TRANSFUSION

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TRANSFUSION PRACTICE

Implementation of a rapid assay of ADAMTS13 activity was associated with improved 30-day survival rate in patients with acquired primary thrombotic thrombocytopenic purpura who received platelet transfusions

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ABSTRACT

BACKGROUND: Platelet transfusions are probably harmful in patients with acquired idiopathic thrombotic thrombocytopenic purpura (aTTP). Introduction of a rapid assay for ADAMTS13 activity should reduce the time to definite diagnosis of aTTP, reduce the amount of inappropriately transfused platelet concentrates and improve mortality and morbidity.

STUDY DESIGN AND METHODS: We collected 265 aTTP patients with severe ADAMTS13 deficiency. Of these, 91 patients were diagnosed by March 2005 (Period 1), when ADAMTS13 activity was measured by VWF multimer assay which took 4-7 days until the result was reported. Another 174 patients were diagnosed after April 2005 (Period 2), when the activity was measured by a chromogenic ELISA which took 1-2 days.

RESULTS: We found no significant differences in 30-day survival rate between the two periods. Overall, 48 patients received platelet transfusions. Mortality was slightly greater between patients with (22.9%) versus without platelet transfusion (17.7%), but not statistically significant. In Period 1, Cox-proportional-hazards regression analysis showed older age (≥60 year) and platelet transfusion administration were independent factors associated with higher risks of 30-day mortality. In contrast, in Period 2, lower Rose-Eldor TTP severity score, and use of plasma exchange and corticosteroid therapy were independent factors associated with higher survival rates while non-administration of platelet transfusions was not.

CONCLUSION: Our results indicate that platelet transfusions are harmful for aTTP patients when the definite diagnosis of severe ADAMTS13 deficiency is delayed. If it can be done as soon as possible, platelet transfusions for severe bleeding or surgical interventions might be allowed with subsequent plasmapheresis.

ABBEREVIATIONS: aTTP = acquired primary thrombotic thrombocytopenic purpura; TMA = thrombotic microangiopathy; VWF = von Willebrand factor; ADAMTS13 = a disintegrin-like and metalloproteinase with thrombospondin type 1 motifs 13; ELISA = Enzyme-Linked ImmunoSorbent Assay; UL-VWFMs = unusually-large VWF multimers

Key words: Platelet transfusion, Thrombotic thrombocytopenic purpura, mortality, ADAMTS13 assay

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INTRODUCTION

Thrombotic thrombocytopenic purpura (TTP) is a life-threatening disease characterized by systemic platelet aggregation[1] and originally defined in 1966 by a classic pentad; thrombocytopenia, microangiopathic hemolytic anemia, renal dysfunction, fever and neurological findings.[2] More recently, TTP is clinically diagnosed by thrombocytopenia and microangiopathic hemolytic anemia and signs of organ dysfunction.[3] The diagnosis of acquired TTP is confirmed by severe deficiency of ADAMTS13 activity (<10% of normal).[3] ADAMTS13 is a metalloprotease that specifically cleaves the peptide bond between Try1605 and Met1606 in the A2 domain of von Willebrand factor (VWF) and also specifically cleaves unusually large VWF multimers (UL-VWFMs), which are the most active forms of VWF.[4] VWF is one of the key protein of primary hemostasis. UL-VWFMs are secreted from endothelial cells and are cleaved into smaller fragments just after secretion by ADAMTS13 under the high shear conditions in microvasculatures. When ADAMTS13 activity is severely decreased, excessive amounts of UL-VWFMs persist in the circulation and bind platelets when stretched under high shear conditions, resulting in platelet aggregation and organ ischemia.

Severe deficiency of ADAMTS13 activity in TTP most often results from autoantibodies against ADAMTS13 (ADAMTS13 inhibitor) in acquired TTP[5,6] or less often from mutations in *ADAMTS13* gene in congenital TTP (Upshaw-Schulman syndrome, USS).[7,8] Plasma exchange is still the most important therapeutic approach for acquired TTP, decreasing mortality from approximately 90% to less than 20%.[2,9] Plasma exchange usually requires placement of central venous catheter for access. Most acquired TTP patients with ADAMTS13 activity <10% show severe thrombocytopenia under 20×10^9/ul.[10] Therefore, prophylactic platelet transfusion is often performed to prevent

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bleeding complications related line insertion. UK TTP guidelines recommend against transfusing platelet transfusions unless there is life-threatening hemorrhage.[11] However, two studies report that empirical evidence of harm from platelet transfusions in patients with TTP is uncertain.[12,13] Platelet transfusion during TTP management remains controversial.

Our laboratory has been functioning as a nationwide referral center for thrombotic microangiopathies (TMAs) in Japan.[14] We established a large Japanese registry of patients with TMA and analyzed their clinical and laboratory information. In this study, we conducted a retrospective analysis of platelet transfusion to patients with acquired primary TTP (aTTP) from our Japanese registry before and after a rapid assay for ADAMTS13 activity level was available. While platelet transfusions are probably harmful in patients with aTTP, introduction of the rapid assay should reduce the time to definite diagnosis of aTTP, reduce the amount of inappropriately transfused platelet concentrates and/or facilitate early treatment of intensive TTP therapy following platelet transfusion, and thus, mortality and morbidity.

MATERIALS AND METHODS

Patients

The inclusion criteria of aTTP in this study are : 1) microangiopathic hemolytic anemia (hemoglobin < 12g/dL), 2) thrombocytopenia (platelet count < 100×10^9/uL), 3) severe deficiency of ADAMTS13 activity (<10% of the normal control), 4) positive anti-ADAMTS13 inhibitors ($\geq$ 0.5 Bethesda unit/ml), 5) no alternative cause such as connective tissue disease, stem cell transplantation, pregnancy, and drugs. The same inclusion criteria were incorporated into our prior study.[14] This study was approved by the

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Ethics Committee of Nara Medical University, and written informed consent was obtained from all patients at each referral hospital.

The severity of TTP were evaluated by the previous validated Rose & Eldor scoring system.[15] This 8-point score system is designed based on neurological findings, renal impairment, thrombocytopenia, and microaogiopathic hemolytic anemia (Supplemental Table 1). Using this system, central nervous system (CNS) dysfunction was judged having confusion, lethargy, behavioral changes, focal neurological deficits, convulsions, stupor, and coma.[15] Renal impairment was diagnosed by blood urea nitrogen (BUN) > 30mg/dl, or serum creatinine >1.5 mg/dl, urine proteinuria >2g/day, or hematuria.[15]

Blood sampling

Before therapeutic approach including plasma exchange, plasma infusion and use of immunosuppressants, blood samples (4.5mL) from each patient were placed into plastic tubes containing 0.5mL of sodium citrate. The plasma was isolated by centrifugation at 3,000 g for 15 minutes at 4 °C, kept in aliquots at -80 °C and sent to Nara Medical University with clinical information.

Assay of plasma ADAMTS13 activity and ADAMTS13 inhibitor

Between March 2000 and March 2005 (termed Period 1), plasma ADAMTS13 activity was measured by a VWF multimers assay that was based on the method of Furlan et al with a slight modification (Figure 1).[16,17] Briefly, patient plasma incubated with purified VWF from pooled normal human plasmas dissolved in urea buffer at 37 °C for 24 hours. Each reaction mixture was separated by SDS-1.2% agarose gel electrophoresis, and the VWF multimers were visualized by Western blotting and luminography as described

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before.[18,19] The result of ADAMTS13 activity analyzed by VWF multimers method was returned to physicians at the referred hospital in 4-7 days.

Subsequently, between April 2005 and December 2013 (Period 2), we analyzed plasma ADAMTS13 activity using a chromogenic ADAMTS13 activity ELISA.[20] Briefly, peptide containing 73 amino acid residues (D1596 to R1668) in A2 domain of VWF, termed VWF73,[21] is used as a substrate. VWF73 is cleaved by ADAMTS13 and detected by monoclonal antibodies that specifically recognized Y1605, which is the C-terminal edge residue cleaved by ADAMTS13.[20] This ELISA is completed within 3 hours in our laboratory. The results analyzed by ADAMTS13 activity ELISA were reported back to referring physicians within 1-2 days after obtaining the plasma. All plasma samples collected in the Initial time-period were re-examined by the ELISA. Patients who had <10% of ADAMTS13 activity by VWF multimers assay but ≥10% by the ELISA were excluded in this study.

Plasma ADAMTS13 inhibitor titers were evaluated either by VWF multimers assay or chromogenic ADAMTS13 activity ELISA using heat-inactivated plasma at 56°C for 30 minutes. One Bethesda unit (BU) is defined as the amount to reduce ADAMTS13 activity to 50% of control levels.[22]

Clinical data

The primary outcome of this study was death from TTP or other causes. The Cox proportional hazard model was used to calculate survivals. Overall survival was defined between the first day when TTP symptom developed and the date of death or the last follow-up. Platelet transfusion was operationally defined as occurring during the inpatient admission for aTTP. One unit of platelet transfusion in Japan was derived from 200 ml of

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peripheral blood. The clinical information and outcome were reported from the referring physician who also sent follow-up plasma for analyzing ADAMTS13 activity and inhibitor. For this study, we investigated outcomes of patients to physicians on March 2015 again as much as possible. The longest follow-up period was 15 years.

Statistical analysis

We included the following variables: age, gender, time period (Period 1 or Period 2), the level of CNS dysfunction, renal impairment, thrombocytopenia, anemia, and fever according to Rose-Eldor TTP severity score, titer of anti-ADAMTS13 inhibitor, presence or absence of plasma exchange, platelet transfusion, corticosteroid administration, additional immunosuppressive therapy, and rituximab administration. All variables were examined for associations with mortality. Data from a first episode of TTP for individual patients were included in the analysis. Categorical variables were compared by Fisher exact test. Analysis of continuous variables was performed by the Mann-Whitney *U* test. Overall survival was estimated by the Kaplan-Meier curves and compared with the Log-Rank test. The Cox proportional hazard model was used to calculate the mortality odds ratio. *P*-values of 0.05 or less were considered statistically significant. All statistical analyses were performed with EZR (Saitama, Japan),[23] which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

We collected a large dataset of medical information on 1,211 patients with TMA from March 1st 2000 to December 31th 2013 (Figure 1). Of these, 904 patients had an etiology other than TTP, and 307 patients met criteria for aTTP. Because of the absence of detailed

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clinical information, another 42 patients were excluded. Overall, 265 aTTP patients with severely decreased ADAMTS13 activity (<10% of normal) were included in this study. Of these, 91 patients were diagnosed before April 1, 2005 (Period 1), when ADAMTS13 activity was measured by VWF multimer assay. Another 174 patients were diagnosed on or after April 1, 2005 (Period 2), when a chromogenic ADAMTS13 activity ELISA was available and days required for an a-TTP diagnosis were much shorter than the Period 1. Overall, 48 patients received platelet transfusion one or more times during the admission for aTTP, 17 in Period 1 and 31 in Period 2 (Figure 1). Of these, 13 patients received platelet transfusion before plasma exchange in Period 1 and 24 in Period 2. Moreover, 40 patients received platelet transfusions before aTTP diagnosis (14 in Period 1, 26 in Period 2), and eight patients received plaltlet transfusions after aTTP diagnosis (3 in Period 1, 5 in Period 2) due to severe bleeding such as tracheal hemorrhage and ovarian hemorrhage.

Patient characteristics and outcome

Table 1 show clinical features and outcomes in overall period (n=265) and the comparison between 91 patients diagnosed in Period 1 and 174 patients in Period 2. The number of patients ≥ 60 years of age was 111 (42%). One hundred forty-six (55%) patients were female. The TTP pentad of CNS dysfunction, renal impairment, thrombocytopenia, anemia, fever was noted in 75%, 50%, 88%, 77%, and 70%, respectively. As for severity of TTP, 54% patients had severity score ≥ 6 and 57% had ADAMTS13:INH > 2 BU/mL. Overall, 92% of the aTTP patients received plasma exchange. Among patients who received plasma exchange, the median (range) of the number and duration of plasma exchanges was 10 (2-60) times and 15 (7-120) days, respectively. Nine patients died before initiation of plasma exchange (five patients in period 1 and four in period 2). Another 12

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patients did not receive plasma exchange because of an indolent clinical course, old age, and problems of hospital facilities. As shown in Table 1, the time from admission to the first plasma exchange therapy was no significant difference. Interestingly, among patients who received platelet transfusions, the days to plasma exchange in deceased patients was significantly longer than those in survivors (31.3 vs 6.6 days, $p<0.05$).

Seventy-seven percent of patients received adjunctive corticosteroids therapy. Additional immunosuppressive (IS) therapies such as cyclophosphamide, vincristine, cyclosporine, and intravenous immunoglobulin were received in 35% of patients. Rituximab, anti CD20 monoclonal antibody, was received in 52 patients.

Platelet transfusions were administered to 48 patients (18%) with between 10 and 115 units. In 265 patients with aTTP, 49 patients died between day 0 and 479. Of these 49 patients, 46 died between day 0 and day 30. The remaining 3 patients died after day 200. Therefore, the survival rate at 30 days in all patients was 83% [95% confidence interval (CI), 77-87%].

Comparison of clinical features between aTTP patients in Period 1 and Period 2

We identified significant differences between clinical features in Period 1 and Period 2 with respect to gender, renal impairment, ADAMTS13 inhibitor titer, and treatment including plasma exchange, corticosteroids, and rituximab treatment (Table 1). In Period 1, rituximab was not commonly used in Japan. The frequency (18.7% vs 17.8%) and volume [20 units (10-100) vs 20 units (10-115)] of platelet transfusion were not different between two periods. We did not find significant difference in survival rate at 30 days between Period 1 and Period 2 ($p=0.18$).

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Univariate and multivariate analysis of risk factors for mortality

Old age (≥60 years old) and treatments of plasma exchange, corticosteroids, additional IS therapy, and Rituximab were significantly associated with increased 30-day mortality based on univariate analysis (Table 2, left). We identified significant differences in therapeutic strategy between Period 1 and Period 2. However, the period in which TTP was diagnosed (Period 1 or Period 2) was not significantly associated with mortality (p=0.18). Moreover, administration of platelet transfusions was not statistically significantly associated with lower 30-day survival rates in the overall time-period (p=0.46).

Multivariate analysis was performed with seven independent variables- age, diagnosing period (Period 1 versus Period 2), CNS dysfunction, plasma exchange, platelet transfusion, corticosteroids, and additional IS therapy in the overall period (Table 2, right). This analysis identified 4 factors predictive of 30-day survival - age less than 60 years of age, no CNS dysfunction, use of plasma exchange, and corticosteroid administration. Moreover, platelet transfusion was not associated with lower risks of survival in the overall period by the analysis of both Log-rank and Cox proportional hazard model (Figure 2 upper, Table 2 left).

Univariate and multivariate analysis of risk factor to aTTP mortality for Period 1 and Period 2

The mortality rate of patients with aTTP was analyzed separately in the two time-periods categorized by the different methods of ADAMTS13 activity assay. In Period 1 using the classic VWF assay, Cox-proportional-hazards regression analysis to evaluate 30-day survival was performed using 5 variables (age, plasma exchange, corticosteroids,

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additional IS therapy, and platelet transfusion). We found that old age (≥ 60 years) and administration of platelet transfusions were independently associated with 30-day mortality ($p<0.05$) (Table 3 upper). Similarly, based on results of the Log-rank test, the 30-day mortality of patients who received platelet transfusion tended to be higher than those who did not receive platelet transfusions ($p=0.051$) (Kaplan-Meier survival curve in Figure 2 bottom).

In Period 2 using the rapid ADAMTS13 activity ELISA, 6 independent factors [old age (≥60 years), Rose-Eldor severity score, use of plasma exchange, use of corticosteroids, additional IS therapy, and administration of platelet transfusion] were evaluated by Cox-proportional-hazards regression analysis. High Rose-Eldor severity score, use of plasma exchange, and use of corticosteroids were identified as independent risk factor for 30-day mortality. Platelet transfusion was not associated with increased risks of mortality in Period 2 by both multivariate analysis and Log-rank test (Table 3 lower, Figure 2 bottom).

DISCUSSION

All patients included in this study had a severe deficiency of ADAMTS13 activity (<10%), which is now commonly used to support a diagnosis of aTTP. A deficiency of ADAMTS13 activity results in accumulation of UL-VWFMs and platelet thrombi in the microvasculature. Under the condition of ADAMTS13 deficiency, theoretically platelet transfusion accelerates to produce platelet thrombi and causes organ damage. Indeed, some cases with aTTP are reported whose clinical symptoms worsened markedly after platelet transfusion.[24-26] However, the study from Oklahoma TTP-HUS Registry reported that clinical evidence of harm from platelet transfusion in patient with TTP is uncertain.[13] In

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addition, Otrock et al[27] reported that platelet transfusion in patients with TTP does not appear harmful in regard to thrombotic complications.

UK TTP guidelines describe that platelet transfusions are contra-indicated in TTP unless there is life-threatening hemorrhage.[11] However, severe hemorrhage is rare in TTP patients, although profound thrombocytopenia is a typical feature.[28] In clinical practice, platelet transfusion is often requested by a surgeon prior to inserting a central venous catheter for access for plasma exchange rather than the risk of severe hemorrhage occurring during or following the procedure. Platelet transfusion before plasma exchange seemed to be more harmful than platelet transfusion after plasma exchange.[29,30] In our study, platelet transfusion was performed in 37 patients prior to plasma exchange (among 48 patients who received platelet transfusion and also underwent plasma exchange).

In 2015, three studies analyzed a large number of TTP patients and concluded that platelet transfusion in TTP is harmful. Goel et al[31] analyzed platelet transfusion in platelet consumption disorders using a nationwide hospital discharge database in United States. In 10,624 hospitalizations for TTP, platelet transfusion is associated with 6 times higher odds of developing arterial thrombosis and 2 times higher odds of acute myocardial infarction.[31] Benhamou et al[32] analyzed in 339 patients with TTP diagnosed by a severe ADAMTS13 deficiency (<10%) and reported that repeated platelet transfusion may be associated with more death and clinical deterioration. A systematic review using 15 studies of 466 patients reported that platelet transfusion compared with no platelet transfusion was associated with a significant increase in mortality.[33] These studies are limited by selection and publication bias, whereas our results are based on patients with aTTP whose data and samples were submitted to a national laboratory in Japan.

In the present study, we did not find a significant difference in 30-day mortality between

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platelet transfused patients and no transfused patients in the overall time-period. However, in Period 1, the survival rate of transfused patients was significantly lower than those of non-transfused patients. In Period 2, a chromogenic ELISA allowed us to send results of ADAMTS13 activity to physicians within 1 to 2 days after receiving plasma samples. This might have contributed to selection of TTP treatment. In fact, the frequencies of therapy use of plasma exchange, corticosteroids administration, and additional IS therapy significantly differed between two periods. However, the frequency and the volume of platelet transfusion were not different significantly between two periods. We hypothesize that physicians could select TTP therapy with confidence since the results of ADAMTS13 activity and inhibitor assays were available shortly after the tests were drawn and shortly after platelet transfusion occurred in Period 2. Our results may indicate that persistent plasma exchange after platelet transfusion together with confirmation of severe ADAMTS13 activity and an aTTP diagnosis reduces risks for aggravated platelet thrombi formation in microvasculatures of patients.

There are some limitations in this study. First, although the assay for ADAMTS13 activity and inhibitor were performed in a single lab, the management strategy was selected by the referring physicians in different hospitals. Therefore, difference of aTTP therapy is likely to affect mortality of aTTP patients. Second, we evaluated severity of TTP using the Rose-Eldor scoring system, which developed prior to identification of the central role of ADAMTS13 activity levels. This system had some weaknesses, for example including proteinuria and hematuria, which are not TTP features. However, comprehensive symptoms including CNS and renal impairment could be evaluated in this system. Finally, this was a retrospective study analyzing a large database in Japan. Data on the outcome of patients during admission for acute phase of aTTP has been collected, but long-term

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follow-up was not performed. For this study, we made the effort to collect the long-term outcome in patients as much as possible. Consequently, the number of patients following over 1 and 5 year was 57 and 11, respectively, and the maximum follow-up period was 15 years. This study analyzed 265 patients with ADAMTS13 activity deficiency. However, only 48 patients received platelet transfusion. Therefore, it is difficult independently to analyze outcomes among patients who received platelet transfusion before plasma exchange was initiated.

Our results indicate that platelet transfusions are harmful for aTTP patients when the definite diagnosis of severe deficiency of ADAMTS13 activity is delayed. However, if the diagnosis can be done as soon as possible, platelet transfusions to the patients required for severe bleeding or surgical interventions might be allowed in conjunction with subsequent plasmapheresis.

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Figure Legends

Figure 1. Patients selection for this study

Our TMA registry enrolled 1,211 patients from March 1st 2000 to December 31th 2013. Of these, 904 patients had another etiology and 42 were excluded due to the lack of detail information. In 265 aTTP patients included in this study, 91 were diagnosed by March 31, 2005 (Initial period), when we determined ADAMTS13 activity by VWF multimer assay. Another 174 patients were diagnosed after April 1, 2005 (Latter period), when a chromogenic ADAMTS13 activity ELISA was available and days required for diagnosis became much shorter than Period 1. Totally 48 patients received platelet transfusion during the admission for aTTP, 17 of them in Period 1 and 31 in Period 2.

aTTP: acquired primary thrombotic thrombocytopenic purpura

Figure 2. The survival rate of aTTP patients with and without platelet transfusion

Kaplan-Meier curve of aTTP patients with and without platelet transfusion in overall period (upper), Period 1 (lower left) and Period 2 (lower right) were shown. Based on the Log-rank test, the mortality of patients with platelet transfusion tended to be higher than those without platelet transfusions in Period 1 (p=0.051).

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Table 1. Clinical features and outcome of the patients in Initial and Latter period

		Overall n=265		Period 1 (n=91)		Period 2 (n=174)		p
Demographics								
	Age ≧60	111	(42%)	40	(44%)	71	(41%)	0.70
	Female	146	(55%)	42	(46%)	104	(60%)	0.04
Clinical symptoms								
	CNS dysfunction	196	(75%)	67	(74%)	129	(75%)	0.88
	Renal impairment	132	(50%)	57	(63%)	75	(44%)	<0.01
	Thrombocytopenia Plt<20×10^9/L	231	(88%)	76	(84%)	155	(90%)	0.12
	Platelet counts, Median (25-75%) (10^9/L)	1	(0.7-1.5)	1	(0.7-1.8)	1	(0.7-1.4)	0.26
	Anemia Hb<9g/dL	202	(77%)	73	(80%)	129	(75%)	0.44
	Fever	185	(70%)	61	(76%)	104	(64%)	0.07
	Severity score≧6	141	(54%)	53	(58%)	88	(51%)	0.30
	ADAMTS13:INH>2BU/mL	150	(57%)	38	(42%)	112	(65%)	<0.01
Treatment								
	Plasma exchange	244	(92%)	79	(87%)	165	(95%)	0.03
	Days to the first plasma exchange, mean (25%-75%)	2	(1-3)	4	(1-9)	3	(1-7)	0.16
	Corticosteroid administration	203	(77%)	63	(69%)	140	(81%)	0.03
	Additional IS therapy	93	(35%)	25	(28%)	68	(40%)	0.06
Platelet transfusion (PT)								
	Number of patient who received PT	48	(18%)	17	(19%)	31	(18%)	0.87
	Number of patient who received PT before PE	37	(77%)	13	(76%)	24	(77%)	1
	Volume of PT (unit), mean (range)	20	(10-115)	20	(10-100)	20	(10-115)	0.57
	Frequency of PT, mean (range)	2	(1-3)	2	(1-4)	2	(1-3)	1
Outcome								
	14 days Overall survival (%), median (CI)	88 (84-92)		87 (78-92)		89 (83-92)		0.18
	30 days Overall survival (%), Median (CI)	83 (77-87)		79 (68-86)		85 (78-89)		0.19

CNS: central nervous system, Plt: platelet count, Hb: hemoglobin, INH: inhibitor, BU: Bethesda Unit, IS: immunosuppressive,

CI: confidence interval

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Table.2 Univariate and multivariate analysis of risk factors in a-TTP patients

		Univariate analysis		Multivariate analysis		
Factor		30days OS	*p*.value	HR	95%CI	p
Age	<60	0.90 (0.84-0.94)	<0.001	1		<0.01
	≧60	0.73 (0.63-0.80)		2.47	1.35-4.53	
Sex	male	0.87 (0.79-0.92)	0.2			
	female	0.79 (0.72-0.85)				
Period	Period 2	0.85 (0.78-0.89)	0.18	1		0.51
	Period 1	0.79 (0.68-0.86)		1.22	0.68-2.18	
CNS dysfunction	No	0.91 (0.81-0.96)	0.07	1		0.09
	Yes	0.80 (0.73-0.85)		2.07	0.90-4.76	
Renal impairment	Yes	0.79 (0.72-0.86)	0.2			
	No	0.86 (0.78-0.91)				
Thrombocytopenia	>20×10^9/l	0.78 (0.59-0.87)	0.53			
	≦20×10^9/l	0.83 (0.78-0.88)				
Anemia	>9g/dl	0.90 (0.79-0.95)	0.13			
	≦9g/dl	0.81 (0.74-0.85)				
Fever	Yes	0.81 (0.75-0.86)	0.38			
	No	0.86 (0.76-0.92)				
ADAMTS13:INH	≦2BU/ml	0.84 (0.75-0.89)	0.57			
	>2BU/ml	0.82 (0.75-0.87)				
Plasma Exchange	No	0.57 (0.34-0.75)	<0.001	1		<0.001
	Yes	0.83 (0.77-0.88)		0.22	0.10-0.48	
Platelet Transfusion	No	0.83 (0.77-0.88)	0.46	1		0.19
	Yes	0.81 (0.66-0.90)		1.59	0.80-3.18	
Steroid Administration	No	0.62 (0.49-0.74)	<0.001	1		<0.001
	Yes	0.89 (0.83-0.92)		0.31	0.17-0.56	
Additional IS therapy	No	0.77 (0.70-0.82)	<0.001	1		0.16
	Yes	0.93 (0.86-0.97)		0.56	0.25-1.27	
Rituximab Administration	No	0.80 (0.74-0.85)	0.04			
	Yes	0.92 (0.80-0.97)				

SR: survival rate, HR: hazard ratio, CI: confidence interval, CNS: central nervous system, INH: inhibitor, BU: Bethesda unit,

IS: immunosuppressive

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Table 3. Multivariate analysis

Period 1

Factor	Hazard.ratio	*p*.value
Age ≧60	4.53 (1.63-12.59)	<0.01
Plasma exchange	0.39 (0.13-1.11)	0.08
Steroid administration	0.39 (0.14-1.08)	0.07
Additional IS therapy	0.37 (0.08-1.83)	0.22
Platelet transfusion	4.04 (1.43-11.40)	<0.01

Period 2

Factor	Hazard.ratio	*p*.value
Age ≧60	1.76 (0.81-3.79)	0.15
Severity score≧6	2.52 (1.09-5.81)	0.03
Plasma exchange	0.22 (0.07-0.69)	<0.01
Steroid administration	0.31 (0.14-0.70)	<0.01
Additional IS therapy	0.59 (0.22-1.55)	0.28
Platelet transfusion	0.68 (0.23-2.01)	0.49

IS: immunosuppressive

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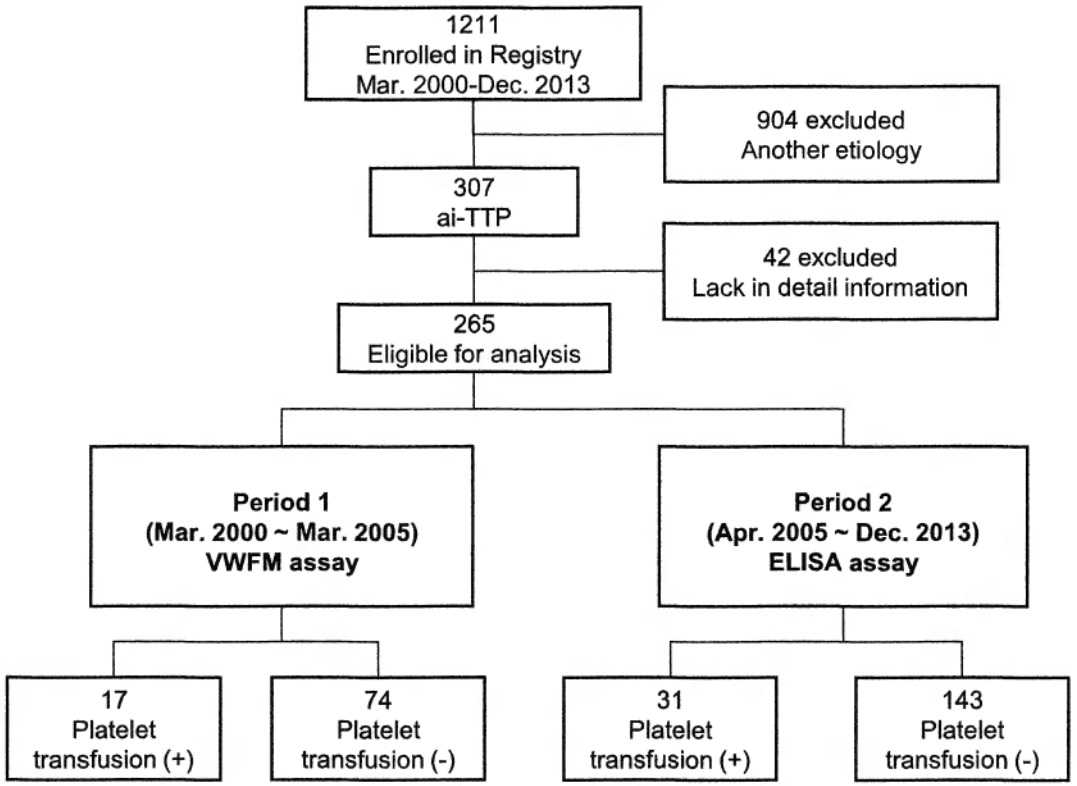
1211
Enrolled in Registry
Mar. 2000-Dec. 2013
904 excluded
Another etiology
307
ai-TTP
42 excluded
Lack in detail information
265
Eligible for analysis
Period 1
(Mar. 2000 ~ Mar. 2005)
VWFM assay
Period 2
(Apr. 2005 ~ Dec. 2013)
ELISA assay
17
Platelet
transfusion (+)
74
Platelet
transfusion (-)
31
Platelet
transfusion (+)
143
Platelet
transfusion (-)

Fig. 1

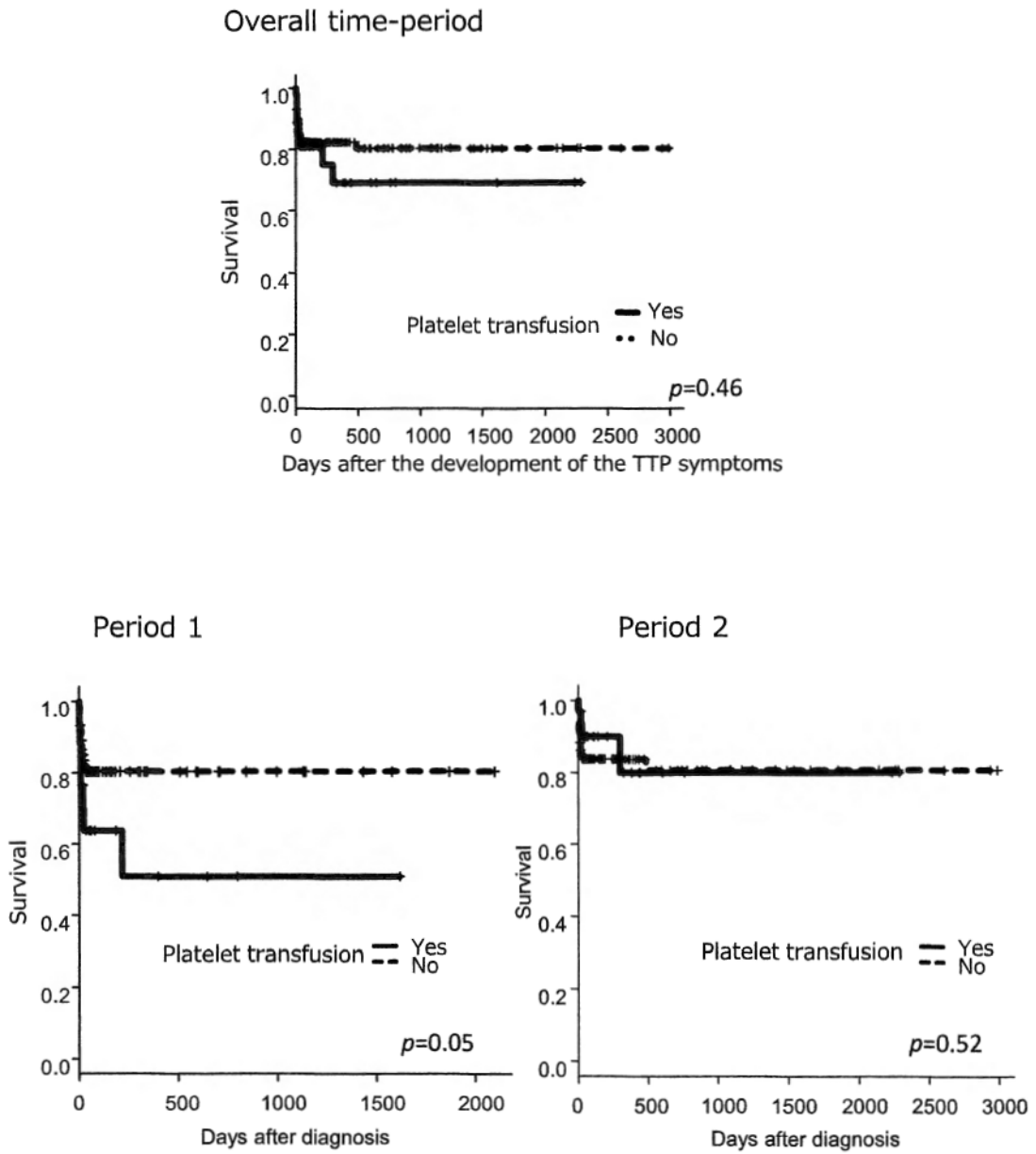
Overall time-period
Survival
1.0
0.8
0.6
0.4
0.2
0.0
Platelet transfusion
Yes
No
p=0.46
0
500
1000
1500
2000
2500
3000
Days after the development of the TTP symptoms
Period 1
Survival
Platelet transfusion
Yes
No
p=0.05
0
500
1000
1500
2000
Days after diagnosis
Period 2
Survival
Platelet transfusion
Yes
No
p=0.52
0
500
1000
1500
2000
2500
3000
Days after diagnosis

Fig. 2

Supplemental Table 1. Severity scoring system of patients with TTP by Rose and Eldor

Level of Abnormality	System Affected			
	Neurologic Findings	Renal Function Impairment	Platelet Count at Presentation ($\times 10^9$/liter)	Hemoglobin Level at Presentation (g/dl)
0	None	None	>100	>12
1	Confusion, lethargy, behavioral changes	30mg/dl<BUN<70mg/dl and/or 1.5mg/dl<creatinine<2.5mg/dl and/or proteinuria>2g/day and/or hematuria	20-100	9-12
2	Focal neurologic deficits, convulsions, stupor, coma	BUN≧70mg/dl and/or creatinine≧2.5mg/dl and/or dialysis	<20	<9

(References 15)

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