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Regular Article

Decreased ADAMTS13 Levels in Patients after Living Donor Liver Transplantation

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ABSTRACT

Introduction: Thrombotic microangiopathy (TMA) is a complication occurring after liver transplantation (LT), and an unusually large multimer (ULM) of Von Willebrand factor (VWF) and ADAMTS13 may play an important role in the onset of TMA during LT.
Material and Methods: Eight-one patients underwent living donor LT (LDLT). Seventeen of those patients had both severe thrombocytopenia and hemolytic anemia with fragmented red cells and were diagnosed as TMA-like syndrome (TMALS).
Results and Conclusions: A significant reduction of ADAMTS13 and an increase of VWF were observed in the patients with TMALS. The ADAMTS13 activity in patients after LDLT was significantly reduced from day 1 to day 21, and it was significantly low in those with TMALS at day 14 and 28. The VWF levels in patients with LDLT were significantly high, and the VWF/ADAMTS13 ratio was significantly increased in patients at 7, 14 and 28 days after LDLT, especially in patients with TMALS at day 14 and 28 after LDLT. High molecular weight multimers of VWF were observed to have increased in patients with LDLT, and the high molecular weight multimers of VWF were further increased in those with mild TMALS but they decreased in those with severe TMA. These findings suggest that ULM- VWF and ADAMTS13 might be associated with the onset of TMA after LT.

Introduction

Thrombotic microangiopathy (TMA) is a microvascular occlusive disorder induced by endothelial damage, primary platelet aggregation and microangiopathic hemolytic anemia (MHA) [1–3]. This complication is a well-recognized disorder that may occur in up to 6% of patients following bone marrow transplantation [4] and shows symptoms similar to thrombotic thrombocytopenic purpura (TTP) [5]. 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% [6–8]. This complication is most prevalent in kidney transplant recipients, but it has also been reported in liver transplant (LT) recipients [9,10].

The possible causative factors of TMA following liver transplantation include calcineurin inhibitors [11,12] and infections [13], including hepatitis C virus [14]. However, the specific pathophysiological mechanism of TMA is still not 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 [15]. ADAMTS13 is a metalloprotease that specifically cleaves the multimeric VWF [16–20]. A severely deficient ADAMTS13 activity (less than 5% of that in normal plasma) is caused by either a mutation of the ADAMTS13 gene [17,21] or by inhibitory antibodies against ADAMTS13 (14-16). UL-VWFM produced in and then quickly released from vascular endothelial cells, has often been found in patient's plasma in familial and nonfamilial TTP [22,23].

Abbreviations: TMA, thrombotic microangiopathy; LT, liver transplantation; VWF, von Willebrand factor; LDLT, living donor liver transplantation; TMALS, thrombotic microangiopathy - like syndrome; MHA, microangiopathic hemolytic anemia; TTP, thrombotic thrombocytopenic purpura; UL-VWFM, unusually large Von Willebrand factor multimer; ULM, unusually large multimer; FRC, fragmented red blood cell; ELISA, enzyme immunosorbent assay; Hb, hemoglobin.

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Table 1
TMALS patients.

Age	Sex	Underlying disease	Day	PLT ($x10^3/\mu l$)	Hb (g/dl)	LDH (g/L)	ADAMTS13 (%)	VWF (%)
66	F	Hepatocellular Carcinoma	14	77	9.4	2002	27.5	578
53	M	Hepatocellular Carcinoma	14	27	7.6	198	1.0	377
54	F	Liver Cirrhosis	6	46	9.1	758	22.5	617
49	M	Liver Cirrhosis	22	44	10.4	1432	16.3	309
54	M	Hepatocellular Carcinoma	15	19	9.7	342	7.5	430
45	F	Primary Biliary Cholangitis	14	33	6.4	6112	1.0	528
51	M	Liver Cirrhosis	7	40	12.4	330	2.5	560
70	F	Hepatocellular Carcinoma	14	81	8.8	409	37.5	526
45	F	Alcoholic Cirrhosis	16	44	9.0	679	18.8	164
60	M	Liver Cirrhosis	14	51	6.9	546	8.8	303
47	F	Primary Biliary Cholangitis	15	64	8.7	596	1.0	561
6	M	Cholestasis	18	62	9.0	641	13.8	321
62	F	Liver Cirrhosis	15	68	4.7	1909	33.8	601
64	F	Primary Biliary Cholangitis	10	24	9.4	458	12.5	550
45	F	Fulminant Hepatitis	18	50	8.3	801	11.3	502
51	F	Liver Cirrhosis	17	43	7.9	589	12.5	241
59	F	Liver Cirrhosis	21	46	9.3	904	18.8	439

TMALS; thrombotic microangiopathy - like syndrome.

VWF is a large glycoprotein which is essential for high-shear stress associated platelet adhesion and aggregation [24]. These UL-VWFMs have been thought to interact with circulating platelets, thus resulting in platelet clumping due to an elevated shear stress [22].

This study measured the ADAMTS13 activity and VWF antigen in the plasma of 81 patients during living donor LT (LDLT) in order to examine the usefulness of a diagnosis of TMA after LT.

Materials and Methods

The ADAMTS13 activity, VWF antigen and fragmented red blood cell (FRC) were measured in 50 healthy volunteers (31 females and 19 males; median age, 31 years; range, 19-51 years) and 81 patients after LDLT (35 females and 46 males; median age, 47 years; range, 0-70 years) from January 1, 2002 to December 31, 2005. 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 TMA-like syndrome (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.

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.

Human plasma was obtained by centrifugation at 3000×g at 4 for 15 min from whole blood that was treated with a 1/10 volume of 3.8% sodium citrate as an anti-coagulant. All plasma specimens were stored at -80 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* [25]. VWF was measured using an IMUBIND vWF ELISA (ADI, CT, USA) using an enzyme immunosorbent assay (ELISA). Plasma UL-VWFM was evaluated by SDS- 0.9% agarose gel electrophoresis followed by Western blotting with luminographic detection [26,27]. The hemoglobin (Hb) levels and platelet counts were measured by automated the fully automated hematology analyzer XE-2100 (Sysmex, Kobe, Japan).

Statistical analysis

The data are expressed as the median (25%tile - 75%tile). The differences between the groups were examined for statistical significance using Mann- Whitney's U test. A P value of less than 0.05 was considered to indicate the presence of a statistically significant difference.

Results

Seventeen patients were diagnosed to have TMALS after LDLT; 4 hepatic carcinoma (16.7%), 7 liver cirrhosis due to viral infection (35%), 3 primary biliary cirrhosis (27.3%), 1 fulminant hepatitis and 1 alcoholic cirrhosis, and 1 cholestasis (16.6%; Table 1 and 2). The mortality at 30 days and 90 days was significantly higher in TMALS (17.5% and 41.2%) than in the patients without TMALS (0% and 5.2%). In the laboratory data of patients with TMA, the platelet count and hemoglobin (Hb) levels were reduced and the LDH (lactate dehydrogenase) and VWF antigen levels were increased. In the plasma levels of ADAMTS13 activity in healthy volunteers, the median value was 108.0% (25% tile - 75% tile; 68.0% - 148.0%). A significant reduction of ADAMTS13 (median; 25% tile – 75% tile) was observed in the patients with TMALS (12.5%; 6.3 -19.7%) in comparison to the healthy volunteers ($p<0.01$).

The laboratory data during LT is shown in Table 3. The platelet count was significantly ($p<0.01$) increased in patients without TMA in day 7 and 14 in comparison to day 0, but was not increased in those with TMALS from day 0 to 28 days. Therefore, the platelet count was significantly lower in patients with TMALS than in those without TMALS (day 0, 1 and 7; $p<0.05$, day 14 and 28; $p<0.01$; Fig. 1). The level of Hb significantly increased in the patients without TMALS on day 1 ($p<0.05$) and on day 7, 14 and 28 ($p<0.01$, respectively) in comparison to day 0, but it was not increased in those with TMALS from 0 day to 28 days. The level of Hb was significantly lower in the patients with TMALS than in those without TMALS on day 14 ($p<0.01$) and on day 28 ($p<0.05$; Fig. 2). The level of T-Bil significantly increased in the patients with TMALS on days 14 and 28 ($p<0.01$) in comparison to day 0 and the level of T-Bil was significantly higher in the patients with TMALS than in those without TMALS ($p<0.01$) on days 7, 14 and 28.

The level of LDH was significantly increased in patients without TMALS at day1 and 7 ($p<0.01$) and day 14 ($p<0.05$), and in patients with TMALS at day 7 and 14 ($p<0.05$) and day 28 ($p<0.01$) in comparison to day 0. The LDH level was significantly higher in patients with TMALS than in those without TMALS on days 14 ($p<0.05$) and 28 ($p<0.01$). There was no significant difference in the creatinine levels on days 0, 1, 7, 14 and 28 in the patients with and without TMALS. No significant difference was observed in the ADAMTS13 levels before the operation among the patients with various underlying diseases (Table 2). The ADAMTS13 activity in patients with TMALS and without TMALS significantly decreased from day 1 to day 28 ($p<0.01$, except on day 28 in those without TMALS; $p<0.05$) in comparison to day 0, and

Table 2
Frequency of TMA and ADAMTS13 activity in various underlying diseases.

	Frequency of TMA	ADAMTS13 activity
hepatic carcinoma	4(16.7%)	96.3% (55.3% - 120.3%)
liver cirrhosis due to viral infection	7(35.0%)	71.3% (53.8% - 98.8%)
Primary biliary cirrhosis	3(27.3%)	52.5% (37.5% - 72.5%)
cholestasis	1(16.6%)	53.8% (31.6% - 100.0%)
biliary atresia	0(0%)	57.5% (50.0% - 68.8%)
Others	2(20.0%)	41.3% (23.4% - 58.8%)

ADAMTS13 activity was shown as median (25%tile -75%tile).

Table 3
Laboratory data during liver transplantation.

		Day 0	Day1	Day7	Day 14	Day 28
PLT ($x10^3/\mu l$)	TMALS	44 (32 - 60)	50 (44 - 67)	58 (41 - 85)	52 (42 - 86)	71 (55 - 122)
	p	p<0.05	p<0.05	p<0.05	p<0.01	p<0.01
	Without TMALS	56 (46 - 77)	69 (52 - 86)	91 (61 - 120)**	180 (121 - 270)**	147 (108 - 226)**
Hb (g/dl)	TMALS	8.6 (7.9 - 9.5)	9.5 (8.6 - 10.7)	9.1 (8.8 - 10.5)	8.3 (7.0 - 9.1)	8.6 (7.0 - 9.8)
	P	NS	NS	NS	p<0.01	p<0.05
	Without TMALS	8.7 (7.8 - 10.2)	9.5 (9.0 - 10.7) *	10.2 (9.4 - 10.8)**	9.9 (8.7 - 10.8)**	9.5 (9.1 - 10.5)**
T-Bil (mg/dl)	TMALS	4.2 (1.8 - 8.2)	4.6 (2.6 - 6.9)	7.6 (4.0 - 9.8)	7.7 (4.6 - 18.0)**	17.8 (6.8 - 22.9)**
	P	NS	NS	p<0.01	p<0.01	p<0.01
	Without TMALS	3.9 (1.7 - 7.0)	4.4 (2.7 - 7.3)	2.8 (1.7 - 6.2)	1.7 (0.9 - 3.9)	1.0 (0.6 - 2.0)
LDH (IU/L)	TMALS	243 (195 – 415)	377 (232 - 505)	368 (264 - 522)*	409 (269 - 1094)*	396 (329 - 544)**
	P	NS	NS	NS	p<0.05	p<0.01
	Without TMALS	219 (191 – 300)	324 (276 - 437)**	326 (260 - 387)**	283 (221 - 347)*	225 (199 - 331)
Creatinine (mg/dl)	TMALS	1.0 (0.7 - 2.2)	1.0 (0.8 - 1.8)	1.1 (0.8 - 1.6)	1.0 (0.9 - 1.6)	1.7 (0.8 - 1.7)
	P	NS	p<0.05	NS	NS	NS
	Without TMALS	0.8 (0.7 - 1.0)	0.9 (0.6 - 1.0)*	0.8 (0.6 - 1.2)	0.9 (0.6 - 1.2)	1.0 (0.7 - 1.5)
ADAMTS13 (%)	TMALS	74.5 (40.0-96.0)	29.0 (14.0-38.0)**	29.0 (18.8-45.0)**	12.5 (4.5-27.0)**	15.5 (5.0-25.0)**
	P	NS	NS	NS	p<0.01	p<0.01
	Without TMALS	61.0(40.8 – 102)	26.5(20.5 - 38.0)**	36.0 (24.5 - 45.0)**	41.0 (23.0 - 51.0)**	49.5 (30.0 – 65.0)*
VWF (%)	TMALS	360 (222 – 432)	225 (178 – 374)	528 (386 - 623)*	431 (306 - 560)	433 (330 - 583)
	P	NS	NS	NS	P<0.01	NS
	Without TMALS	414 (193 – 568)	274 (133 - 434)**	522 (398 - 643)**	532 (388 – 632)**	459 (354 - 594)
ADAMTS13/ VWF	TMALS	4.6 (2.7 - 12.1)	8.0 (5.0 - 15.0)	19.9 (10.1 - 41.9)**	22.2 (14.6 - 51.0)**	40.1 (14.6 - 78.9)**
		NS	NS	NS	P<0.05	P<0.01
	Without TMALS	5.1(2.7 - 11.3)	8.0(5.3 - 13.0)*	14.8(10.2 - 22.4)**	14.0(9.6 - 20.9)**	8.7(5.4 – 16.9)**

NS; not significant, P<0.05 and p<0.01 show difference between patients with TMALS and those without, *; p<0.05 in comparison to day 0, **; p<0.01 in comparison to day 0. Data shows median (25%tile - 75%tile).

it was significantly lower in patients with TMALS than in those without TMALS on days 14 and 28 ($p<0.01$; Fig. 3).

The VWF antigen levels in patients with LT were significantly higher before the operation in comparison to healthy volunteers and they significantly decreased at 1 day in those without TMALS ($p<0.01$), but they thereafter significantly increased again at day 7 and 14 in those without TMALS ($p<0.01$) and at day 7 in those with TMALS ($p<0.05$) in comparison to day 0. The VWF antigen levels were significantly higher in those without TMALS than those with TMALS at day 14 ($p<0.01$; Fig. 4). The ratio of VWF/ADAMTS13 significantly increased in both groups at 7, 14, and 28 days ($p<0.01$) after the operation, and those ratios were also significantly higher in patients with TMALS than in those without TMALS at day 14 ($p<0.05$) and 28 ($p<0.01$; Fig. 5).

In the VWF multimer analysis, the high molecular weight multimers of VWF increased in the patients without TMALS at 7 and 14 days after the LDLT, and the high molecular weight multimers of VWF, furthermore, increased in the patients with mild TMALS, while the multimers decreased in the patients with severe TMALS (Fig. 6).

Discussion

In this study, 17 patients (21.0%) were diagnosed to have TMALS after LDLT. The most common underlying disease associated with TMALS was liver cirrhosis due to viral infection, but there was no significant difference in the frequency of TMA among various underlying diseases. The ratio of complications with TMALS was markedly high in comparison to that of TMA in the previous reports [6–8], because, almost all of the current patients had severe hepatic dysfunction before the LDLT and the transplanted liver was not full-sized in LDLT. It follows that the improvement in the liver function requires more time and that complications frequently occur. FRC and reticulated platelets [28] are routinely examined by an automated hematology analyzer XE-2100, thus suggesting that a highly sensitive diagnosis for TMALS could be carried out in this study. A significant reduction of ADAMTS13 activity and increase of VWF antigen were observed in these patients with TMALS, 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,

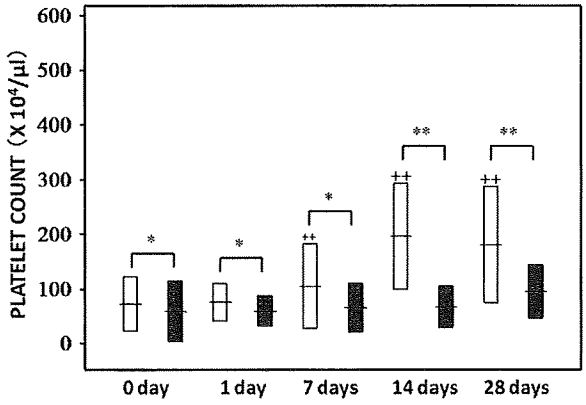

Fig. 1. Platelet counts during LDLT. White bars; LDLT patients without TMALS, Close bars; LDLT patients with TMALS. *; p<0.05, **; p<0.01. ++; p<0.05 in comparison to day 0.

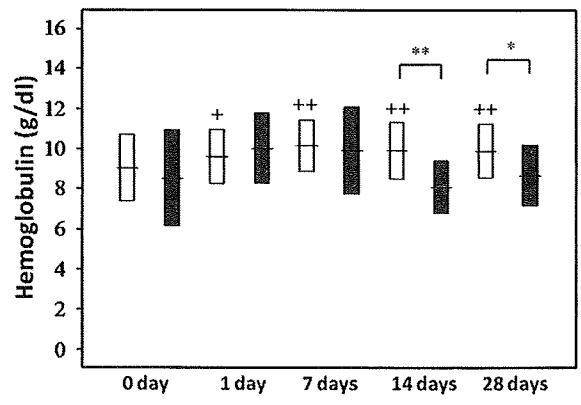

Fig. 2. Hemoglobin levels during LDLT. White bars; LDLT patients without TMALS, Close bars; LDLT patients with TMALS. *; p<0.05, **; p<0.01. +; p<0.05 in comparison to day 0. ++; p<0.05 in comparison to day 0.

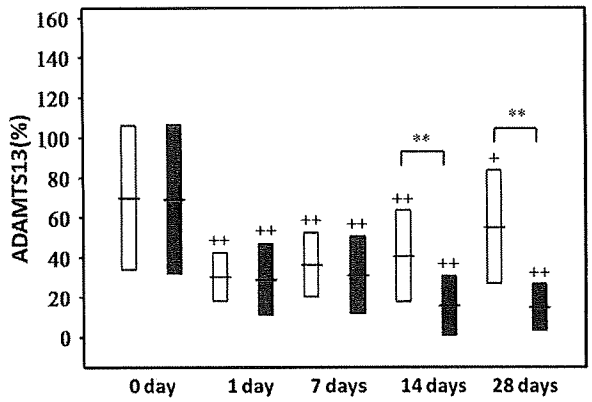

Fig. 3. ADAMTS13 activity during LDLT. White bars; LDLT patients without TMALS, Close bars; LDLT patients with TMALS. **; $p<0.01$. +; $p<0.05$ in comparison to day 0. ++; $p<0.05$ in comparison to day 0.

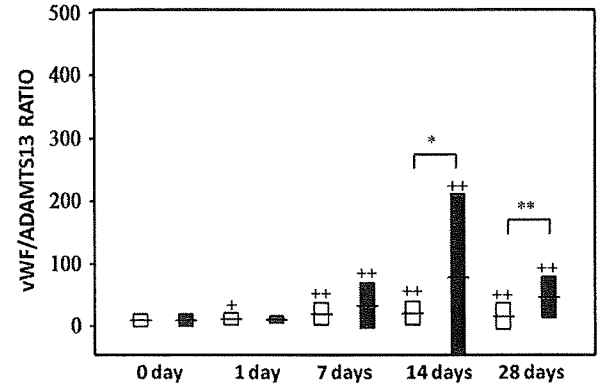

Fig. 5. VWF/ADAMTS13 Ratio during LDLT. White bars; LDLT patients without TMALS, Close bars; LDLT patients with TMALS. *; $p<0.05$, **; $p<0.01$. +; $p<0.05$ in comparison to day 0. ++; $p<0.05$ in comparison to day 0.

renal failure, and fever [5,29]. UL-VWFM, and significant reduce of ADAMTS13 have often been found in patients' plasma in familial and non-familial TTP [22,23]. The ADAMTS13 levels were affected by the production from the liver and consumption or reduction of ADAMTS13 might occur during LT. The VWF levels decrease in persons with blood type "O" [30]. The activity of ADAMTS13 was also low in the patients undergoing hematopoietic stem cell transplantation. A decreased activity has been reported in patients with hepatic veno-occlusive disease (VOD) after stem cell transplantation [31]. These findings suggest that a reduced amount ADAMTS13 may therefore be a risk factor for the onset of VOD or TMA after LDLT.

The platelet counts and Hb levels in the patients with TMALS did not significantly increase on days 7 and 14 in comparison to those without TMALS, and they were also significantly lower in the patients with TMA than in those without TMALS. The levels of LDH and T-Bil significantly increased in the patients with TMALS on day 14 and those were significantly higher in patients with TMALS than in those without TMALS. Indeed, the onset of TMALS frequently occurred on about day 14. The ratio of VWF/ADAMTS13 significantly increased at 7, 14 and 28 days after the LDLT, especially in the patients with TMALS. The plasma ADAMTS13 levels before the operation were not related to the onset of TMA, thus suggesting that the reduction of ADAMTS13 depends on the LDLT. The ratio of VWF/ ADAMTS13 was closely related to the laboratory data, such as the platelet count, Hb, LDH and T-Bil, thus suggesting that decreased ADAMTS13 and UL-VWFM might be related to the onset of TMA after LDLT.

TMA occurs more commonly in cadaveric transplants, thus suggesting that prolonged cold ischemia time and reperfusion injury may cause endothelial injury [32,33]. However, TMA also can occur in LDLT and, to date, there have been sporadic reports of TMA in LDLT recipients [9,10,34,35]. In a VWF multimer analysis, the high molecular weight multimers of VWF were observed to increase in patients without TMALS on days 7 and 14 after the LDLT, and the high molecular weight multimers of VWF, which might be called UL-VWFM, also increased in patients with TMALS while the multimers decreased in patients with severe TMALS. These findings correlate with those from a previous report [15] and suggest that UL-VWFM may cause the consumption of platelets and vascular endothelial cell injuries. These pathophysiological states are similar to those in TTP, thus suggesting that UL-VWFM and decreased ADAMTS13 might thus be associated with the onset of TMA during LT.

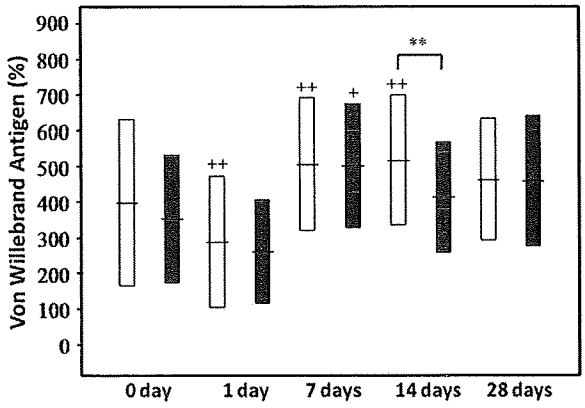

Fig. 4. VWF antigens during LDLT. White bars; LDLT patients without TMALS, Close bars; LDLT patients with TMALS. **; $p<0.01$. +; $p<0.05$ in comparison to day 0. ++; $p<0.05$ in comparison to day 0.

Conflict of interest statement

All authors disclose no financial and personal relationship with other people or organizations that could inappropriately influence their work.

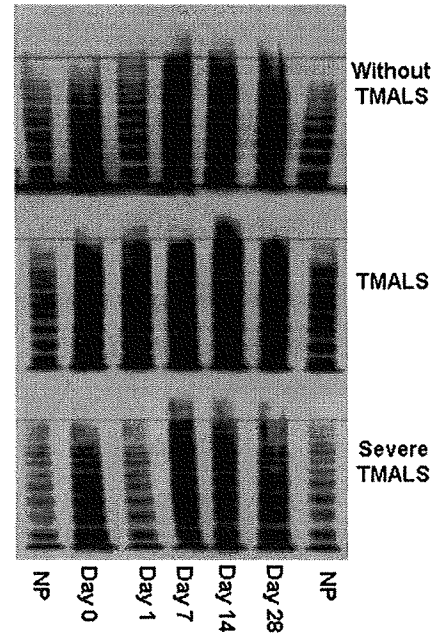

Fig. 6. VWF multimer analysis in mild or severe patients with TMALS, and patients without TMA after LDLT. Plasma VWFM was evaluated by SDS- 0.9% agarose gel electrophoresis followed by Western blotting with luminographic detection. NP; normal plasma. The multimers over the upper line are suggested to be UL-VWFM.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Registry of 919 Patients with Thrombotic Microangiopathies across Japan: Database of Nara Medical University during 1998-2008

Yoshihiro Fujimura and Masanori Matsumoto

Abstract

Background Thrombotic microangiopathies (TMAs) are pathological conditions characterized by generalized microvascular occlusion by platelet thrombi, thrombocytopenia, and microangiopathic hemolytic anemia. Two typical phenotypes of TMAs are hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP). Severe deficiency of plasma ADAMTS13 activity (ADAMTS13: AC) is more specific for TTP, but not for HUS. Since 1998, our laboratory has functioned as a nationwide referral center for TMAs by analyzing ADAMTS13.

Methods Of 1,564 patients tested from 426 hospitals, 919 were positive for TMA. Levels of ADAMTS13: AC and the ADAMTS13 neutralizing autoantibody (ADAMTS13: INH) were determined by chromogenic act-ELISA and/or by classic von Willebrand factor multimer assay.

Results TMA patients consisted of two groups: severe (less than 3% of normal control) and non-severe deficiency of ADAMTS13: AC. Both groups were divided into congenital (n=65) and acquired (n=854) TMA. Of the former, 41 had congenital deficiency of ADAMTS13: AC, while the remaining 24 had disease of unknown etiology. The 854 patients with acquired TMA could be largely grouped into three categories: idiopathic TTP (n=284), idiopathic HUS (n=106), and secondary TMAs (n=464). The secondary TMAs were observed in heterogeneous patient groups and were associated with drugs, connective tissue diseases, malignancies, transplantation, pregnancy, *E. coli* O157: H7 infection, and other factors. All of the patients with acquired severe ADAMTS13: AC deficiency were positive for ADAMTS13: INH.

Conclusion Although TMAs are highly heterogeneous pathological conditions, one-third of TMA patients have severe deficiency of ADAMTS13: AC. Platelet transfusions to such patients are contraindicated. Rapid ADAMTS13: AC assays are therefore prerequisite to appropriately treat TMA patients.

Key words: TMA, TTP, HUS, USS, ADAMTS13, VWF

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Introduction

Thrombotic microangiopathies (TMAs) are pathological conditions that are characterized by microangiopathic hemolytic anemia, vast microvascular occlusions caused by platelet thrombi (common renal involvement), and thrombocytopenia (1). Two typical phenotypes of TMAs are hemolytic-uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP), both of which are life-threatening diseases. HUS is characterized by the aforementioned three clinical signs (classic 'triad'), while TTP is characterized by a classic 'pentad,' which includes the 'triad' as well as fever and neurological signs; however, the two diseases are often indistinguishable. Further, these TMAs must be differentiated from disseminated intravascular coagulation (DIC) or consumptive thrombohemorrhagic disorders (2).

In 1996, a metalloprotease that specifically cleaves von Willebrand factor (VWF) was identified in normal plasma (3, 4), and 5 years later this enzyme was purified,

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cloned, and termed ADAMTS13 (a disintegrin-like metalloproteinase with thrombospondin type 1 motifs 13) (5-8). ADAMTS13-producing cells were initially identified within the liver, and then more specifically as hepatic stellate cells (9), but now it is known that ADAMTS13 is also present in platelets (10), vascular endothelial cells (11), and kidney podocytes (12). Since the discovery of ADAMTS13, severe deficiency of ADAMTS13 activity (ADAMTS13: AC) has been thought to be a unique feature of TTP, and can be caused by genetic mutations or by acquired autoantibody (ADAMTS13: INH) to this enzyme; however, these alterations are not observed in HUS patients (13, 14). It is notable that a minor population of TTP patients with the 'pentad' of symptoms has almost normal or only slightly reduced ADAMTS13: AC (15). In this regard, Tandon et al (16) reported in 1994 that approximately 80% of the patients with acquired TTP had anautoantibody against CD36. In those days, however, this finding could not be directly linked to the pathogenesis of TTP. Recently, Davis et al (17) have shown that recombinant (r)-human ADAMTS13 specifically binds to r-human CD36 in vitro. CD36 is expressed in endothelial cells, platelets, and monocytes, and has been reported to bind thrombospondin-1 (18). ADAMTS13, after secretion into the circulation, is assumed to efficiently cleave unusually large VWF multimers (UL-VWFMs) released from vascular endothelial cells as a solid-phase enzyme by binding to the cell surface. It is currently unclear whether anti-CD36 autoantibodies block ADAMTS13 binding to vascular endothelial cells, but if so, this may interfere with the efficient cleavage of UL-VWFMs by ADAMTS13 and result in TTP.

In contrast to TTP, HUS is rarely induced by genetic mutations in complement regulatory factors (factors B, H, and I, and membrane cofactor protein or CD46). HUS can also be acquired, typically following acute enterocolitis due to shigatoxin-producing *Echerichia coli* O157: H7 infection, but also rarely due to autoantibody against factor H (19).

Since 1998, our laboratory at Nara Medical University has functioned as a nation-wide referral center for TMAs via assaying ADAMTS13: AC in a large Japan-wide patient population with thrombocytopenia suspected of being TMA. As of the end of 2008, we have established a registry of 919 patients with TMAs, and have analyzed their clinical and laboratory information. Here, we describe the results of this study, and discuss the divergence of TMAs among patient groups with masked or unmasked thrombocytopenia.

Materials and Methods

Patients

Between July 1998 and December 2008, plasma samples from 1,564 patients with thrombocytopenia suspected of TMAs were referred to our laboratory with clinical and laboratory information from 426 medical institutions across Japan. All subjects provided informed consent to participate in this study. The study protocol was approved by the Ethics Committee of Nara Medical University Hospital.

Blood sampling

Before therapeutic approaches including plasma infusion, plasma exchange, and the use of immunosuppressants, whole blood samples (~5 mL) were taken from each patient into plastic tubes containing 1/10 volume of 3.8% sodium citrate. The plasma was separated by centrifugation at 3,000 × g for 15 min at 4℃, kept in aliquots at -80℃ until testing, and sent to our laboratory.

Assays of plasma ADAMTS13: AC and ADAMTS13: INH

Until March 2005, ADAMTS13: AC was determined by classic VWFM assay (3) with a detection limit of 3% of the normal control (20). Thereafter, a chromogenic ADAMTS 13-act-ELISA with a detection limit of 0.5% of the normal control was developed (21), and replaced the VWFM assay. Measurement of plasma levels of ADAMTS13: AC by these assays were highly correlated (R^2=0.72, $p<0.01$) and provided similar results for mean ± SD in healthy individuals (102.4±23.0% vs. 99.1±21.5%), as shown previously (21, 22). Thus, we re-examined the plasma of 724 of the 774 TMA patients determined by the VWFM assay by act-ELISA, and the latter data were used in this study. For 50 TMA patients we were unable to re-examine by act-ELISA, the VWFM assay data were used. We have therefore tentatively categorized plasma levels of ADAMTS13: AC of <3%, 3%-<25%, and 25%-<50% of the normal as severe, moderate, and mild deficiency, respectively.

Plasma ADAMTS13: INH titers were also evaluated either by classic VWFM assay or chromogenic ADAMTS13-act-ELISA using heat-inactivated plasma at 56℃ for 30 minutes (13, 14). One Bethesda unit (U) is defined as the amount necessary to reduce ADAMTS13: AC to 50% of control levels (23). Titers greater than 0.5 Bethesda U/mL were classified as inhibitor positive.

Diagnostic criteria for TMAs

According to previous reports (2, 24, 25), TMAs were defined as having all of the following: (i) microangiopathic hemolytic anemia (hemoglobin ≤12 g/dL), Coombs test negative, undetectable serum haptoglobin (<10 mg/dL), more than 2 fragmented red cells (schistocytes) in a microscopic field with a magnification of 100, and concurrent increased serum lactate dehydrogenase (LDH) above institutional baseline; (ii) thrombocytopenia (platelet count ≤100× 10^9/L); and (iii) a variable severity of organ dysfunction (renal or neurological involvement) devoid of the stigmata of DIC (26).

A differential diagnosis of HUS or TTP based on routine laboratory data is usually difficult. As a rule, plasma levels of ADAMTS13: AC were first determined on all patients suspected of TMAs, and patients with severe deficiency of ADAMTS13: AC were classified as TTP regardless of clini-

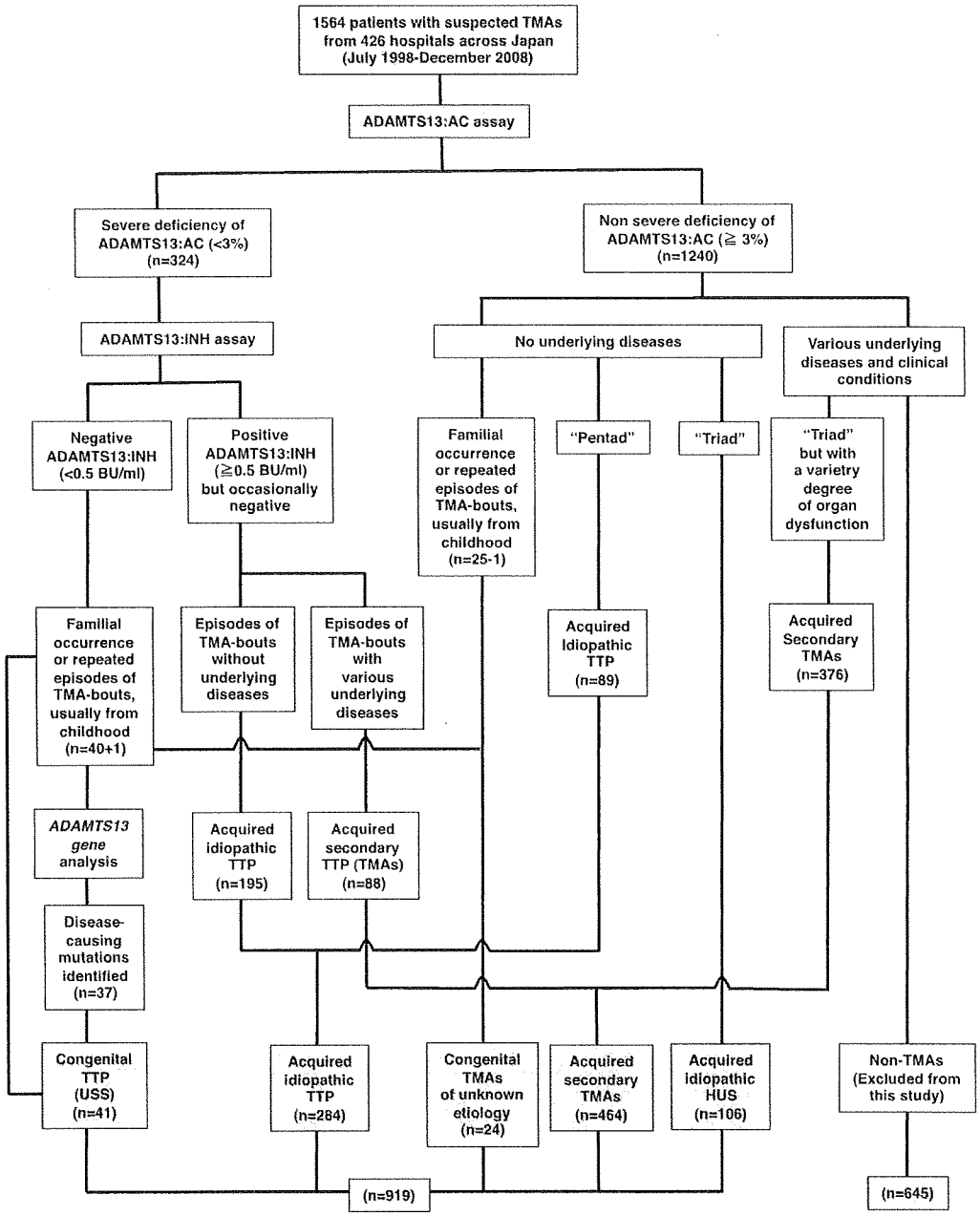

Figure 1. Flow chart of categorization of patients with suspected thrombotic microangiopathies (TMAs) based on ADAMTS13 analysis. Of 1,564 patients with suspected TMAs, 324 had severe deficiency of ADAMTS13 activity and 1,240 did not. In the former category, 40 patients were categorized as USS and 284 as acquired TTP. In the latter category, 24 patients were categorized as congenital TMAs of the unknown etiology, 570 as acquired TMAs, and one patient as USS with moderately reduced plasma ADAMTS13:AC (3.4%), to whom frequent plasma infusions had been made to prevent further aggravation of cerebral infarction. The remaining 645 patients did not have TMAs and were therefore excluded from this study.

cal signs. Second, the patients were grouped as HUS or TTP based on the 'triad' or 'pentad' of clinical signs. This protocol appeared to be important, because our registry includes patients with hereditary deficiency of ADAMTS13: AC or congenital TTP (Upshaw-Schulman syndrome, USS), which generally have less severe clinical signs (often isolated thrombocytopenia) than acquired TTP.

Results and Discussion

A flow chart of patient categorization based on ADAMTS 13 analysis is shown in Fig. 1. Of the 1,564 patients referred to our laboratory, 324 (minor population) had severe deficiency of ADAMTS13: AC and 1,240 (major population) did not. In the population with severe ADAMTS13: AC de-

Table 1. Plasma Levels of ADAMTS13: AC and ADAMTS13: INH in 919 Patients with Thrombotic Microangiopathies (TMAs) Registered at Nara Medical University during July 1988- December 2008

	Congenital TMAs		Acquired TMAs											Total
			Idiopathic		Secondary									
					Drug-induced									
	Upshaw-Schulman syndrome (USS) (n=41)	Unknown etiology (n=24)	Thrombotic thrombocytopenic purpura (TTP) (n=284)	Hemolytic-uremic syndrome (HUS) (n=105)	Ticlopidine (n=22)/ Clopidogrel (n=1)	Mitomycin C (n=10)	Pegylated-interferon (n=1) / Sildenafil (n=1)	Conective tissue diseases and their allied diseases (CTDs/ADs) (n=221)	Malignancies (n=61)	Hematopoietic stem cell transplant-ation (HSCT) (n=54)	Pregnancy (n=15)	E. coli O157: H7 infection (n=32)	Others (Liver cirrhosis, etc) (n=46)	(n=919)
ADAMTS13:AC (%)	(n=41)	(n=24)	(n=284)	(n=105)	(n=22/n=1)	(n=10)	(n=1/n=1)	(n=221)	(n=61)	(n=54)	(n=15)	(n=32)	(n=46)	(n=919)
<3	40	0	195	0	19	0	2	46	5	0	4	0	13	324
3 ~ <25	1	4	72	20	2	2	0	66	23	18	4	5	16	233
25 ~ <50	0	9	14	48	1	5	0	66	22	24	4	17	6	216
≧50	0	11	3	38	1	3	0	43	11	12	3	10	11	146
ADAMTS13:INH (U/ml)	(n=41)	(n=23)	(n=282)	(n=43)	(n=22/n=1)	(n=7)	(n=1/n=1)	(n=187)	(n=26)	(n=15)	(n=8)	(n=17)	(n=23)	(n=697)
≧2	0	0	120	0	15	0	0	28	5	0	3	0	9	180
0.5 ~ <2	0	0	129	2	6	0	2	80	8	4	2	1	8	242
<0.5	41	23	33	41	2	7	0	79	13	11	3	16	6	275

( ) Sample number determined

ficiency, 40 patients were categorized as USS and 284 as acquired TTP, and no patients with DIC or septic DIC were included. In the population without severe ADAMTS13: AC deficiency, 24 patients were categorized as congenital TMAs of unknown etiology, 570 as acquired TMAs, and only one patient (GG in Table 2) as USS with moderately reduced plasma ADAMTS13: AC (3.4%), to whom frequent plasma infusions had been made to prevent further aggravation of cerebral infarction. Thus, a diagnosis of USS in this patient GG was made after identifying the disease-causing mutations (C1024R/C1024R) in exon 24 by *ADAMTS13* gene analysis. These data will be published elsewhere in detail. The remaining 645 patients did not have TMAs, and were therefore excluded from this study; this group included 64 patients with DIC or septic DIC.

Congenital TMAs

Patients with repeated TMA episodes usually starting in early childhood with or without familial occurrence are usually considered as congenital TMAs; these patients are largely separated into the following two categories, on the basis of plasma levels of ADAMTS13: AC and ADAMTS 13: INH.

1. Upshaw-Schulman syndrome (USS)

USS is alternatively termed congenital TTP and is characterized by severe deficiency of ADAMTS13: AC due to genetic mutations (27). Forty-one patients (25 females and 16 males) belonging to 36 different families, were placed in this category (Table 2). All of these patients were negative for ADAMTS13: INH. USS is inherited in an autosomal recessive fashion, and therefore, the parents of patients are asymptomatic carriers with significantly reduced plasma levels of ADAMTS13: AC. The female-to-male ratio in the USS patient population is theoretically one-to-one, but our results indicate an apparent female predominance (25 to 16). Of the 41 patients, 17 (41%) had a history of exchange blood transfusions during the newborn period, and 32 (78%) had a history of thrombocytopenia during childhood. For the remaining 9 (22%), it was unclear whether their platelet counts had been checked during that period.

ADAMTS13 gene analysis was performed for 38 USS patients, and the disease-causing mutations were identified in 37 of the 38. Of the 37 genotyped patients, 8 were homozygotes and 29 were compound heterozygotes [one *de novo* mutation (28)] for *ADAMTS13* gene mutations. Of the 8 homozygous patients, the parents of 6 had consanguineous marriages.

2. Congenital TMAs of unknown etiology

Patients in this category were characterized by repeated TMA episodes with predominant renal involvement from early childhood, and often with familial occurrence. Twenty-four patients belonging to 12 families were identified, but the etiology of TMAs in these patients remained completely unclear.

In this regard, it is well known that gene mutations in complement regulatory cofactors (factor H, factor I, factor B, and CD46 or membrane cofactor protein) cause excessive complement activation by impairing C3b inactivation, resulting in severe hemolysis, which triggers TMA episodes. Therefore, these patients are commonly termed 'congenital atypical HUS' (19). It is possible that among the patients of this category in this study, some disease might be related to gene mutations of complement regulatory cofactors, but at the time such analysis had not been done in Japan. As a first step toward such analysis, we determined the plasma levels of factor H antigen by immunoassay in our patients, and did not observe reduced levels in any patients (data not shown).

Table 2. Registration of 41 Japanese Patient with Upshaw-Schulman Syndrome (USS)

No	Patient	Year of birth	Sex	Exchange blood transfusion during newborn period	Thrombocytopenia during childdhood	Plasma ADAMTS13:AC (%)	*ADAMTS13 gene* mutations
1	A	1999	M	+	+	< 0.5	C-Hetero
2	B	1986	F	+	+	< 0.5	Homo
3	C	1972	M	-	+	< 0.5	Homo
4	D	1978	F	+	+	< 0.5	C-Hetero
5	E	1985	M	+	+	< 0.5	C-Hetero
6	F	1993	M	+	+	0.6	C-Hetero
7	G	1987	F	+	+	< 0.5	C-Hetero
8	H	1951	M	-	-	0.6	C-Hetero
9	I	1972	M	-	+	< 0.5	C-Hetero
10	J-3	1977	F	-	+	< 0.5	C-Hetero
11	J-4	1979	M	-	+	< 0.5	C-Hetero
12	K-3	1976	F	-	+	< 0.5	C-Hetero
13	K-4	1978	F	+	+	< 0.5	C-Hetero
14	L-2	1967	F	-	-	< 0.5	C-Hetero
15	L-3	1972	F	-	+	< 0.5	C-Hetero
16	M-3	1969	F	-	-	< 0.5	C-Hetero
17	M-4	1971	F	-	-	< 0.5	C-Hetero
18	N	1986	F	+	+	< 0.5	C-Hetero
19	O-4	1958	F	-	-	< 0.5	C-Hetero
20	P	1971	M	-	+	< 0.5	C-Hetero
21	Q (1)	1983	M	+	+	< 0.5	C-Hetero
22	Q (2)	1988	M	+	+	< 0.5	C-Hetero
23	R-5	1982	F	-	+	< 0.5	C-Hetero
24	S	1982	F	-	+	0.9	※
25	T	1981	F	-	+	< 0.5	C-Hetero
26	U	1990	F	+	+	< 0.5	Homo
27	V	1983	F	+	+	< 0.5	C-Hetero
28	W-4	1990	F	-	+	< 0.5	C-Hetero
29	X-5	1963	F	-	-	< 0.5	※
30	Y	1960	F	-	+	< 0.5	C-Hetero
31	Z-3	1971	F	-	+	< 0.5	Homo
32	AA	1987	F	-	-	< 0.5	※
33	BB	1947	M	-	-	< 0.5	Homo
34	CC-5	2004	M	+	+	< 0.5	C-Hetero
35	DD	2007	F	-	+	< 0.5	C-Hetero
36	EE	2003	M	+	+	< 0.5	Homo
37	FF	1991	F	+	+	< 0.5	Homo
38	GG	1931	M	-	-	3.4	Homo
39	HH	2004	F	+	+	< 0.5	C-Hetero
40	II	1977	F	+	+	< 0.5	※
41	JJ	1977	M	-	+	< 0.5	C-Hetero

C-Hetero: Compound heterozygotes, Homo: Homozygotes, ※: Not determined.

Acquired TMAs

Patients with acquired TMAs are characterized by the following: 1) usually no familial occurrence, 2) presence or absence of underlying diseases or medications associated with TMAs, and 3) common sudden onset of TMA episodes during adulthood. Patients with acquired TMAs are grouped as primary (idiopathic) or secondary, and then further separated into categories as follows, based on the results of ADAMTS 13: AC and ADAMTS13: INH assays.

1. Idiopathic TMAs

The patients in this group lack apparent underlying diseases or medications related to TMA episodes. Idiopathic TMAs can be further categorized into TTP and HUS subgroups. Idiopathic TTP (n=284) included two patient populations: 1) patients (n=195) with severe deficiency of ADAMTS13: AC, commonly positive for ADAMTS13: INH, and 2) patients (n=89) with clinical 'pentad' signs, regardless of plasma ADAMTS13: AC levels. Distribution of plasma ADAMTS13: AC is shown in Table 1. Detailed analysis of the clinical and laboratory features of these patients will be published elsewhere.

In contrast, idiopathic HUS (n=106) consisted of one patient population with clinical 'triad' signs, without severe deficiency of ADAMTS13: AC. Two patients of this category exhibited low levels of ADAMTS13: INH (0.5-<2 BU/mL).

2. Secondary TMAs

Secondary TMAs develop in the setting of various clinical conditions, such as infection, medication, and various underlying diseases. For instance, acquired TMAs are often associated with connective tissue diseases, and also treatment using several specific drugs. In these patients, clinical signs are often highly variable, so diagnostic differentiation of TTP or HUS appears to be insignificant.

(1) Drug-induced TMAs

A significant number of drugs have been associated with TMAs, including anti-platelet thienopyridine derivative drugs, antineoplastic drugs such as mitomycin C, and quinine (29). We have no experience with quinine-associated TMAs, but observed two suspected drug-associated TMAs:

one with sildenafil (Viagra) and the other with pegylated-interferon. Thus, drug-induced TMAs will be discussed in the following 3 subgroups.

a) Thienopyridine derivative-induced TMAs

Ticlopidine (TC) and clopidogrel (CL) are two typical thienopyridine derivatives (30). We identified 22 patients with TC-induced TMAs and one with CL-induced TMA. Nineteen of the 22 patients with TC-TMAs (86%) had severe ADAMTS13: AC deficiency and were positive for ADAMTS13: INH. The mechanism by which TC induces TMAs is still unclear, but it is speculated that TC becomes active in circulation and binds to ADAMTS13, forming a hapten-carrier complex. Antibodies formed against such a complex may be specific for the hapten, the combination hapten-carrier site, or the carrier alone, in a similar fashion to alpha-methyldopa, which may cause the development of anti-red cell antibodies. In approximately 90% of patients with TC-induced TMAs, the onset of TMA episodes occurred within 40 days of treatment (30). The frequency of TC-induced TMAs is estimated to be one per 1,600 to 5,000 patients. In contrast, only one female patient with CL-induced TMA, who developed TMA episodes 4 days after treatment, has been reported in Japan (31). This patient had slightly reduced plasma ADAMTS13: AC (34%), and was negative for ADAMTS13: INH. The pathogenesis of CL-induced TMAs is unclear, but recent studies suggest that ADAMTS13 is released from the liver into circulation, binds to endothelial cell surfaces, and efficiently cleaves UL-VWFMs. Thus, if endothelial cell injuries are present, ADAMTS13 cannot effectively cleave UL-VWFMs; this may lead to TMA episodes. In this regard, Zakarija et al (32) recently addressed two mechanistic pathways in TMAs related to thienopyridine derivatives.

b) Mitomycin C-induced TMAs

Ten patients with mitomycin C (MMC)-induced TMAs were identified. None had severe deficiency of ADAMTS13: AC, and all were negative for ADAMTS13: INH. Previous reports (33) suggest that MMC-induced TMAs develop with a frequency of 4-15% of the patients treated with this drug. The pathophysiology of MMC-TMAs is not well understood, but it is assumed that MMC may cause vascular endothelial cell injuries.

c) TMAs associated with other drugs

We observed two other TMA patients with severe deficiency of ADAMTS13: AC and positive ADAMTS13: INH. Both of these patients were assumed to have drug-associated TMA. One patient was a 62-year-old male with chronic hepatitis C. This patient developed TMA a month after long-term treatment with pegylated-interferon; the detailed clinical course of this patient was previously reported (34). The other patient with possible drug-induced TMA was a 65-year-old male who had taken sildenafil. The patient had taken sildenafil once several months prior to development of TMA, and then he had taken the drug twice within the 2 weeks prior to TMA. Two days after his third intake of sildenafil, the patient developed a low-grade fever, hemolytic anemia (hemoglobin 10.3 g/dL and reticulocyte 3.9%), thrombocytopenia (11,000/μL), and hematuria. ADAMTS13 analysis identified severe deficiency of ADAMTS13: AC (<3%) and ADAMTS13: INH positivity (1.5 Bethesda U/mL). The patient was treated by oral administration of the anti-platelet drug dipyridamole without plasma exchange. Since then, he has recovered, and his ADAMTS13: AC returned to normal range 3 months later.

(2) Connective tissue diseases and their allied diseases (CTD/AD)-associated TMAs

A close relationship between systemic lupus erythematosus (SLE) and TTP was first described in 1939 (35). It is now known that TMAs are frequently associated with CTDs with a frequency of 1-6% of the patient population (36). We have recently reported that severe deficiency of ADAMTS 13: AC and positive ADAMTS13: INH was predominantly detected in patients with rheumatoid arthritis (RA)- and SLE-associated TMAs, via the analysis of 127 patients with CTD-associated TMAs, whose samples were collected between 1998-2006 (37).

In this study, we included other miscellaneous autoimmune diseases, such as antiphospholipid syndrome (APS), as listed in Table 3, in the analysis. Thus, we examined 221 patients with CTD/AD-associated TMAs (Tables 1, 3), of whom 46 (21%) had severe deficiency of ADAMTS13: AC with positive ADAMTS13: INH, while the remaining 175 (79%) had mild-to-moderate deficiency. We presume that the high prevalence of TMA episodes in patients with CTD/AD is closely related to high plasma levels of VWF over the low levels of ADAMTS13: AC (37). Anatomical changes of the microvasculature, namely narrowed vessel cavities due to the proliferation of vascular endothelial cells, result in altered circulation hemodynamics and contribute to the formation of platelet thrombi at sites of vascular injury.

(3) Malignancy-associated TMAs

Sixty-one patients were classified into this category, which largely consisted of 2 groups: one group of patients with hematological malignancies (n=30) and the other group with malignant solid tumors (n=31) (Table 2).

Of the hematological malignancies, lymphoma was the most frequently seen (n=16), and four of the 16 patients had severe deficiency of ADAMTS13: AC with positive ADAMTS13: INH. The clinical course of one patient with intravascular lymphoma (IVL)-associated TMA was previously reported (38). In this case, the aggravation of TMA was dependent on the treatment efficacy of chemotherapy during the early stage of disease progression, but in the later stage was dependent on rituximab after several relapses during a 4-year observation period (39).

Of 31 patients with malignant solid tumor-associated TMAs, stomach cancer (n=10) was most commonly seen,

Table 3. Details of Underlying Diseases or Clinical Conditions of Patients with Acquired Secondary Thrombotic Microangiopathies (TMAs)

Connective tissue diseases and their allied diseases (CTDs/ADs) (n=221)

Systemic lupus erythematosus (SLE)*	92
Systemic sclerosis (SSc)	51
Polymyositis (PM)／Dermatomyositis (DM)	14
Rheumatoid arthritis (RA)**	10
Mixed connective tissue disease (MCTD)	9
Sjögren syndrome	9
Periarteritis nodosa (PN)	7
ANCA***-related nephritis	7
Antiphospholipid syndrome (APS)	4
Anti-GBM**** antibody positive nephropathy	1
Overlap syndrome	3
Vasculitis	3
Wegner syndrome	2
Pemphigoid	2
Goodpasture syndrome	1
Adult Still's disease	1
Amyloidosis	1
Sarcoidosis	1

SLE* (+APS, n=6; +Sjögren, n=4; +RA, n=1)
RA** (+Amyloidosis, n=1)
ANCA***(anti-neutrophil cytoplasmic antibody)
GBM**** (glomerular basement membrane)

Malignancies (n=61)

Haematopoietic malignancies	**n=30**
Lymphoma*	16
Myelodysplastic syndrome (MDS)	7
Multiple myeloma	2
Acute myeloid leukemia (AML)	2
Acute lymphocytic leukemia (ALL)	2
Chronic myeloid leukemia (CML)	1
Solid tumor	**n=31**
Stomach cancer	10
Pancreas cancer	3
Lung cancer	2
Ovarian tumor	2
Neuroblastoma	2
Colon cancer	1
Oesophageous cancer	1
Breast cancer	1
Vater's papilla cancer	1
Pharyngeal cancer	1
Uterus cancer	1
Rectum cancer	1
Medulloblastoma	1
Bile duct cancer	1
Bladder cancer	1
Ewing's sarcoma	1
Histiosarcoma	1

Lymphoma*(Non-Hodgkin's lymphoma, n=7; Intravascular lymphoma, n=6; Burkitt lymphoma, n=3)

Hematopoietic stem cell transplantation (HSCT) (n=54)

Bone marrow transplantation (BMT)*	22
Peripheral blood stem cell transplantation (PBSCT)**	22
Cord blood tranplantation (CBT)***	10

BMT*(unrelated, n=8; related, n=2, unclear, n=11)
PBSCT** (autologous, n=8; allogenic, n=13)
CBT*** (unrelated, n=10)

Others (n=46)

Liver disease	**n=16**
Liver transplantation*	7
Liver cirrhosis**	3
Hepatitis C	1
Alcoholic hepatitis	1
Veno-occlusive disease (VOD)	1
Liver insufficiency	1
Fulminant hepatitis	1
Autoimmune hepatitis	1
Infections	**n=10**
Pneumonia	3
Influenza	2
Human immunodeficiency virus (HIV)	1
Pyothorax	1
Liver abcess	1
Sepsis	1
Meningitis	1

Miscellaneous	**n=20**
Malignant hypertension	5
Diabetes mellitus	2
Renal transplantation	2
Schonlein-Henoch purpura	1
Duchane type muscular dystrophy	1
Amyotrophic lateral sclerosis (ALS)	1
Interstrial peumanoia	1
Eoshisophilic syndrome	1
Hyperthyroidism	1
Anorexia nervosa	1
Myasthenia gravis	1
Perforation of duodenal ulcer	1
Encephalitis	1
Ulcerative colitis	1

Liver transplantation* (Hepatitis B, n=1; Biliary atresia , n=1; Primary biliary cirrhosis, n=1; Liver cirrhosis, n=1; unclear, n=3)
Liver cirrhosis** (Hepatitis C, n=1; Alcoholic cirrhosis, n=1, unclear, n=1)

but a variety of organs were involved as listed in Table 3. One patient with Vater's papilla cancer showed severe deficiency of ADAMTS13: AC with the presence of ADAMTS 13: INH.

(4) Hematopoietic stem cell transplantation (HSCT)-associated TMAs

Fifty-four patients with TMAs were classified in this category. Of these, 22 were associated with bone marrow transplantation, 22 with peripheral blood SCT, and 10 with cord blood SCT (Table 3). The pathogenesis of TMAs in this category is highly complicated by pre-conditioning regimens of chemotherapies and body irradiation, as well as post-transplantation complications, such as bacterial or viral infections and graft-versus-host disease (GVHD). It was remarkable that none of the patients in this category had severe deficiency of ADAMTS13: AC, and all were negative for ADAMTS13: INH, as previously reported by others (40).

(5) Pregnancy-associated TMAs

TMA episodes are sometimes precipitated by pregnancy and postpartum, and require a rapid differential diagnosis from other thrombocytopenic status, such as ITP, pregnancy toxemia, eclampsia, and HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome. The category of pregnancy-associated TMAs was present in two clinical settings: USS (congenital TTP) and acquired TTP. We have recently reported that pregnancy induced isolated thrombocytopenia or TTP in all female patients with USS examined, and their babies were stillborn or premature when the appropriate plasma therapy was not performed (41).

In the present study, we were able to identify 15 patients with acquired TMAs associated with pregnancy or postpartum. Of these, eight patients developed TMA episodes at 10-40 weeks of gestation, six patients developed TMA episodes soon after delivery, and one patient developed TMA episodes 3 months postpartum. Four of the 15 patients (27%) had severe deficiency of ADAMTS13: AC with positive ADAMTS13: INH (Table 1). These results suggest that the pathogenesis of acquired pregnancy-associated TMAs may be multifactorial, a different setting from USS.

(6) Escherichia coli O157 H7-associated TMAs:

Shigatoxin (1 and 2), composed of one A-subunit of 33 kDa and five B-subunits of 7 kDa each, is produced by the *E. coli* O157: H7 strain. Shigatoxin binds to a receptor, termed globotriaosyl ceramide, which is richly expressed in glomerular endothelial cells. After binding, shigatoxin is internalized and it induces endothelial cell apoptosis; this releases significant levels of UL-VWFMs into the circulation, resulting in platelet thrombi within microvasculatures. Hence, *E. coli* O157: H7-associated TMAs appear to be induced independent of plasma levels of ADAMTS13: AC. Thirty-two patients with TMAs were in this category, and in fact none of them had severe deficiency of ADAMTS13: AC. However, 22 patients had slightly reduced ADAMTS13: AC (Table 1). The reason underlying this is unclear, but we postulate either that ADAMTS13 is partially consumed to cleave the increased plasma VWF or that shigatoxin directly targets ADAMTS13-producing cells.

(7) TMAs associated with other causes

Forty-six TMA patients, who did not fit the aforementioned categories, were classified in this category (Table 3). Because of high heterogeneity in this category, it was subcategorized into patients with liver diseases (n=16), those with infections (n=10), and miscellaneous causes (n=20).

We have reported that numerous liver diseases are associated with reduced plasma ADAMTS13: AC. Notably, plasma levels of ADAMTS13: AC decline in parallel to the progression of liver cirrhosis (42). More interestingly, several patients with advanced liver cirrhosis had severe deficiency of ADAMTS13: AC with positive ADAMTS13: INH. These patients were assumed to have cryptic clinical signs of TMA; therefore, the term 'subclinical TTP' was introduced. In addition, we have reported on recipients of liver transplants with early allograft dysfunction who showed severe thrombocytopenia accompanied by a marked reduction of ADAMTS13: AC one or two days after transplantation, but without any apparent clinical features of TMAs (43). This observation has been confirmed by two recent reports (44, 45), but the mechanism has not yet been addressed.

Viral or bacterial infections can trigger TMA episodes, but the mechanism has not yet been addressed. Most recently, influenza has been revisited by researchers, due to a close relationship between influenza and TMA originally reported in 1980 (46). It is now known that influenza vaccine may induce TTP or disease relapse (47). We have two patients with influenza A-associated TMAs, and one of them had severe deficiency of ADAMTS13: AC with positive ADAMTS13: INH. Influenza virus or vaccination often worsens underlying diseases or conditions, including diabetes mellitus, pregnancy, and ongoing hemodialysis, resulting in multiorgan failure (MOF). Is it possible that such MOF is caused by microcirculatory disturbances, resembling the pathogenesis of TTP.

Human immunodeficiency virus (HIV) infection is also a known trigger of TMAs (48). In our registry, only one HIV-positive patient with severe deficiency of ADAMTS13: AC with positive ADAMTS13: INH was identified.

Finally, the TMAs that fell into the miscellaneous subcategory are too variable to address in this report. The details of some of these patients will be reported in detail elsewhere by referral physicians.

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A 9-MONTH-OLD INFANT WITH ACQUIRED IDIOPATHIC THROMBOTIC THROMBOCYTOPENIC PURPURA CAUSED BY INHIBITORY IgG-AUTOANTIBODY TO ADAMTS13

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□ *Although acquired idiopathic thrombotic thrombocytopenic purpura (ai-TTP) is rare in children, the authors present the case of a 9-month-old boy with ai-TTP showing severe deficiency of ADAMTS13 activity by its inhibitory IgG-autoantibody (4.8 Bethesda units/mL). Plasma exchange therapy was clinically effective but transient. Deficient activity of ADAMTS13 with the presence of its inhibitor persisted for 7 months after the initial diagnosis. However, other laboratory findings improved gradually with steroid (pulse) therapy. The hitherto insufficiently characterized clinical settings of ai-TTP during early childhood underscore the importance of measuring ADAMTS13 activity and its inhibitors for differential diagnosis in patients with thrombocytopenia of unknown etiology.*

Keywords acquired TTP, ADAMTS13, infancy, von Willebrand factor-cleaving protease

Thrombotic thrombocytopenic purpura (TTP) is prominent in disorders with thrombotic microangiopathy characterized by hemolytic anemia, thrombocytopenia, and organ dysfunctions such as neurological

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abnormalities or renal insufficiency. Recent reports have described that von Willebrand factor (vWF) cleaving protease, designated as ADAMTS13, plays important roles in TTP pathophysiology. The lack of ADAMTS13 activity causes the accumulation of unusually large vWF multimers in the plasma, resulting in the disseminated platelet thrombi characteristic of TTP [1, 2]. Previous reports described that 18–72% of clinically diagnosed TTP patients had severe deficiency of ADAMTS13 activity [3].

Decreased activity of ADAMTS13 in patients with TTP might be associated with either inherited or acquired mechanisms. Hereditary TTP, known as Upshaw–Schulman syndrome (USS), results from ADAMTS13 gene mutations [4, 5]. In contrast, acquired TTP—either idiopathic or secondary to drugs, pregnancy, or diseases such as infections, cancers, and autoimmune diseases—is caused mostly by autoantibody against ADAMTS13 [6, 7].

While approximately one-third of USS cases with an inherent deficiency of ADAMTS13 are diagnosed in adolescence or adulthood after passing early childhood [8], acquired TTP in children, including infant patients, has been reported in the relevant literature. This report describes a young infant with acquired idiopathic TTP caused by IgG autoantibody against ADAMTS13. Clinical findings for this patient suggest that assays of ADAMTS13 activity and its inhibitor are indispensable for differential diagnosis with USS and other thrombocytopenic diseases during childhood.

CASE REPORT

In January 2005, a 9-month-old boy with petechial hemorrhage was referred to the Tokyo Medical and Dental University Hospital for suspected idiopathic thrombocytopenic purpura (ITP). He showed nonimmune hemolytic anemia as well as thrombocytopenia. Subsequent examinations revealed that his plasma ADMATS13 activity by vWF multimer assay [6] was markedly decreased (<3%). Moreover, inhibitor activity was detected in the titer of 4.8 BU/mL. This inhibitor activity resided on the purified IgG (data not shown). Based on these findings, he was diagnosed as having acquired idiopathic TTP. Treatment with plasma exchange (PE) performed at the National Center for Child Health and Development was effective to decrease the inhibitor activity (0.2 BU/mL) and increase the serum ADAMTS13 activity to 62.8%, engendering the improvement of his anemia and thrombocytopenia. However, this effect of 6 courses of PE was transient: after about 1 month, the inhibitor activity rebounded to the higher titer at 10 BU/mL with recurrence of low ADAMTS13 activity (<3%) and hematological abnormalities. No effect of administration of fresh frozen plasma (FFP) was observed, nor any obvious sign of renal insufficiency. After PE treatment, as his platelet count decreased rapidly to the critical level lower than 10×10^9/L, presenting the increased risk of hemorrhage, attending

physicians chose to administer low doses of continuous platelet infusion under close observation and started treatment with oral prednisolone (PSL) (2 mg/kg/day). His platelet counts were constantly higher than 1.0×10^9/L after beginning these treatments.

At admission to our hospital in March 2005, he had no petechiae, hepatomegaly, or neurological abnormality. His mental status was normal. His peripheral blood examination showed severe thrombocytopenia (1.6×10^9/L), mild anemia (Hb 7.9 g/dL), and elevation of reticulocyte counts (16.1%). In fact, RBC fragmentation was found in his peripheral blood smear (0.16%). Biochemical examination revealed mild elevation of lactate dehydrogenase (672 IU/L) and indirect bilirubin (0.88 mg/dL), in addition to decreased haptoglobin: lower than 10 mg/dL. Both blood urea nitrogen (14.2 mg/dL) and serum creatinine (0.19 mg/dL) values were within normal ranges. Results of both direct and indirect Coombs tests were negative. Hemostatic examination showed that PT, APTT, and FDP were within normal ranges, but a slightly elevated value of D-dimer was observed. *Escherichia coli* O157 was not detected in his stool culture. His ADAMTS13 activity remained at an undetectable level (<3%). Furthermore, the inhibitor of ADAMTS13 was detected in serum with the titer of 4.3 BU/mL. Moreover, ADAMTS13 gene analysis in this patient revealed no disease-causing mutations for USS (data not shown).

During his stay at our hospital, he received pulse therapy using methylprednisolone (mPSL) (30 mg/kg/day for 3 days). Although the effect of this therapy was not apparent initially, platelet counts of his peripheral blood increased gradually over the 7 weeks subsequent to pulse therapy. Thereafter, the recovery of ADAMTS13 activity with a concomitant disappearance of its inhibitory activity was observed after approximately 5 and 7 months, respectively, following pulse therapy and initial treatment (Figure 1). Oral PSL was stopped when the recovery of ADAMTS13 activity was detected.

DISCUSSION

We report here an infant case with acquired TTP caused by anti-ADAMTS13 autoantibody. Based on findings of low ADAMTS13 activity with serum inhibitors, our patient was considered to have an acquired form of TTP. Only 2 cases diagnosed as acquired TTP during the first year of life have been reported in the relevant literature [9, 10] (Table 1). Ashida et al. reported a 9-month-old girl with high titer of ADMAMTS13 inhibitor (200 Bethesda units/mL), who was treated successfully with mPSL pulse therapy following PE. Schneppenheim et al. also reported an 11-month-old boy with recurrent thrombocytopenia who responded to corticosteroids. For our patient, however, we were unable to conclude simply that steroid therapy was effective because low ADAMTS13 activity with inhibitors was sustained for a certain time after hematological improvement was achieved.

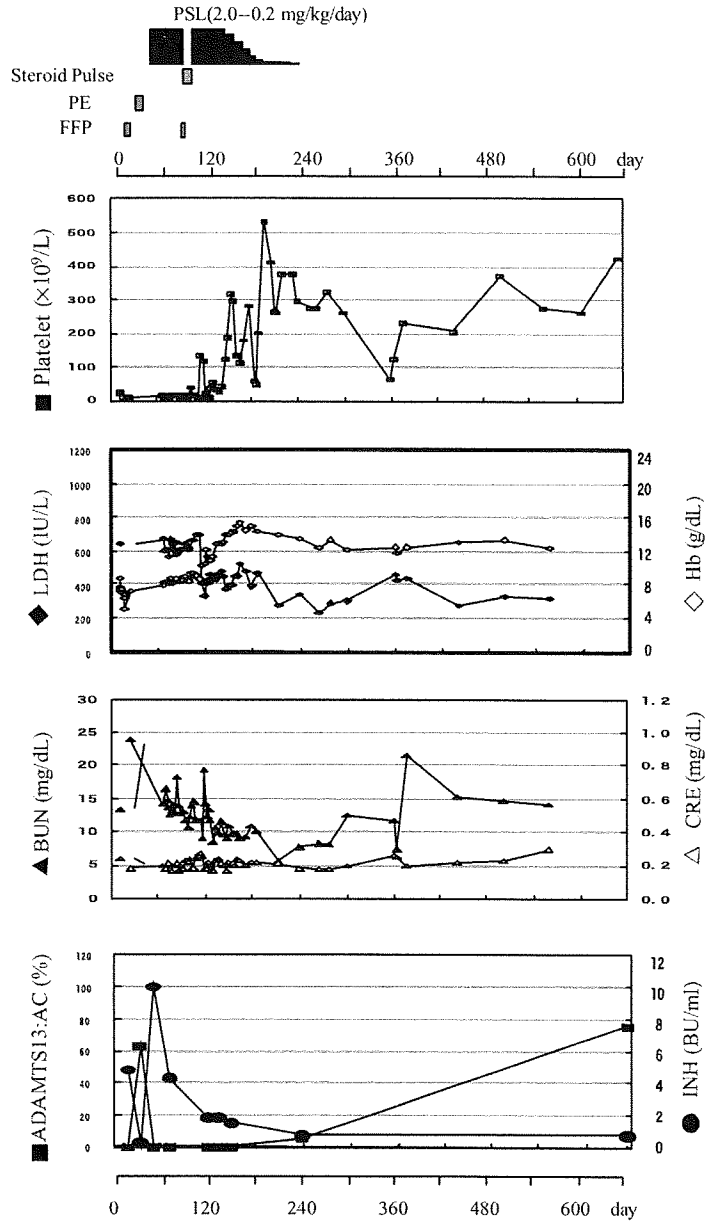

FIGURE 1 Clinical course of the present case of acquired TTP in infancy. ADAMTS13 activity and its autoantibodies were examined using frozen plasma stocked in −80°C with sensitive chromogenic ADAMTS13-act-ELISA method described in a prior study [16]. *Abbreviations*: PSL, prednisolone; FFP, fresh frozen plasma; INH, inhibitors; BU, Bethesda unit.

Neurological abnormalities and renal insufficiency are the hallmarks of acquired TTP in adults. These symptoms are worsened by platelet transfusions through expanding thrombus formation [11]. However, our patient, with a low titer of antibodies, showed no such symptoms at onset or

TABLE 1. Infants with acquired TTP showing anti-ADAMTS13 autoantibody

Patient no.	Sex	Age (months)	Renal impairment	CNS complication	Initial diagnosis	ADAMTS13 activity (%)	Inhibitor (BU/mL)	Ref.
1	F	9	Hematuria	Hemiconvulsion	TTP	<3	200	[9]
2	M	11	(−)	(−)	ITP	<2	(+)	[10]
Present case	M	9	(−)	(−)	ITP	<3	4.8	

Note. M, male; F, female; CNS, central nervous system; ITP, idiopathic thrombocytopenic purpura; TTP, thrombotic thrombocytopenic purpura; BU, Bethesda unit.

worsening in the hospital. In contrast, an infant TTP with high titer of antibodies showed both neurological and renal symptoms [9]. Results show that clinical symptoms become more severe in patients with high titer of inhibitor [12]. Therefore, results suggest that the titer of inhibitors might be a critical factor determining the severity of clinical manifestations of acquired TTP in infancy.

It is noteworthy that, during approximately 3 months, our patient showed hematological improvement despite the sustained low ADAMTS13 activity and the presence of inhibitor, thereby indicating that low ADAMTS13 activity does not necessarily worsen thrombotic microangiopathy. This finding in our patient might resemble the clinical picture of a subset of patients with USS who might be asymptomatic during infancy despite the inherent impairment of ADAMTS13 function [8, 13].

The classical 'pentad' of TTP is known to be fully present in only a minority of patients [3], indicating a limitation of diagnosis based solely on symptoms and routine examinations. Childhood TTP might be diagnosed initially as hemolytic anemia, Evans syndrome, or ITP [10, 14]; the assessment of ADAMTS13 would be of value for differential diagnosis of these diseases. Recently, rapid assays for measuring ADAMTS13 activity have been developed [15, 16]. Therefore, the assessment of ADAMTS13 activity and its inhibitor would be of value as routine laboratory tests for differential diagnosis of thrombocytopenia of unknown etiology during childhood.

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