of the platelets. Decrease of ADAMTS13 levels in patients with CTD implicates increase in the risk of the hypercoagulable conditions in patients with

Table 2. ADAMTS13, VWF, and VWFpp Levels in Patients With Limited-Type and Diffuse-Type SSc.

Median (2.5%-97.5%)			*P* Value
ADAMTS13, %	Limited	87.3 (31.2-140)	.45
	Diffuse	73.5 (27.5-127)	
VWF, U/dL	Limited	145 (64.0-367)	.84
	Diffuse	145 (98.0-344)	
VWFpp, U/dL	Limited	136 (38.0-301)	.21
	Diffuse	145 (89.0-333)	

Abbreviations: ADAMTS13 indicates a disintegrin-like and metalloproteinase with thrombospondin type I motifs 13; VWF, von Willebrand factor; VWFpp, VWF propeptide; SSc, systemic sclerosis.

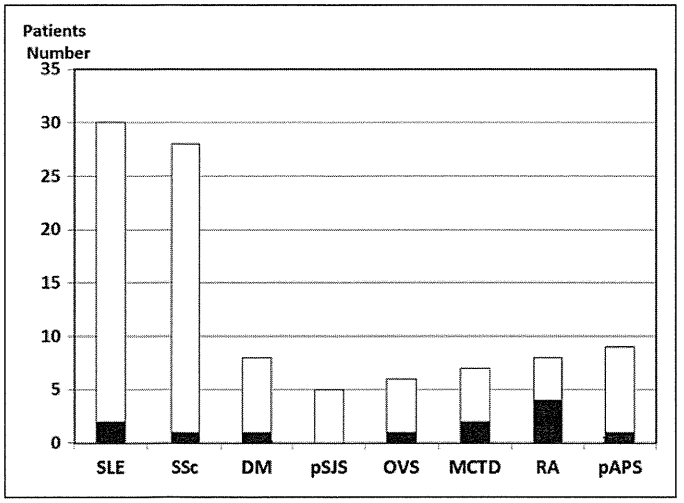

Figure 5. Clinical profiles of patients having CTD with and without increased VWF/VWFpp or decreased ADAMTS13 activity. Closed bars, with reduced ADAMTS13 activity (less than 50%) and elevated VWF or VWFpp (more than 200 U/dL); open bars, without reduced ADAMTS13 activity (less than 50%) and elevated VWF or VWFpp (more than 200 U/dL). ADAMTS13 indicates a disintegrin-like and metalloproteinase with thrombospondin type I motifs 13; VWF, von Willebrand factor; CTD, connective tissue disease; VWFpp, VWF propeptide.

CTD by activation of the platelets. The antiplatelet agents have been indicated for the threat condition for the ischemic disorders in patients with CTD.[29] Decreased ADAMTS13 levels have been reported in the patients with TMA,[11] DIC,[26] and liver disease.[30] Interestingly, the ADAMTS13 activity levels of the patients with CTD in the present study were significantly decreased. The main causes of the reduction of the ADAMTS13 activity are consumption or presence of the inhibitors of ADAMTS13 including TTP and liver injuries. The patients with CTD frequently develop TTP, especially patients with SLE.[31] Therefore, various conditions that inhibit ADAMTS13 activity may frequently develop in patients with CTD. In contrast, the ADAMTS13 activity was not decreased in the patients with primary APS, indicating no significant association with TTP. The patients having APS with reduced ADAMTS13 levels are rarely reported except the SLE cases with APS.[32] In the present study, no significant differences were detected in the ADAMTS13 activity levels among the CTD subgroups; however, the study population was too small to prove this result. Significant low level, less than 10%, of ADAMTS13 activity commonly present in patients with TTP, however, this was only observed in 4 of the patients having CTD with TTP in the present study. Reduced ADAMTS13 activity has been reported in patients with SLE.[32] This might be resulted from lower incidence of typical TTP and severe SLE in the present study compared with previous reports.[12,31]

The serum VWF and VWFpp antigen levels, which are the markers of the vascular endothelial cell injury, were significantly elevated in the patients with CTD as well as the patients with primary APS. This suggests the presence of a common mechanism in CTD and APS: the vascular endothelial cell injury.

Because of the size of the population and the severity of CTD, the differences in the VWF and VWFpp levels in CTDs might be influenced. The VWFpp levels were significantly high in patients having CTD with TTP or thrombosis, which suggests that TTP and thrombosis induce vascular endothelial cell injuries in patients with CTD. Consistent with previous reports, the VWFpp level was more sensitive to thrombosis than the VWF level.[24,25] In addition, there was no significant

Table 3. LDH, PT, APTT, and LA Levels in Patients With and Without Elevated VWF/VWFpp and Reduced ADAMTS13 Activity.

		Median (2.5%-97.5%)	*P* Value
LDH, IU/L	With elevated VWF/VWFpp and reduced ADAMTS13	1338 (207.0-2016)	<.001
	Without elevated VWF/VWFpp and reduced ADAMTS13	206.0 (127.7-1833)	
PT, second	With elevated VWF/VWFpp and reduced ADAMTS13	11.0 (10.6-13.9)	<.05
	Without elevated VWF/VWFpp and reduced ADAMTS13	10.7 (9.69-13.0)	
APTT, second	With elevated VWF/VWFpp and reduced ADAMTS13	32.2 (28.6-43.6)	<.001
	Without elevated VWF/VWFpp and reduced ADAMTS13	27.90 (23.35-40.83)	
LA	With elevated VWF/VWFpp and reduced ADAMTS13	0.98 (0.80-1.1)	<.05
	Without elevated VWF/VWFpp and reduced ADAMTS13	0.80 (0.70-0.96)	

Abbreviations: LDH, lactate dehydrogenase; PT, prothrombin time; APTT, activated partial thromboplastin time; LA, lupus anticoagulant (Russell viper venom time: dRVVT); ADAMTS13 indicates a disintegrin-like and metalloproteinase with thrombospondin type I motifs 13; VWF, von Willebrand factor; VWFpp, VWF propeptide.