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LETTER TO THE EDITOR
Limited Renal Prophylaxis in Regular Plasmatherapy for Heritable ADAMTS13 Deficiency

To the Editor: Germline mutations of *ADAMTS13* cause chronic relapsing-thrombotic thrombocytopenic purpura (TTP), called Upshaw–Schulman syndrome. Affected patients with the rare disease may develop renal impairment with a need for dialysis unless thrombotic microangiopathy is controlled [1]. Plasma exchange is the standard therapy for disease control. There is little information about the long-term effects of plasmatherapy on renal disease. We report the outcomes of patients who underwent >15-years' regular plasma infusion.

Three unrelated patients were treated in our hospitals (Table I) [2]. All presented the classical triad of neonatal jaundice, hemolytic anemia, and thrombocytopenia requiring whole-blood exchange transfusion. Chronic relapsing-TTP was diagnosed in Pt-1, Pt-2, and Pt-3 at age 16 months, 7 years, and 1 month, respectively. Pt-1 suffered a varicella-induced TTP at age 7 months. On-demand infusion of plasma-derived intermediate purity factor VIII concentrates, Confact F (Kaketsuken, Kumamoto, Japan) or Conco-eight (Green Cross Corp., Osaka, Japan) was the first line treatment [3,4]. In Japan, they were replaced by monoclonal antibody-purified/recombinant FVIII products, therefore, not appropriate replacement. Pathogen-inactivated plasma was unavailable. Regular infusion of fresh-frozen plasma (FFP) was then started to control repetitive bouts. No IgG-antibody against ADAMTS13 was detected in any patient.

The FFP prophylaxis and current renal functions are shown in Table I. At the time of infusion, all patients showed petechiae, thrombocytopenia at around 10×10^9/L, and non-severe hemolysis represented by changing hemoglobin, total bilirubin, and lactate dehydrogenase levels, along with proteinuria and hematuria/hemoglobinuria. However, stable hematological responses led to no hospitalization in three patients. Two patients had anti-hepatitis C virus (HCV) antibody, but not HCV RNA or abnormal transaminase levels. Pt-1 had occasional infusion reactions including anaphylaxis. No immunologic disorders developed. Serum concentrations of creatinine ranged normally in Pt-1 and Pt-2, but increased in Pt-3 beyond 20 years of prophylaxis. All had <90 ml/min/1.73 m^2 of the estimated glomerular filtration rate (eGFR), that was calculated by the formula [5]. Pt-3 had high urine protein to creatinine ratio. At the time of FFP infusion, Pt-3 always showed hemoglobinuria, hematuria, proteinuria, and granular casts, Pt-1 had proteinuria only, and Pt-2 showed neither. Pt-1 accepted to undergo the renal biopsy at age 9 years, histopathology of which demonstrated a few microthrombotic glomeruli but no significant inflammation (Supplemental Fig. 1).

One third of patients with heritable ADAMTS13 deficiency have stroke and mostly relapse if untreated, and half of them suffer from neurologic sequelae [6]. Plasma prophylaxis has not been demonstrated to prevent the end-stage renal failure in childhood [7]. The 20 years' prophylaxis controlled TTP bouts without apparent inhibitors, but did not prevent the development of some renal impairment. Our patients shared the genotype, severity, and treatment response [2,8]. Pt-3 had symptomatic urine at each infusion compared with two others. Anti-thrombotic effects require at least 5% of plasma ADAMTS13 activity [9]. Our protocol might insufficiently replace the activity to protect from chronic kidney injury. Nevertheless, the early prophylaxis in Pt-1 might sustain higher GFR. Long-term renal prophylaxis using ADAMTS13 products may overcome the limit of plasmatherapy, as in the joint prophylaxis in hemophiliacs [10].

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Abbreviations: ADAMTS13, a disintegrin-like and metalloproteinase with thrombospondin type 1, motifs 13; eGFR, estimated glomerular filtration rate; HCV, hepatitis C virus; FFP, fresh-frozen plasma; TTP, thrombotic thrombocytopenic purpura

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TABLE I. Long-Term Fresh-Frozen Plasma Prophylaxis and the Renal Outcomes of Patients With Heritable ADAMTS13 Deficiency

		ADAMTS13			Plasma prophylaxis				Renal function at present replacement				Urinalysis		
Pt	Sex	Activity (%)	Mutations derived from father and mother	Age at clinical diagnosis	Age at start (years)	Dose (ml/kg)	Interval (days)	Adverse events	Age at study (years)	Cr (mg/dl)	Cystatin C (mg/L)	eGFR (ml/min/1.73m^2)	Urine P/Cr (g/gCr)	RBC/Hb	Casts
1	f	<0.5	p.C754Afs, p.C754Afs	16 months	2	14.7	14	HCV, anaphylaxis	22	0.72	0.63	85	0.18	No	No
2	f	<0.5	p.W1081X, p.R193W	7 years	12	5.3	21	HCV	29	0.71	0.86	73	0.07	No	No
3	f	<0.5	p.Y1074Afs, p.Y1074Afs	1 month	11	7.1	18	No	32	1.22	1.70	43	0.66	Positive	Granular

Patient (Pt)-1, Pt-2, and Pt-3 correspond to U3, V3, and T4 in the reported Japanese cohort of Ref. [2]. The first diagnosis and treatment of Pt-3 was also reported in Ref. [4]. Pt-1 was born to healthy parents who were first cousins. Pt-2 and Pt-3 had unrelated healthy parents. Cr, creatinine; HCV, hepatitis C virus; eGFR, estimated glomerular filtration ratio; Urine P/Cr, urine protein to creatinine ratio, Normal range; creatinine: 0.40–0.70 mg/dl, cystatin C (a biomarker for GFR): 0.53–0.83 mg/L, eGFR: >90 ml/min/1.73 m^2, Urine P/Cr: <0.15 g/gCr.

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ADAMTS13 activity and genetic mutations in Japan

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Keywords
ADAMTS13, genetic mutation, thrombotic thrombocytopenic purpura, TTP

Summary
Thrombotic thrombocytopenic purpura (TTP), a life threatening disease, can be induced by congenital or acquired deficiency of plasma metalloprotease ADAMTS13. Since the publication of the first genetic analysis in patients with congenital ADAMTS13 deficiency in 2001, more than 100 genetic defects in the ADAMTS13 gene have been reported worldwide. Genetic analysis in patients with ADAMTS13 deficiency has greatly contributed to the understanding of the etiology of TTP. A rapid and quantitative assay method for the plasma ADAMTS13 activity was developed recently in 2005 and opened a new area of TTP research – namely genetic research using a general population to evaluate age and gender differences of ADAMTS13 activity as well as phenotype – genotype correlations of genetic polymorphisms and estimation of a homozygote or a compound heterozygote ADAMTS13 deficiencies. The Japanese general population study included 3616 individuals with an age between 30 – 80 years confirming other studies that while ADAMTS13 activity decreased with age, VWF antigen increased and VWF antigen levels are lowest in blood group O indviduals, whereas ADAMTS13 activity levels were not associated with the ABO blood group. 25 polymorphisms with a minor allele frequency of more than 0.01 were found, among them 6 missense mutations and 19 synonymous mutations, except P475S missense polymorphisms that was only idenitified in an East Asian population, characterized by reduced ADAMTS13 activity. Prevalence of congenital ADAMTS13 deficiency in the Japanese population was estimated about one individual in 1.1 x 10^6 to be homozygote or compound heterozygote for ADAMTS13 deficiency. So far more than 40 mutations in Japanese congenital TTP patients were found, but R193W, Q449*, C754Afs*24 (c.2259delA) and C908Y were identified in more than four patients suggesting the precipitaion of these mutations in the Japanese population.

Schlüsselwörter
ADAMTS13, Mutation, thrombotisch-thrombozytopenische Purpura, TTP

Zusammenfassung
Die thrombotisch-thrombozytopenische Purpura (TTP), eine lebensbedrohliche Erkrankung, kann durch kongenitalen oder erworbenen Mangel an Metalloprotease ADAMTS13 im Plasma ausgelöst werden. Seit 2001 die erste genetische Analyse bei Patienten mit kongenitalem ADAMTS13-Mangel publiziert wurde, sind weltweit mehr als 100 Gendefekte im ADAMTS13-Gen beschrieben worden. Die Genanalyse bei Patienten mit ADAMTS13-Mangel hat viel zum Verständnis der Ätiologie von TTP beigetragen. Ein schnelles und quantitatives Testverfahren wurde 2005 für die Aktivität von ADAMTS13 im Plasma entwickelt und damit ein neues TTP-Forschungsgebiet begründet – und zwar die genetische Erforschung alters- und geschlechtsbedingter Unterschiede bei der ADAMTS13-Aktivität in der Allgemeinbevölkerung, von Zusammenhängen zwischen Phäno- und Genotyp bei genetischen Polymorphismen sowie der Bewertung des homozygoten bzw. kombinierten heterozygoten ADAMTS13-Mangels. In der japanischen Bevölkerungsstudie an 3616 Personen im Alter von 30 bis 80 Jahren wurden die Ergebnisse anderer Studien bestätigt, dass zwar die ADAMTS13-Aktivität mit dem Alter abnimmt, das VWF-Antigen jedoch ansteigt, und dass die Konzentration des VWF-Antigens bei Personen mit Blutgruppe 0 am niedrigsten ist, während das ADAMTS13-Aktivitätsniveau keinen Zusammenhang mit der Blutgruppe AB0 aufwies. Es wurden 25 Polymorphismen mit einer Allelfrequenz über 0,01 gefunden, darunter 6 Missense-Mutationen und 19 synonyme Mutationen, außer dem P475S-Missense-Polymorphismus, der ausschließlich in einer ostasiatischen Population identifiziert wurde und durch reduzierte ADAMTS13-Aktivität gekennzeichnet ist. Bei der Prävalenz des kongenitalen ADAMTS13-Mangels in der japanischen Bevölkerung schätzt man, dass 1 von 1,1 x 10^6 homozygot oder kombiniert heterozygot für einen ADAMTS13-Mangel ist. Bislang wurden mehr als 40 Mutationen bei japanischen Patienten mit kongenitaler TTP entdeckt; jedoch fand man R193W, Q449*, C754Afs*24 (c.2259delA) und C908Y bei mehr als vier Patienten und nimmt daher an, dass diese Mutationen gehäuft in der japanischen Bevölkerung vorkommen.

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VWF cleavage by ADAMTS13

A disintegrin-like and metalloprotease with thrombospondin type 1 motif-13 (ADAMTS13) is a plasma metalloprotease that cleaves a specific Tyr-Met bond in the A2 domain of von Willebrand factor (VWF). The gene, which consists of 29 exons, is located on chromosome 9q34.2, and is only 129 kb distant from the ABO blood group gene.

ADAMTS13 is 1427 amino acid residues in length and consists of a

- signal peptide, a propeptide,
- reprolysin-like metalloprotease domain,
- disintegrin-like domain,
- thrombospondin type 1-like domain,
- cysteine-rich domain,
- spacer domain,
- seven additional thrombospondin type 1-like domains, and
- two CUB domains (1–3).

ADAMTS13 is mainly synthesized in stellate cells in the liver and secreted into plasma (4, 5). In humans, the plasma concentration of ADAMTS13 (6, 7) ranges from

- 0.5 to 1.0 μg/ml in Japanese and
- 0.74 to 1.42 μg/ml in Austrians.

The molecular weight of plasma ADAMTS13 was estimated to be 150 kDa by SDS-polyacrylamide gel electrophoresis (6). Thus, an ADAMTS13 plasma concentration of about 1 μg/ml is equivalent to about 6.7 nmol/l, which is less than the human plasma concentration of factor VII (10 nmol/l). Approximately 3% of ADAMTS13 in human plasma is bound to plasma VWF through its C-terminal domains (8, 9). Some mouse strains have a C-terminally truncated ADAMTS13 mutant that is probably unable to bind to VWF. Mice having a C-terminally truncated ADAMTS13 mutant show less antithrombotic activity under certain pathophysiological condition in vivo (10). Therefore, the ADAMTS13-VWF complex in plasma is functionally important for the antithrombotic activity.

The plasma half-lives of ADAMTS13 were determined as 3.3 and 2.1 days in patients with congenital ADAMTS13 deficiency following the last plasma exchange (11). The half-lives of 3.3 and 2.1 days represent the lowest known clearance rates of proteases in circulating human plasma. Plasma ADAMTS13 can bind to Lys-plasminogen in vitro but the physiological significance of the resulting compound is unknown (12).

> VWF, a plasma glycoprotein of 2050 amino acid residues in length, is a large multimeric plasma glycoprotein that mediates platelet adhesion at sites of vascular injury.

VWF mediates initial platelet adhesion to the injured vessel wall by forming a bridge between subendothelial collagen and platelets in circulation (13). VWF is synthesized primarily in endothelial cells and secreted into plasma as an ultra-large (UL-VWF) multimer (>20000 kDa), some of which remains attached to the endothelial surface.

The UL-VWF multimer possesses a strong platelet aggregation ability and can spontaneously bind and aggregate platelets to generate widespread microthrombi in circulation, leading to a life-threatening disease, thrombotic thrombocytopenic purpura (TTP) (14–16). Under normal conditions, the UL-VWF multimer can be depolymerized by ADAMTS13. Congenital or acquired deficiency of ADAMTS13 leaves the UL-VWF multimer intact, leading to TTP. Platelets and coagulation factor VIII can accelerate the VWF cleavage by ADAMTS13 (17, 18). The plasma concentration of VWF is about 10 μg/ml, which is 10-fold higher than the concentration of ADAMTS13.

VWF has a discrete domain structure that can be clearly observed by an electron microscope (19). In the central region of the polypeptide chain, VWF has three successive A domains, A1-A3, with a high sequence identity. Each A domain is about 90 amino acid residues in length. The A1 domain has a binding region for platelet glycoprotein Ib and the A3 domain has a binding site for subendothelial collagen (13). The A1 and A3 domains have a disulfide bond that forms a link between the N- and C-terminal regions. The A2 domain, however, does not have a corresponding disulfide bond and instead has a vicinal disulfide bond in the C-terminal region. The crystal structure of the A2 domain suggested that the C-terminal half of the domain can be easily unfolded so that the scissile bond, Tyr1605-Met1606, for ADAMTS13 is exposed (20). As to how ADAMTS13 specifically cleaves this unique single peptide bond in the A2 domain of VWF, new information has been accumulating.

> The basic concept for the cleavage is that the scissile bond in the A2 domain is cryptic and sequestered and the shear stress partially unfolds the A2 domain, resulting in exposure of this bond (21, 22).

ADAMTS13 is constitutively active in plasma and the exposed scissile bond in VWF can be easily cleaved by ADAMTS13. Thus, the shear stress-dependent substrate-binding mechanism of ADAMTS13 in vivo is very unique.

VWF73 as a minimal substrate for ADAMTS13

Although identification of the proteolytic cleavage site, the Tyr1605-Met1606 bond, in VWF was reported more than two decades ago (23), a plasma assay for the ADAMTS13 activity was a laborious and time-consuming work (24, 25). In order to develop a fast, quantitative, and synthetic substrate for the VWF-cleaving activity of ADAMTS13, a minimal sequence specifically recognized and cleaved by ADAMTS13 should be determined in VWF.

To identify the region in VWF required for ADAMTS13 cleavage, we expressed a series of deletion mutants of the A2 domain and found that a 73-amino-acid fragment from Asp1596 to Arg1668 was essential for the cleavage of the Tyr1605-Met1606 bond by ADAMTS13 (26). We named this fragment VWF73. A 64-amino-acid fragment from Asp1596 to Arg1659 was not efficiently cleaved by ADAMTS13. We determined the solution structure of the ^{1}H and ^{15}N double-labeled substrates VWF73 and VWF64, each of which included a C-terminal 6xHis tag, by nuclear magnetic resonance. The results

indicated an extended structure for both peptides, suggesting an induced-fit substrate recognition mechanism (27).

VWF73 has all the characteristics of an ADAMTS13 substrate. We developed a chemically modified VWF73 for use in a fluorescence resonance energy transfer (FRET) assay for ADAMTS13 activity. This substrate, FRETS-VWF73, quantitatively and reproducibly yielded the activity of plasma ADAMTS13 within one hour (24), which constitutes a remarkable improvement in rapidity and accuracy over the previous assays (28, 29). Using this assay, we were able to show that patients with congenital TTP exhibited severely decreased (<5% of the reference value) or undetectable ADAMTS13 activities. A slightly modified method using FRETS-VWF73 can quantitatively detect ADAMTS13 activity of less than 5% of the reference value (30).

> An enzyme immunoassay of ADAMTS13 activity using a monoclonal antibody that specifically recognizes Tyr1605, the C-terminal residue of the cleaved A2 domain, has been developed and a chromogenic ADAMTS13-act-ELISA using a glutathione-conjugated VWF73 peptide as the substrate has also been reported (25).

These assays are utilized for the clinical diagnosis of TTP.

ADAMTS13 activity in the Japanese population

The Suita Study is an epidemiological study consisting of Japanese residents between the ages of 30 and 79 years who were randomly selected from the municipality population registry and stratified into groups by sex and age in 10-year increments. We have used FRETS-VWF73 to measure the plasma ADAMTS13 activity in 3616 individuals from this general population with age ranged from 30 to 80 years.

- When the mean of all plasma ADAMTS13 activity values was set at 100%, the mean activity of men (93 ± 24%, n = 1687) was significantly lower than that of women (106 ± 27%, n = 1929) (31).
- The plasma ADAMTS13 activity tended to decrease with age, especially after age 60, in both men and women. The mean ADAMTS13 activity value was
  - 110% for subjects in their 40s,
  - 109% for those in their 50s,
  - 101% for the 60s,
  - 93% for the 70s, and
  - 85% for the 80s.

We also measured the plasma VWF antigen level in this population. The VWF antigen level increased with age, as reported previously. Because of the combined effects of the increase in VWF antigen level and the decrease in ADAMTS13 activity, the ratio of VWF antigen-to-ADAMTS13 activity was dramatically increased with age (31). This may partly explain the prothrombotic state of elderly men and women. As the FRETS-VWF73 assay itself was not affected by VWF concentration in plasma samples (0–160 ug/ml) (31), the reduced ADAMTS13 activity in the plasma of elderly subjects was not considered to be due to the assay-dependent artifactual phenomenon. In fact, when age-adjusted VWF antigen level was compared among quartiles of ADAMTS13 activity in the population, no significant association between VWF antigen and ADAMTS13 activity levels was observed in men or in women.

The AB0 blood group is a well-known genetic determinant for plasma VWF antigen levels:

> Individuals with blood group 0 have a lower VWF level than those with non-0 groups (32).

The AB0 blood group gene is located approximately 129 kb from the ADAMTS13 gene, and this may suggest a possible correlation between the two genes. In our population, the individuals with blood group 0 exhibited a significantly lower VWF antigen level than those with non-0 groups, as shown in previous studies. In contrast, the plasma ADAMTS13 activity was not associated with the AB0 blood group (31). This was consistent with the observation that ADAMTS13 antigen levels were not associated with AB0 blood group in 387 male Dutch individuals (33). The results are also consistent with the fact that VWF (34), but not ADAMTS13 (35), contains AB0 blood group-related N-linked oligosaccharides.

Control plasma for ADAMTS13 assay

The level of ADAMTS13 activity in the general population varied widely, ranging from approximately 40% to 240% of the normal level (▶Fig. 1). In general, the plasma ADAMTS13 activity is expressed as a percentage of the activity in commercially available or locally prepared, pooled normal plasma (control plasma). Therefore, if there is a wide range of ADAMTS13 activity among the control plasma samples be-

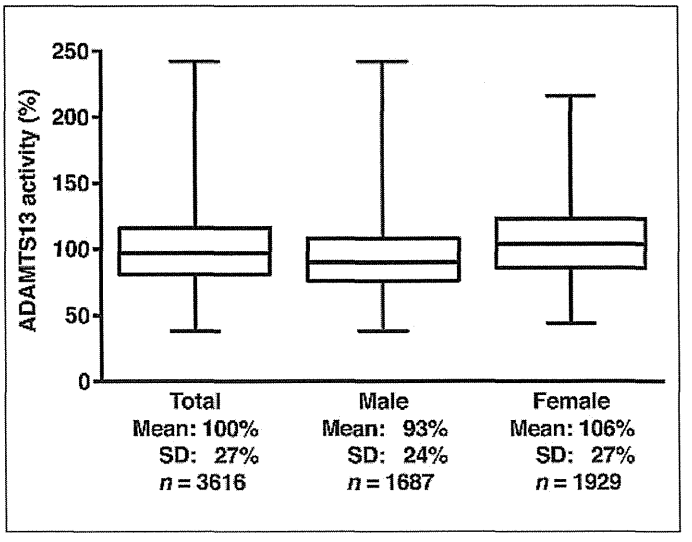

Fig. 1 Plasma ADAMTS13 activity in a Japanese population. Box-and-whisker plots of the plasma ADAMTS13 activities in men, women, and all subjects are shown. The mean of all values in total subjects was set at 100%. The top and bottom of the box are the 75th and 25th percentiles. The whiskers go down to the smallest value and up to the largest.

fore pooling, this can create a serious problem for ADAMTS13 measurement. It is easy to assume that the control plasma samples prepared from a relatively small number of individuals would show large deviation. In order to estimate the ideal number of individuals for the preparation of the control plasma, we randomly selected the ADAMTS13 activity values from 10 individuals (5 men and 5 women) in the general population cohort consisting of 3616 individuals, and repeated this selection 10 times to obtain the mean ± 2 standard deviation. The results of the 10-times repeated selection showed that 80% was the value of the mean – 2 standard deviations and 125% was the value of the mean + 2 standard deviations. These results indicated that the control plasma randomly prepared from 10 individuals represented a wide variation of activity and thus was not suitable for use as a control.

When we selected the activities of 20 individuals (10 men and 10 women), the activities of the mean + and – 2 standard deviations were narrowed down to 89% and 113%, respectively, which might have been sufficient for diagnostic purposes. When we selected 40 individuals or 100 individuals, the activity ranges were reasonably narrowed and were useful for the rigorous analysis of the ADAMTS13 activity (40 individuals: mean ± 2 standard deviations, 91–108%; 100 individuals: mean ± 2 standard deviations, 94–104%). As described, sex and age influence plasma ADAMTS13 activity. Therefore, the control plasma for plasma ADAMTS13 activity can be prepared from at least 20 individual plasma samples in consideration of age and sex.

Currently, a new project, "*Development of the WHO 1st International Standard for ADAMTS13 in Plasma*" led by the VWF Subcommittee of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis has been initiated by Dr. Johanna Kremer Hovinga.

Genotype-phenotype correlation of polymorphisms

The ADAMTS13 gene contains several genetic missense polymorphisms (3, 36, 37) some of which may influence the VWF cleaving activity. Since low plasma ADAMTS13 and high VWF levels are related to ischaemic stroke and myocardial infarction (38–40), missense polymorphisms in the ADAMTS13 gene could be important. Twenty missense polymorphisms of the ADAMTS13 gene have been listed (37), and some of their possible structural defects have been examined in silico (41).

To identify genetic polymorphisms in the Japanese population, we sequenced the ADAMTS13 gene in 346 individuals and identified 25 polymorphisms with a minor allele frequency of > 0.01 (42):

- 6 were missense polymorphisms and
- 19 were synonymous mutations.

We further genotyped six missense polymorphisms in a large Japanese cohort consisting of 3616 individuals whose plasma ADAMTS13 activities had been measured. We found that the minor allele frequencies were

- 0.192 for Q448E (c.1342C>G),
- 0.05 for P475S (c.1423C>T),
- 0.048 for S903L (c.2708C>T),
- 0.027 for T339R (c.1016C>G),
- 0.027 for P618A (c.1852C>G) and
- 0.022 for G1181R (c.3541G>A).

The T339R and P618A polymorphisms were in absolute linkage disequilibrium. When we examined the association of these polymorphisms with plasma ADAMTS13 activity, the ADAMTS13 activity of Q448E heterozygotes (QE) and minor allele homozygotes (EE) was significantly higher than that of major allele homozygotes (QQ):

- QQ: 97.6% ± 25.9%;
- QE: 104.2% ± 27.4%;
- EE: 105.7% ± 27.5%.

In contrast, the ADAMTS13 activity of P475S heterozygotes (PS) and minor allele homozygotes (SS) was significantly lower than that of major allele homozygotes (PP):

- PP: 101.4% ± 26.6%;
- PS: 87.2% ± 23.3%;
- SS: 73.3% ± 20.3% (42).

Four other missense polymorphisms did not affect the ADAMTS13 activity.

Tab. 1 Non-synonymous mutations identified in four segregated groups with different ranges of ADAMTS13 activity

group (average activity)	mutation	predicted damage	references
maximum (183%)	L19F	benign	newly identified
	R268Q		
median (97.6%)	Q723K		
	N1321S		
2nd minimum (53.1%)	I380T		
	Y1074Afs*46	–	causative for congenital ADAMTS13 deficiency (46)
	R1274C	possibly damaging	newly identified
minimum (47.1%)	F324L	probably damaging	
	F418L		
	I673F	possibly damaging	causative for congenital ADAMTS13 deficiency (47)
	Q773*	–	newly identified
	Y1074Afs*46	–	causative for congenital ADAMTS13 deficiency (46)
	R1095Q	probably damaging	newly identified

*stop codon

As described, the AB0 blood group gene is located near the ADAMTS13 gene. T339R and P618A were associated with the blood group A allele and P475S and S903L tended to be associated with the blood group 0 allele in our study (42).

The P475S missense polymorphism is ethnic specific, having only been identified in an East Asian population. The reduced plasma ADAMTS13 activity in individuals with the P475S mutation is consistent with the finding that the recombinant ADAMTS13-P475S mutant showed approximately 70% of the activity of the wild-type ADAMTS13 (43). To further elucidate the molecular basis of the reduced activity of the ADAMTS13-P475S mutant, we recently determined the enzymatic parameters of ADAMTS13-MDTCS (residues 75–685) and MDTCS-P475S and solved the crystal structure of the P475S mutant of the ADAMTS13-DTCS domain (44). MDTCS-P475S exhibited a reaction rate similar to that of wild-type MDTCS but showed twofold lower affinity for FRETS-VWF73, indicating that Pro475 is involved in formation of the substrate-binding exosite. The crystal structures showed that the conformation of the P475S-containing loop was significantly different between the mutant and the wild-type. This explains the higher susceptibility of the enzymatic activity of MDTCS-P475S to environmental conditions such as denaturants and high temperature. MDTCS-P475S can moderately cleave shear-treated VWF.

Incompatible evidences between in vivo and in vitro studies are accumulating on one of the missense polymorphisms, P618A. PolyPhen-2, an in silico tool which predicts the possible impact of an amino acid substitution on the structure and function of a human protein, predicted P618A as a damaging mutation. The crystal structure of the S domain of ADAMTS13 showed that Pro618 adopted the *cis* conformation (41), and the substitution of Pro618 with Ala, which cannot adopt the *cis* conformation, may cause structural distortion. Indeed, a transient expression study of the ADAMTS13-P618A mutant showed lower levels of activity and antigen in the conditioned media of HEK293 cells (45). However, as described, the P618A mutation was not associated with plasma ADAMTS13 activity in the general population. This inconsistent observation should be properly addressed in the future experiments.

ADAMTS13 deficiency in a Japanese population

The ADAMTS13 activity-genotype analysis based on ~3200 individuals enabled us to estimate the frequency of congenital ADAMTS13 deficiency. We selected 128 individuals according to their plasma ADAMTS13 activity:

- 32 individuals of the "minimum" activity group (average activity, 47.1%),
- 32 individuals of the "second minimum" activity group (average activity, 53.1%),
- 32 individuals of the "median" activity group (average activity, 97.6%), and
- 32 individuals of the "maximum" activity group (average activity, 183%).

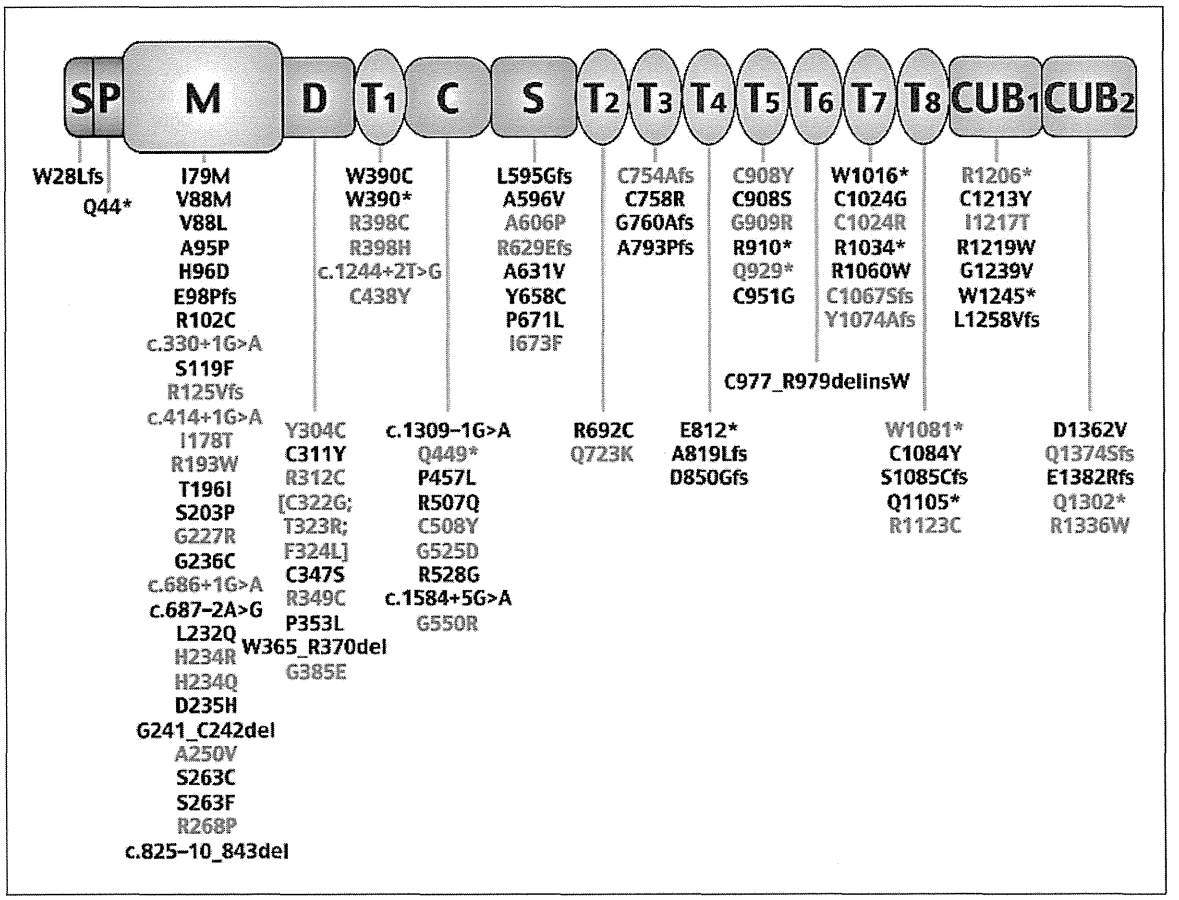

Fig. 2
ADAMTS13 gene mutations responsible for congenital TTP (*stop codons). The description of protein sequence mutation follows the recommendation of the Human Genome Variation Society (www.hgvs.org/mutnomen/recs-prot.html). Mutations in red were identified in Japanese patients.

Sequence analysis of the ADAMTS13 gene in these individuals showed that 14 individuals had rare non-synonymous mutations: seven individuals in the minimum activity group, three individuals in the second minimum activity group, two individuals in the median activity group, and two individuals in the maximum activity group (▶Tab. 1). In particular, three of the subjects had causative mutations for congenital ADAMTS13 deficiency, Y1074Afs*46 (46) and I673F (47). These data indicated that 2 of every 32 individuals had a mutation that does not cause a functional defect of ADAMTS13. Therefore, it would be a reasonable assumption that five individuals in the minimum activity group and one individual in the second minimum activity group would be heterozygotes carrying a mutation with a functional defect. If this assumption is valid, 6 out of 3200 individuals would be heterozygotes for ADAMTS13 deficiency. This estimation suggested that ~ 1 individual in 1.1×10^6 should be a homozygote or a compound heterozygote for ADAMTS13 deficiency. If a part of homozygous/compound heterozygous mutation carriers would die during the neonatal period, the prevalence in the surviving population may be lowered. It has been reported that the E1382Rfs*6 mutation (the E1382R frameshift mutation giving rise to the stop codon at six amino acid residues thereafter) due to the 4143insA mutation is frequent among patients with congenital ADAMTS13 deficiency in Northern and Central European countries (48). The estimation of the prevalence of patients with congenital ADAMTS13 deficiency may be biased due to insufficient sample sizes, ethnicity, lethality, and other factors.

ADAMTS13 mutations in congenital TTP

Since the publication of the first genetic analysis in patients with Upshaw-Schulman syndrome in 2001 (3), more than 100 genetic defects in the ADAMTS13 gene have been reported worldwide (36, 37, 49). The genetic variants that lead to TTP are very broadly distributed, occurring everywhere from the N-terminal signal peptide to the C-terminal CUB domain. The missense mutations are most frequent (about 60%), but other non-synonymous mutations such as frameshift mutations (small deletions or insertions), nonsense mutations, abnormal splicing, and insertions/deletions, are also detected (▶Fig. 2).

> We have so far identified more than 40 genetic mutations in Japanese patients with congenital TTP.

Most of the mutations were found in a single patient, but four mutations, i.e., R193W, Q449*, C754Afs*24 (c.2259delA), and C908Y, were identified in more than four patients, suggesting the accumulation of these mutations in a Japanese population (46).

Acknowledgments

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Conflict of interest

The National Cererbral and Cardiovascular Center where TM and KK (inventors) belong has an awarded patent on the use of reagent, FRETS-VWF73. MM is al clinical advisory board for Alexion Pharmaceuticals, and has a patent on the use of chromogenic ADAMTS13 activity assay using the nonoclonal antibody, which specifically recognizes TYR1605 within VWF-A2 domain, exposed by ADAMTS13 cleavage. YF is a clinical advisory board for Baxter Bioscience and for Alexion Pharmaceuticals, and has a patent on the use of chromogenic ADAMTS13 activity assay using the nonoclonal antibody, which specifically recognizes TYR1605 within VWF-A2 domain, exposed by ADAMTS13 cleavage.

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CASE REPORT Open Access

Long term follow up of congenital thrombotic thrombocytopenic purpura (Upshaw-Schulman syndrome) on hemodialysis for 19 years: a case report

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Abstract

Background: Thrombotic thrombocytopenic purpura (TTP) is frequently associated with renal abnormalities, but there have been few reports about renal abnormalities in patients with hereditary TTP. In particular, little is known about the long-term prognosis of patients with childhood-onset congenital TTP.

Case presentation: We report a Japanese patient with congenital TTP (Upshaw–Schulman syndrome) who was followed for 19 years after initiation of hemodialysis when he was 22 years old. At the age of 6 years, the first episode of purpura, thrombocytopenia, and proteinuria occurred without any precipitating cause. He underwent living-related donor kidney transplantation from his mother, but the graft failed after 5 months due to recurrence of TTP. Even after resection of the transplanted kidney and resumption of regular hemodialysis, TTP became refractory to infusion of fresh frozen plasma (FFP). Therefore, splenectomy was performed and his disease remained in remission for 10 years. However, TTP recurred at the age of 39 years. Plasma activity of ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type I domain 13) was less than 3%, while ADAMTS13 inhibitor was not detected (< 0.5 Bethesda units/mL). The patient died suddenly after hemodialysis at the age of 41 years. Subsequent genetic analysis of this patient and his parents revealed two different heterozygous mutations of ADAMTS13, including a missense mutation in exon 26 (c.3650T>C causing p.I1217T) inherited from his father and a missense mutation in exon 21 (c.2723G>A causing p.C908Y) inherited from his mother. The former mutation has not been detected before in Japan, while the latter mutation is common in Japan. A retrospective review showed that serum C3 levels were consistently low while C4 levels were normal during follow-up, and C3 decreased much further during each episode of TTP.

Conclusion: Congenital TTP was diagnosed from the clinical, biochemical, and genetic findings. Infusion of FFP controlled each thrombotic episode, but the effect was limited and of short duration. Review of the complement profile in this patient suggested that a persistently low serum C3 level might be associated with refractory TTP and a worse renal prognosis.

Keywords: Congenital thrombotic thrombocytopenic purpura, ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type I domain 13), Chronic hemodialysis, Complement activation, C3, Alternative pathway

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Background

Thrombotic thrombocytopenic purpura (TTP) is a rare disorder characterized by thrombocytopenia and microangiopathic hemolytic anemia. Congenital TTP has been reported to be associated with severe deficiency of the plasma activity of ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type I domain 13), which is reduced to <5% of normal by mutation of the ADAMTS13 gene, and this is known as the Upshaw–Schulman syndrome (USS) [1,2]. Deficiency of ADAMTS13 activity can also be caused by inhibitory antibodies targeting ADAMTS13, leading to acquired TTP. ADAMTS13 is a metalloproteinase that specifically cleaves multimeric von Willebrand factor (VWF) [2], while VWF is a large glycoprotein that is essential for platelet adhesion and aggregation under high shear stress conditions [3]. ADAMTS13 is mainly synthesized in the liver by stellate cells [4,5]. In addition, it is expressed by the podocytes and endothelium of the renal glomeruli, where podocyte-derived ADAMTS13 might have a local protective effect in the high shear stress glomerular microcirculation [6].

TTP is often associated with renal abnormalities and there have been some reports about such abnormalities in TTP patients, but few about hereditary TTP. In particular, there is little information about the long-term prognosis of patients with childhood-onset congenital TTP [7]. Here, we report a Japanese man with congenital TTP confirmed by genetic analysis, who was followed up for 19 years after initiation of hemodialysis.

Case presentation

A 22-year-old man was admitted to our hospital for renal transplantation. He was the third of five children of non-consanguineous parents. There was no history of severe neonatal jaundice. Purpura of the lower extremities, thrombocytopenia, and proteinuria occurred without any precipitating cause at the age of 6 years, and hemolytic uremic syndrome (HUS) was diagnosed. This episode subsided spontaneously without treatment, but there were repeated recurrences and his renal function deteriorated gradually. In 1990, at the age of 22 years, hemodialysis was started for end-stage renal disease (ESRD) along with the occurrence of cerebral infarction. After 4 months, living-related kidney transplantation was performed with his mother as the donor. Immunosuppressive therapy included prednisolone (70 mg daily), cyclosporine (420 mg daily), antilymphocyte globulin (1 g daily), and azathioprine (100 mg daily). At 7 days after surgery, he developed thrombocytopenia (23.1 to $1.8 \times 10^4/\mu L$) and hemolytic anemia (Hb: 10.3 to 8.2 g/dL), along with an increase of serum creatinine (1.1 to 2.1 mg/dL), lactate dehydrogenase (LDH: 208 to 785 IU), and total bilirubin (0.4 to 2.2 mg/dL). Haptoglobulin was decreased to 3.4 mg/dL. Serum levels of C3 and C4 were also decreased (C3: 63.0 to 51.7 mg/dL, normal range; 83 to 177 mg/dL, C4: 34.4 to 22.9, normal range; 15 to 45 mg/dL). Activation of HUS was suspected to have been caused by cyclosporine, so it was switched to deoxyspergualin (200 mg daily). After methylprednisolone pulse therapy (500 mg/day for 3 days) and infusion of fresh frozen plasma (FFP) (800 mL × 5 days), HUS subsided temporarily. However, there was frequent relapse of HUS, so azathioprine was changed to mizoribine and muromonab-CD3 was administered. Plasma exchange or infusion FFP was effective for terminating each episode of HUS. After 50 days, cerebral hemorrhage occurred, followed by gastrointestinal bleeding at 90 days. Then HUS recurred with thrombocytopenia and hemolytic anemia, which was refractory to plasma exchange or infusion of FFP, and his renal function deteriorated gradually. In May 1991, removal of the kidney graft was performed and hemodialysis was restarted. Examination of the resected kidney showed thrombi, endothelial cell swelling, and numerous red blood cells in the glomeruli and small arteries (Figure 1). After nephrectomy, jejunal bleeding was treated by transcatheter arterial embolization of an arteriovenous malformation in the superior mesenteric artery territory.

Even after hemodialysis was resumed, transient ischemic attacks and cerebral infarction occurred every time his platelet count decreased spontaneously, subsiding in response to infusion of FFP. However, TTP became refractory to FFP in 1998. Because indium platelet scintigraphy showed high uptake in the spleen and his platelets had a short lifespan (1.76 days), splenectomy was performed in order to prevent excessive platelet destruction. Thereafter, thrombotic episodes requiring the infusion of FFP did not occur for 10 years until 2008. During this remission period, the serum level of C3 was always lower than normal and serum C4 was normal, while the C3 level decreased much further with each episode of TTP. When cerebral infarction with thrombocytopenia occurred again at the age of 39 years, plasma ADAMTS13 activity was less than 5% of normal, as measured by the FRETS-VWF73 assay [8], while ADAMTS13 inhibitor was negative (<0.5 Bethesda units/mL) [9]. USS was diagnosed because he had severe deficiency of ADAMTS13 activity without any detectable inhibitor in conjunction with appropriate clinical criteria. Although the thrombotic episodes subsided following infusion of FFP, he died suddenly after hemodialysis in 2010 at the age of 41 years. After the patient's death, we measured plasma ADAMTS13 activity and inhibitor in his parents using a chromogenic ELISA [10]. Both of them had ADAMTS13 activity around 30% of normal and the inhibitor was negative.

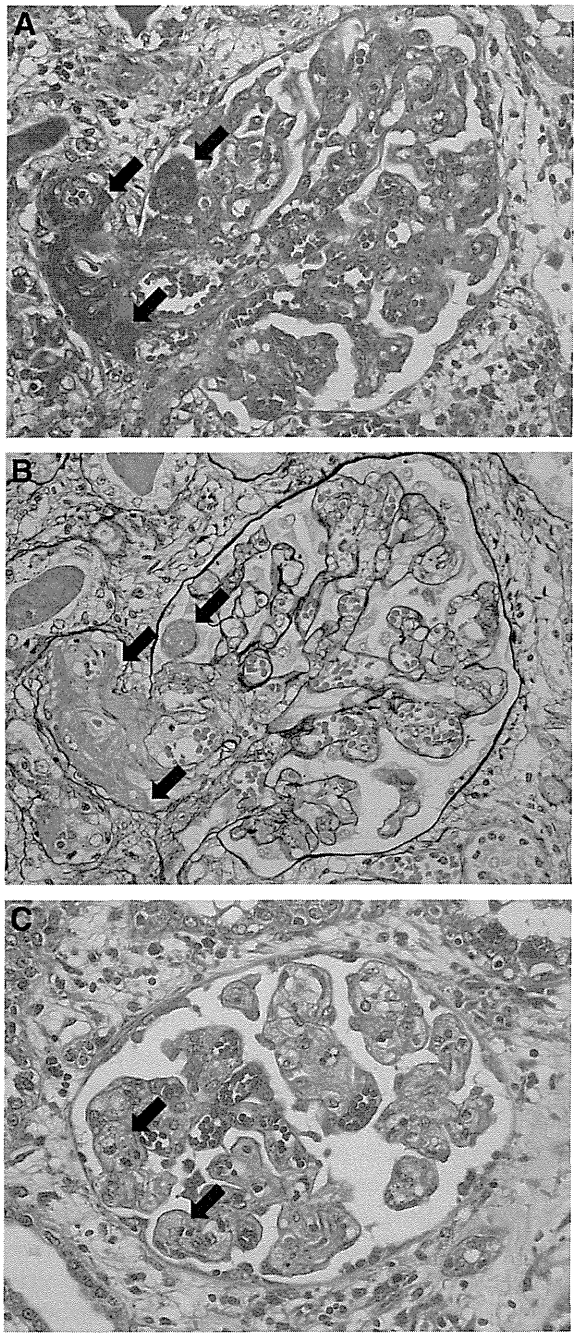

Figure 1 Renal histopathological findings. A, B: Fibrin thrombi in small arteries (arrows) and a glomerulus containing numerous red blood cells (**A**: Heidenhain's azan trichrome stain, **B**: Periodic acid methenamine silver stain × 400). **C**: Endothelial cell swelling (arrows) (Heidenhain's azan trichrome stain × 400).

Genetic analysis

After obtaining consent from his parents, genetic analysis of the patient and parents was performed with the approval of the Ethics Committees of Nara Medical University, the National Cerebral and Cardiovascular Center, and Toranomon Hosipital. Genetic analysis of the patient was carried out at the National Cerebral and Cardiovascular Center using DNA extracted from the resected spleen. For his parents, analysis was performed at the Department of Blood Transfusion Medicine of Nara Medical University.

It was demonstrated that the patient had compound heterozygous mutations of ADAMTS13, comprising a missense mutation in exon 26 (c.3650T>C causing p. I1217T) that was inherited from his father and a missense mutation in exon 21 (c.2723G>A causing p.C908Y) inherited from his mother. A diagnosis of congenital TTP (USS) was confirmed by these findings (Figure 2).

Discussion

It is widely recognized that TTP is associated with renal abnormalities, with renal failure occurring secondary to damage caused by microthrombi that develop because of decreased plasma ADAMTS13 activity. The common renal manifestations of TTP are proteinuria and hematuria. Acute renal failure (ARF) affects 11% of patients with severe congenital TTP and often recurs with exacerbation of this disease [7]. Although ARF requiring dialysis was reported to be less frequent (0–9.7%) in four series of patients with acquired TTP [11-13], the percentage of patients with congenital TTP who need regular dialysis is unclear. Tsai et al. [7] reported that five out of nine patients with USS progressed to ESRD requiring dialysis, and three of them had episodes of ARF. Therefore, repeated episodes of ARF may be associated with progression to ESRD.

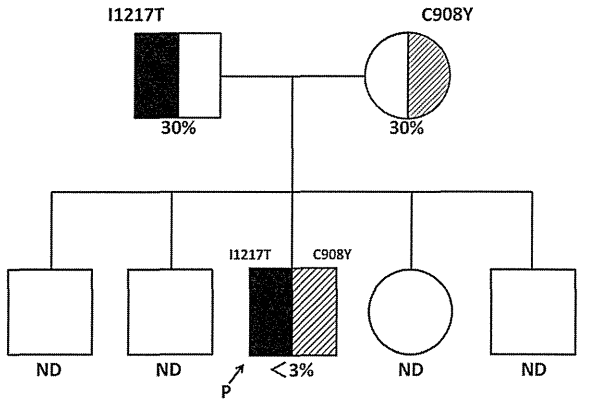

Figure 2 Pedigree of the index patient with genetic haplotypes and plasma activity of ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type I domain 13). Squares represent males and circles represent females. Plasma ADAMTS13 activity (%) is shown under the circles and squares. Mutations of the ADAMTS13 gene are shown as one-letter amino acid abbreviations numbered from the initial Met codon. The arrow indicates the index patient. The mother and father of the index patient are both asymptomatic carriers. Abbreviations P: patient, ND: not determined.

Because infusion of plasma is effective for acute exacerbation of congenital TTP, plasma exchange is the standard treatment. In patients with relapsing and/or refractory TTP, splenectomy can be effective. The mechanism is assumed to be that splenectomy decreases autoantibody production by removing a large reservoir of B lymphocytes [14], which is a reasonable explanation for patients with acquired TTP and elevated levels of ADAMTS13 inhibitor. However, Snider et al. [15] reported a patient with relapsing and refractory congenital TTP who remained in complete clinical remission for 4 years after splenectomy. In our patient, remission of TTP persisted for 10 years after splenectomy, but the effect was limited. The mechanism by which splenectomy improves congenital TTP is unknown, although it is possible that a state like idiopathic thrombocytopenia purpura (ITP) might have coexisted with TTP in our patient because his short platelet lifespan was compatible with ITP. Since TTP remained in remission for 10 years after splenectomy without the need for FFP, this case shows that splenectomy can be a useful option for relapsing/refractory congenital TTP. There has only been one previous case report of renal transplantation for chronic renal failure in a patient with congenital TTP, and the graft showed early failure due to disease recurrence [16]. In our case, the graft also failed due to chronic relapsing TTP only 5 months after transplantation. Therefore, renal transplantation may not a feasible option for ESRD in patients with congenital TTP.

Several mutations of the ADAMTS13 gene have been reported in congenital TTP. It is thought that specific ADAMTS13 mutations are more common among certain ethnicities [17]. Fujimura et al. [17] evaluated 43 USS patients in Japan and found ADAMTS13 mutations that were specific to Japanese individuals with congenital TTP. The present patient had p.C908Y with maternal inheritance, which is one of the common ADAMTS13 mutations found in Japanese patients [17]. However, the patient also had p.I1271T (inherited from his father) and this has not been reported before in Japanese patients, was although it is consistent with the missense mutation reported by Park et al. [18] in a Korean patient who had congenital TTP complicating moyamoya disease. Fujimura et al. [17] reported that two out of 43 patients with congenital TTP progressed to ESRD requiring dialysis. One of them was homozygous for c.414 + 1G > A, while the other was heterozygous for c.1885delT (paternal inheritance) and p.C908Y (maternal inheritance). However, these mutations were also detected in some of their TTP patients without progression to dialysis. In fact, five of the 43 patients had the p.C908Y mutation that was detected in our case, but only one of them progressed to dialysis during follow-up. Therefore, as Tsai et al. [7] concluded, the relation between ADAMTS13 mutation and the renal prognosis remains uncertain [17].

With regard to the occurrence of renal impairment in this patient, it may be important to focus on the complement system. Ruiz-Torres et al. [19] studied thrombotic microangiopathy patients with congenital ADAMTS13 deficiency and patients with ADAMTS 13 inhibitors, and they reported that four of out of six patients (66%) showed a moderate decrease of C3 in the acute phase, which was indicative of complement activation and consumption. They hypothesized that platelet microthrombi caused activation of the alternative pathway in patients with ADAMTS13 deficiency. Moreover, Noris et al. [20] reported 2 sisters who had the same compound heterozygous ADAMTS13 mutations, while one sister also had a heterozygous mutation of the gene encoding complement factor H, a plasma factor that inhibits activation of the alternative pathway. The second sister had severe disease, with renal involvement requiring chronic dialysis, and eventually died of a stroke. She had subnormal serum C3 levels and normal C4 levels. In addition, one of the four congenital TTP patients reported by Ruiz-Torres et al. had a subnormal C3 level even in remission and her serum creatinine level was 5.73 mg/dL, suggesting ESRD. Considering these reports, some patients with congenital TTP may have persistently low C3 levels that may be associated with a worse renal prognosis. The findings in our case seem to support this hypothesis. If a persistently depressed C3 level and normal C4 level, indicating selective activation of the alternative pathway, is one of the causes of severe TTP, the anti-C5 monoclonal antibody eculizumab may be an effective treatment for refractory TTP. In fact, Chapin et al. [21] reported that eculizumab was effective for refractory TTP, so use of eculizumab might have been a good treatment option in our case.

Conclusion

We encountered a male patient with congenital TTP who remained on hemodialysis for 19 years. His ADAMTS13 gene had two mutations, which were p.I1217T (the first report of this mutation in Japan) and p.C908Y (common in Japan). Infusion of FFP was effective for controlling thrombotic episodes, but the improvement was limited and of short duration. The profile of complement components in this patient suggests an association of persistently low serum C3 level with refractory TTP and a worse renal prognosis.

Consent

Written informed consent was obtained from the patient's parents for the genetic analyses, as well as for publication of this case report and any accompanying images. We could not obtain written consent from the patient himself because he was already dead when we wrote this paper.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
KM contributed to analyzing and interpretation of data and writing the manuscript. YU contributed to analyzing and interpretation of data and writing the manuscript. MM contributed to analyzing and interpretation of data and writing the manuscript. KS contributed to managing the patient and assessing data. RH contributed to managing the patient and assessing data. EH contributed to managing the patient and assessing data. MY contributed to managing the patient and assessing data. NH contributed to managing the patient and assessing data. TS contributed to managing the patient and assessing data. JH contributed to managing the patient and assessing data. NS contributed to managing the patient and assessing data. KO contributed to analyzing and interpretation of pathological findings. KK contributed to analyzing the ADAMTS13 gene of patient. TM contributed to analyzing the ADAMTS13 gene of patient. YF contributed to analyzing and interpretation of data and writing the manuscript. KT contributed to analyzing and interpretation of data and management the patient. All authors read and approved the final manuscript.

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Regular Article

Behavior of ADAMTS13 and Von Willebrand factor levels in patients after living donor liver transplantation

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ABSTRACT

Introduction: Thrombotic microangiopathy (TMA) is one of the important complications occurring after liver transplantation (LT), and it is suggested that a Von Willebrand factor (VWF) and ADAMTS13 may play an important role in the onset of TMA and poor outcome after LT.
Materials and Methods: In 81 patients after living donor LT (LDLT), 17 patients who had both severe thrombocytopenia and hemolytic anemia with fragmented red cell were diagnosed as TMA- like syndrome (TMALS) and 10 patients died.
Results: ADAMTS13 activities were slightly low, and plasma levels of VWF and VWF propeptide (VWFpp) antigens and the ratio of VWFpp/VWF were significantly high before LDLT. ADAMTS13 activities were significantly reduced from day 1 to day 28 after surgery, and plasma levels of VWF antigen slightly decreased on day 1 and plasma levels of VWFpp continued to be high. The ratio of VWFpp/VWF was significantly high on day 1 after surgery.
The mortality was high in the patients with TMALS and the frequency of TMALS was high in non-survivors. VWF levels were significantly low and the ratio of VWFpp/VWF was significantly high in those with TMALS on day 1 after surgery. The ADAMTS13 activity was significantly low, and the VWFpp and the VWFpp/ADAMTS13 ratio were significantly high in non-survivor on day 28 after surgery.
Conclusion: These findings suggest that VWF and ADAMTS13 might therefore play an important role in the onset of TMA and poor outcome after LT. The VWFpp may therefore be a more useful marker for the diagnosis of TMALS than VWF.

Introduction

Thrombotic microangiopathy (TMA) is a microvascular occlusive disorder induced by endothelial damage, primary platelet aggregation and microangiopathic hemolytic anemia (MHA) [1–3]. TMA stands out as an infrequent but severe life-threatening complication, often requiring intense therapy [2]. According to previous reports, TMA develops after solid-organ transplantation with an incidence of 0.5 to 3% [4–6]. It has also been reported in liver transplant (LT) recipients [7,8]. Possible causative factors of TMA following liver transplantation include calcineurin inhibitors [9] and infections [10], including hepatitis C [11]. However, the specific pathophysiological mechanism of TMA is not yet fully understood.

Recently, the kinetics of ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type I domain 13) and unusually large Von Willebrand factor (VWF) multimer (UL-VWFM) have been reported to be good indicators for the occurrence of adverse events after LT [12,13]. ADAMTS13 is a metalloprotease that specifically cleaves the multimeric VWF [14–18]. A severely deficient ADAMTS13 activity (less than 5% of that in normal plasma) is caused by either a mutation of the ADAMTS13 gene [15,19] or by inhibitory antibodies against ADAMTS13 [20]. UL-VWFM produced in and then quickly released from vascular endothelial cells, has often been found in patient's plasma in familial and non-familial TTP [21,22]. VWF is a large glycoprotein which is essential for high-shear stress associated platelet adhesion and aggregation [23]. These UL-VWFMs

Abbreviations: ADAMTS13, a disintegrin and metalloprotease with thrombospondin type I domain 13; VWF, Von Willebrand factor; VWFpp, VWF propeptide; UL-VWFM, unusually large Von Willebrand factor multimer; LDLT, living donor liver transplantation; TMA, Thrombotic microangiopathy; TMALS, TMA-like syndrome; MHA, microangiopathic hemolytic anemia; TTP, thrombotic thrombocytopenic purpura; VOD, veno-occlusive disease.

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have been thought to interact with circulating platelets, thus resulting in platelet clumping due to an elevated shear stress [21].

The pre-pro VWF, which is synthesized in endothelial cells and megakaryocytes, undergoes intracellular modifications including signal peptide cleavage, C-terminal dimerization, glycosylation, sulfation, and N-terminal multimerization [24]. Then proteolysis occurs in the trans-Golgi where the VWF propeptide (VWFpp) is cleaved but remains stored together with mature VWF in alpha-granules (megakaryocytes) and Weibel-Palade bodies (endothelial cells). After the secretion of VWFpp and VWF into plasma from endothelial cells due to several physiological or pathological stimuli, VWFpp dissociates from VWF [25,26]. Elevated VWFpp is reported in patients with TMA [27].

In this study, we measured the ADAMTS13 activity, VWFpp and VWF antigen in the plasma of 17 patients with TMA-like syndrome (TMALS) and 23 without TMALS during living donor LT (LDLT) in order to examine the usefulness of a diagnosis of TMA after LT.

Materials and methods

Eighty one patients (35 females and 46 males; median age, 47 years; range, 0–70 years) after LDLT from January 1, 2002 to December 31, 2005 were examined the association with TMALS. The underlying diseases of the LDLT patients were 24 with hepatic cell carcinoma, 20 with liver cirrhosis due to viral infection, 11 with primary biliary cirrhosis, 10 with hepatitis due to other causes, 6 with cholestatic disease due to other cause, 7 with biliary atresia, and 3 with other diseases. The diagnosis of TMALS is mainly based on thrombocytopenia due to consumption and hemolytic anemia due to the microangiopathy and, in addition, it also includes the laboratory data and clinical symptoms such as liver dysfunction, neurological dysfunction, renal failure, or fever. Seventeen patients (9 females and 8 males; median age, 53 years; range, 6–70 years,) were diagnosed as TMALS (onset after LDLT; median, day 15, range, day 6 – day 22) from 81 patients after LDLT. No other thrombotic event during LDLT. The mortality was evaluated on days 90 after surgery [13]. The study protocol was approved by the Human Ethics Review Committee of Mie University School of Medicine and a signed consent form was obtained from each subject. The immunosuppression protocol consisted of tacrolimus and low-dose steroids. The target trough level for tacrolimus in whole blood was 10–15 ng/mL during the first 2 weeks, approximately 10 ng/mL during the next 2 weeks, and 5–10 ng/mL thereafter. Methylprednisolone, 10 mg/kg body weight, was administered intravenously immediately before perfusion of the graft portal vein. Methylprednisolone in a dose of 1 mg/kg was given intravenously daily on postoperative days (PODs) 1–3 and a dose of 0.5 mg/kg daily on PODs 4–6. 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 per day. Patients whose liver function was stable were weaned off the steroid at around 3–6 months postoperatively.

In all of the 17 patients who developed TMALS, the three factors of ADAMTS13 activity, VWF antigen and VWF propeptide (VWFpp) could be measured in pre- and postoperatively, while among the remaining 64 patients who did not developed TMALS we could measure the three factors in 23 patients (9 females and 14 males; median age, 56 years, range 0–69 years). To obtain the normal control levels of these three factors we measured them in 50 healthy volunteers (HV; 31 females and 19 males; median age, 31 years; range, 19–51 years).

Human plasma was obtained by centrifugation at 3000 ×g at 4 °C for 15 min from whole blood that was treated with a 1/10 volume of 3.8% sodium citrate as an anti-coagulant. This plasma was stored at −80 °C before the assay.

ADAMTS13 was measured using a FRETS-VWF73, which was chemically synthesized by the Peptide Institute, Inc. (Osaka, Japan) according to the method of Kokame et al. [28]. VWF and VWFpp levels were measured with a VWF&Propeptide assay kit (GTI DIAGNOSTICs, Waukesha, USA). The hemoglobin (Hb) levels and platelet counts were measured by automated the fully delete hematology analyzer XE-2100 (Sysmex, Kobe, Japan).

Statistical analysis

The data are expressed as the median (25, 75% tile). The differences between the two groups on days 1 and 28 after LDLT were examined using the Mann-Whitney's U test. The difference of mortality between the groups with and without TMALS and the frequency of TMALS between the groups of survivor and non-survivor were analysed by the Chi square test. The differences between the groups of before LDLT (day 0) and after LDLT on days 1, 7, 14 and 28 were examined for statistical significance using the Wilcoxon signed-rank test and Bonferroni's multiple comparison. And the difference of VWF plasma levels and VWFpp/VWF ratio between the groups with and without TMALS were analysed by the Mann-Whitney's U test. A *P* value of less than 0.05 denoted the presence of a statistically significant difference.

Results

Plasma ADAMTS13 activities were significantly low before LDLT (65.0%: 50.6, 98.8%, $p<0.01$) in comparison to HV (109%: 95.0, 124%) and significantly reduced on day 1 (29.7%: 22.2, 37.8%, $p<0.01$), day 7 (31.3%: 21.3, 41.9%, $p<0.01$), day 14 (20.0%; 8.44, 45.0%, $p<0.01$) and day 28 (25.0%; 13.8, 42.5%, $p<0.01$) after surgery (Fig. 1-A). Plasma levels of VWF antigen before LDLT (308 U/dl: 169, 379 U/dl, $p<0.01$) on day 1 (154 U/dl: 106, 229 U/dl, $p<0.01$), day 7 (368 U/dl: 291, 408 U/dl, $p<0.01$), day 14 (304 U/dl: 254, 421 U/dl, $p<0.01$), day28 (312 U/dl: 250, 409 U/dl, $p<0.01$)after surgery were significantly higher in comparison to those in HV (85.0 U/dl; 77.2, 102 U/dl). (Fig. 1-B). Plasma levels of VWFpp before (257 U/dl; 221, 314 U/dl, $p<0.01$), on day 1 (236 U/dl; 186, 279 U/dl, $p<0.01$), day 7 (251 U/dl; 213, 304 U/dl, $p<0.01$), day 14 (211 U/dl; 181, 269 U/dl, $p<0.01$) and day 28 (236 U/dl; 185, 292 U/dl, $p<0.01$) after surgery were significantly higher in comparison to those in HV (85.0 U/dl; 62.5, 102 U/dl) (Fig. 1-C). The ratio of VWFpp/VWF was significantly higher on day 1 after surgery (1.40; 1.13, 1.88, $p<0.01$) than before surgery(1.02; 0.91, 1.38) and HV (1.12; 0.935, 1.27, $p<0.01$), and significantly lower on day 7 after surgery (0.91; 0.85, 1.1, $p<0.01$) than before surgery (Fig. 1-D). The ratio of VWF/ADAMTS13 was significantly higher on day 7 (9.86; 7.27, 15.7, $p<0.01$), on day 14 (12.2; 8.43, 31.1, $p<0.01$) and on day 28 after surgery (14.0; 7.70, 27.8, $p<0.05$) than before surgery (4.13; 2.43, 7.73). The ratio of VWFpp/ADAMTS13 was significantly higher on day 1 (7.15; 5.32, 10.7, $p<0.05$), on day 7 (7.81; 5.81, 11.7, $p<0.01$) and on day 14 after surgery (9.22; 5.17, 16.3, $p<0.05$) than before surgery (4.10; 2.37, 6.27) (Fig. 2-A and B).

There was no significant difference in the age, sex, and underlying diseases between the patients with and without TMALS, but the mortality of the patients with TMALS was higher than those without (Table 1). On day 1 after surgery, there was no significant differences in the ADAMTS13 or, VWFpp levels and VWF/ADAMTS13 ratio or VWFpp/ADAMTS13 ratio between the two groups, but VWF levels (137 U/ml; 96, 186 U/ml vs. 184 U/ml; 160, 244 U/ml, $p<0.01$) were significantly lower and the ratio of VWFpp/VWF (2.00; 1.74, 2.67 vs. 1.20; 1.06, 1.33, $p<0.001$) were significantly higher in those with TMALS than in those without (Fig. 3-A and B). There was also no significant difference in the age, sex and underlying diseases between survivors and non-survivors, but the frequency of TMALS was higher than in non-survivors than in survivors ($p<0.01$). However there was no significant difference in ADAMTS13, VWF, VWFpp, the ratio of VWFpp/VWF, VWF/ADAMTS13 and VWFpp/ADAMTS13 between survivors and non-survivors on day 1 after surgery, but the ADAMTS13 activity ($p<0.05$) was significantly lower, VWFpp ($p<0.01$) and VWFpp/ADAMTS13 ratio ($p<0.05$) were significantly higher in non-survivor than survivor on day 28 after surgery (Table 2).

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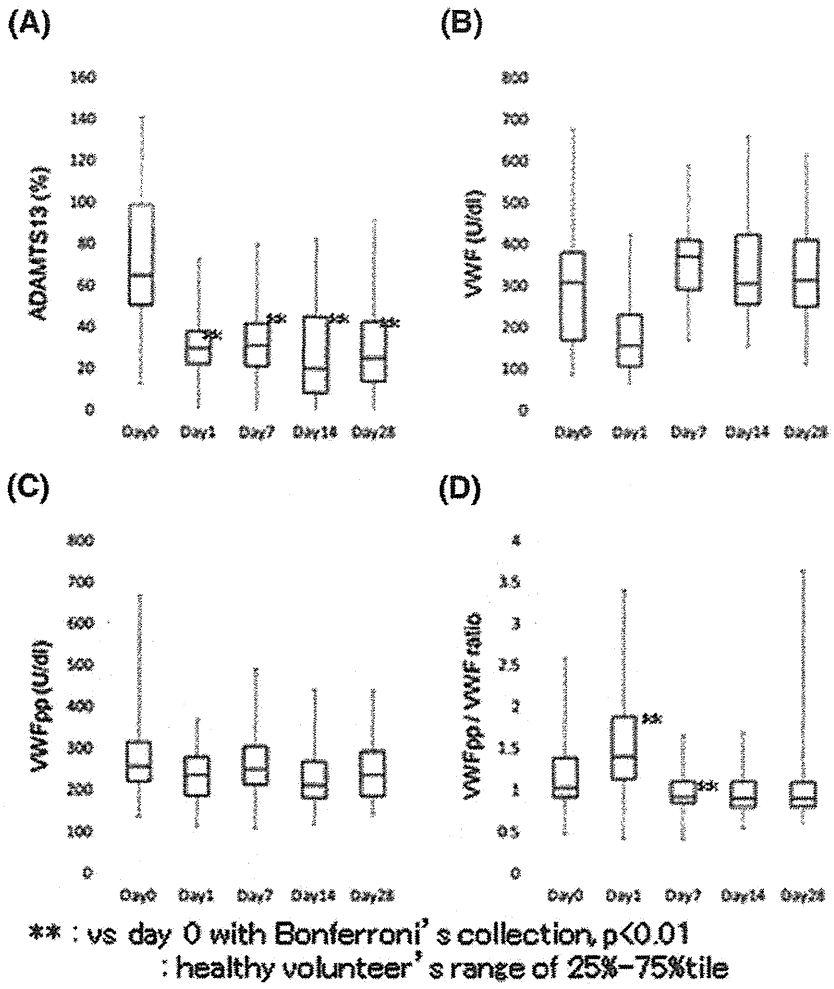

Fig. 1. ADAMTS13 activities, the VWF and VWFpp levels and the VWFpp/VWF ratio during LDLT. A) ADAMTS13 activities B) VWF C) VWFpp and D) the VWFpp/VWF ratio.

Discussion

The significant reductions of ADAMTS13 activity and increases of VWF antigen were previously reported in the patients with TMALS [13], thus suggesting that this pathophysiological state is similar to TTP. TTP, a life-threatening syndrome characterized by thrombocytopenia and microangiopathic hemolytic anemia, is often associated with neurological dysfunction, renal failure, and fever [20] and also, UL-VWFM, and significant reduce of ADAMTS13 have often been found in patients plasma in familial and non-familial TTP [20,21].

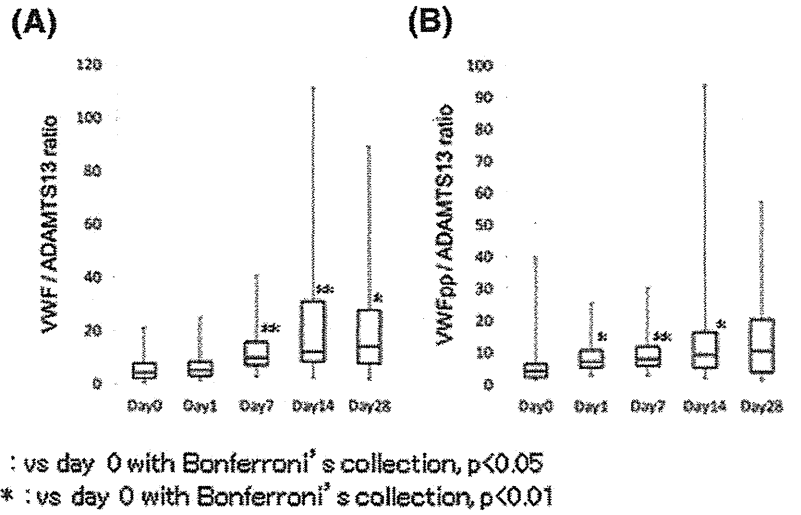

Fig. 2. VWF/ADAMTS13 and VWFpp/ADAMTS13 ratio during LDLT. A) VWF/ADAMTS13 B) VWFpp/ADAMTS13.

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Table 1
The study subjects.

	Without TMALS (n=23)	With TMALS (n=17)	Survivor (n=32)	Non-survivor (n=8)
Age (years)	56 (0–69)	53 (6–70)	53.5 (0–70)	56 (45–66)
Sex (F : M)	9:14	9:8	14:18	4:4
Infection or HCC	13/23 (56.5%)	11/17 (64.7%)	20/32 (62.5%)	4/8 (50.0%)
Mortality	3/23 (13.0%)**	5/17 (29.4%)**	-	-
TMALS	-	-	12/32 (37.5%)**	5/8 (62.5%)**

**: p<0.01.
TMA:Thrombotic microangiopathy.
HCC:hepatocellular carcinoma.
TMALS:TMA-like syndrome.

Table 2
The ADAMTS13, VWF and VWFpp levels and the VWFpp/VWF, VWF/ADAMTS13 and VWFpp/ADAMTS13 ratio in survivors and non-survivors.

	Survivor (n=32)	Non-survivor (n=8)	
Day1			
ADAMTS13 (%)	28.8 (22.2, 35.3)	35.6 (26.8, 55.9)	NS
VWF (U/dl)	154 (102, 227)	167 (129, 253)	NS
VWFpp (U/dl)	236 (189, 288)	238 (161, 261)	NS
VWFpp/VWF ratio	1.37 (1.19, 1.90)	1.58 (1.03, 1.76)	NS
VWF/ADAMTS13 ratio	5.07 (3.27, 9.47)	5.31 (3.60, 7.09)	NS
VWFpp/ADAMTS13 ratio	7.37 (5.82, 11.4)	5.60 (4.17, 9.55)	NS
Day28			
ADAMTS13 (%)	30.0 (16.3, 43.1)	9.38 (6.25, 15.3)	P<0.05
VWF (U/dl)	312 (244, 407)	327 (263, 395)	NS
VWFpp (U/dl)	193 (164, 269)	326 (274, 333)	P<0.01
VWFpp/VWF ratio	0.895 (0.775, 1.02)	1.00 (0.84, 1.40)	NS
VWF/ADAMTS13 ratio	10.7 (6.80, 23.3)	32.5 (15.6, 46.7)	NS
VWFpp/ADAMTS13 ratio	5.72 (3.66, 14.5)	20.6 (20.2, 32.5)	P<0.05

Day1 : Day1 after surgery.
Day28 : Day28 after surgery.
ADAMTS13 : a disintegrin and metalloprotease with thrombospondin type I domain 13.
VWF: Von Willebrand factor.
VWFpp : VWF propeptide.

Slightly low ADAMTS13 activities and markedly high plasma levels of VWF and VWFpp antigens suggest that hypercoagulable and consumptive states exist in the recipients.

Markedly high VWFpp/VWF ratio indicates over-synthesis and release of VWF from vascular endothelial cells and the consumption of VWF in the recipients with terminal liver diseases. Significant reduction of ADAMTS13 activities from day 1 to day 28 after surgery suggests that almost all of our patients had severe hepatic dysfunction before the LDLT and the transplanted liver is not full-sized in LDLT. It follows that the improvement in the liver function requires more time and that complications frequently occur. The ADAMTS13 levels were affected by the production from the liver and the consumption due to cleavage of UL-VWFM [2]. The activity of ADAMTS13 was also low in patients with hematopoietic stem cell transplantation. A decreased activity was reported in patients with hepatic veno-occlusive disease (VOD) after stem cell transplantation [29]. These findings suggest that a reduced amount ADAMTS13 may therefore be a risk factor for the onset of VOD or TMA after LDLT.

As the plasma levels of VWFpp continued to be high, overproduction of VWF persist during more than 28 days after the surgery. The slight reduction of VWF antigen and significant increase of VWFpp/VWF indicates that marked consumption of VWF occurs on day 1 after LDLT surgery. While the ratio of VWF/ADAMTS13 was significantly higher on day 7 after surgery than before surgery, the ratio of VWFpp/ADAMTS13 was significantly higher on day 1 after surgery than before surgery. We suggest that VWFpp is more useful marker than VWF as reflecting the state after LDLT. And VWFpp is reported to be useful marker for the diagnosis of TMA and for the prediction of poor outcome [27]. TMA also can occur in LDLT and, to date, there have been sporadic reports of TMA in LDLT recipients [9,10,30,31]. In a VWF multimer analysis, high molecular weight multimers of VWF were observed to increase in patients with LDLT, while the multimers decreased in patients with severe TMALS [13]. Our findings show that TMALS might be high risk for survival and significant reduction of VWF on day 1 after surgery might play an important role in the onset of TMALS. Furthermore, both markedly low levels of ADAMTS13 and markedly high levels of VWFpp on day 28 after surgery might be related to death. Therefore, the VWFpp/ADAMTS13 ratio was significantly higher in non-survivor in comparison to the VWF/ ADAMTS13 ratio. There are many risk factors such as the antithrombin, protein C, protein S and FVIII levels for thrombosis [32]. Therefore performing examinations in order to identify these thrombophilic factor may be important in patients with TMALS.

Finally, VWF and ADAMTS13 might thus play an important role in the onset of TMALS after LDLT and related to the outcome. The VWFpp may be a more useful marker for the diagnosis of TMALS than VWF.

Conflict of interest statement

The authors have no conflicts of interest to declare in respect to this manuscript.

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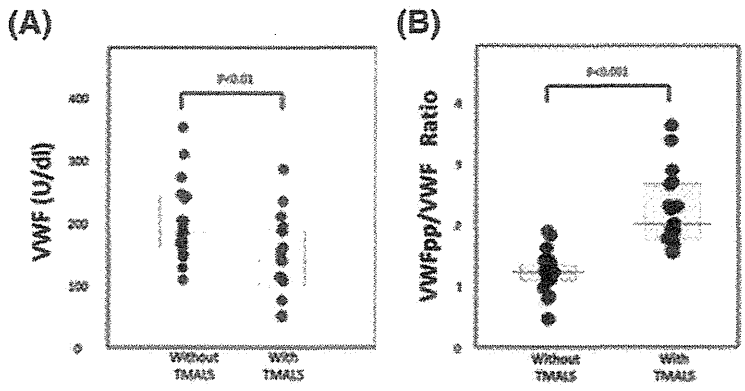

Fig. 3. Plasma levels of VWF and the VWFpp/VWF ratio in the patients with and without TMALS. A) VWF B) the VWFpp/VWF ratio.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

A Prospective Analysis of Disseminated Intravascular Coagulation in Patients with Infections

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Abstract

Objective Disseminated intravascular coagulation (DIC) is often associated with infection and a poor outcome. In this study, useful markers for predicting poor outcomes were examined.

Methods The frequency of DIC and organ failure, outcomes and hemostatic markers were prospectively evaluated in 242 patients with infections.

Results Seventy-seven patients were diagnosed with DIC, 36 of whom recovered from the condition. The rate of DIC or resolution of DIC was highest in the patients with sepsis and lowest in the patients with respiratory infections. Mortality tended to be high in the patients with respiratory infections. The DIC score, sepsis-related organ failure assessment (SOFA) score, prothrombin time (PT) ratio and thrombin-antithrombin complex level were significantly high in the patients who did not recover from DIC. The age, DIC score, SOFA score, PT ratio and levels of thrombomodulin and plasminogen activator inhibitor (PAI)-I were significantly high in the non-survivors. Factors related to a poor outcome included resolution of DIC, the SOFA score, age and the PT ratio. Factors related to resolution of DIC included the SOFA score and age, while factors related to the SOFA score included the levels of PAI-I, leukocytes, fibrinogen, D-dimer and platelets.

Conclusion The outcomes of septic patients primarily depend on the SOFA score and the resolution of DIC, which are related to organ failure.

Key words: DIC, infection, SOFA score, SIRS, outcome

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Introduction

Disseminated intravascular coagulation (DIC) is frequently observed in patients with infections (1, 2). A hypercoagulable state and microvascular dysfunction, including decreased levels of antithrombin (AT) and protein C (PC) and elevated levels of plasminogen activator (PA) inhibitor-I (PAI-I) and thrombomodulin (TM), are frequently present in patients with infectious DIC (3-5). PAI-I inhibits PA, which activates plasminogen to plasmin. Therefore, hypercoagulation without hyperfibrinolysis is usually observed in patients with infections. In addition, bleeding symptoms are rare, whereas organ failure is often observed in patients with septic DIC (6, 7). The outcomes of DIC are poorer in patients with infections than in patients with leukemia (4). The poor outcome of infectious DIC is dependent on organ failure. The degree of organ failure is evaluated according to the

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