Thrombosis Research 133 (2014) 440–444

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Regular Article

# Elevated plasma levels of soluble platelet glycoprotein VI (GPVI) in patients with thrombotic microangiopathy

Yoshiki Yamashita [a], Katsuki Naitoh [b], Hideo Wada [c,*], Makoto Ikejiri [c], Takeshi Mastumoto [d], Koshi Ohishi [d], Yoshitaka Hosaka [b], Masakatsu Nishikawa [e], Naoyuki Katayama [a]

[a] Departments of Hematology and Oncology, Mie University Hospital and Mie University Graduate School of Medicine, Tsu, Japan
[b] Biology Laboratory, Discovery Research, Mochida Pharmaceutical CO., LTD. Shizuoka, Japan
[c] Molecular and Laboratory Medicine, Mie University Hospital and Mie University Graduate School of Medicine, Tsu, Japan
[d] Blood transfusion, Mie University Hospital and Mie University Graduate School of Medicine, Tsu, Japan
[e] Clinical Research Support Center, Mie University Hospital and Mie University Graduate School of Medicine, Tsu, Japan

ARTICLE INFO

*Article history:*
Received 15 July 2013
Received in revised form 7 November 2013
Accepted 19 November 2013
Available online 1 December 2013

*Keywords:*
TMA
Platelet activation
ADAMTS13
sGPVI

ABSTRACT

*Background:* Thrombotic microangiopathy (TMA) is caused by various conditions, such as decreased a ADAMTS13 level, activated or injured vascular endothelial cells or activated platelets. This study examined the soluble platelet glycoprotein VI (sGPVI) levels in patients with TMA to evaluate the activation of platelets in thrombotic states.
*Materials and Methods:* The plasma levels of sGPVI, ADAMTS13 activity, von Willebrand factor (VWF) and VWF propeptide (VWFpp) were measured in patients with TMA.
*Results:* The plasma levels of sGPVI were significantly higher in postoperative patients, patients with TMA and those with disseminated intravascular coagulation (DIC) than in those without thrombosis. The plasma levels of sGPVI were the highest in patients with TMA without markedly reduced ADAMTS13 and those were significantly reduced after plasma exchange.
*Conclusion:* The measurement of sGPVI level is therefore considered to be important for the diagnosis and evaluation of TMA.



## Introduction

Thrombotic microangiopathies (TMAs) are defined by the association of acute mechanical hemolytic anemia, thrombocytopenia, and visceral ischemic manifestations related to the formation of platelet thrombi in the microcirculation [1–3]. TMAs are caused by various conditions, such as markedly decreased a ADAMTS13 (a disintegrin and metalloprotease with thrombospondin type I domain 13) level and unusually large multimers of Von Willebrand factor (ULM-VWF) [4], activated or injured vascular endothelial cells, activated platelets, or etc. ADAMTS13 [5] is a zinc metalloprotease that specifically cleaves ULM-VWF at the Tyr (1605)- Met(1606) boundary located in the A2 region of VWF [6,7], suggesting that ULM-VWF cause multiple platelet thrombi due to TMA. The diagnosis of TMA has improved remarkably by developing the method of ADAMTS13 measurement [8,9].

In the activated or damaged vascular endothelial cells, plasma levels of soluble thrombomodulin [10], P-selectin [11], VWF and VWF propeptide (VWFpp) [12] were reported in patients with TMA. After the secretion of VWFpp and VWF into plasma from endothelial cells in response to several physiological or pathological stimuli, VWFpp dissociates from VWF [13,14]. It is reported that VWFpp is more useful for the diagnosis of TMA than VWF or TM [11,15].

Platelet glycoprotein VI (GPVI), a type I transmembrane glycoprotein of immunoreceptor family, is constitutively associated and expressed with the Fc receptor γ-chain (FcRγ), an immunoreceptor tyrosine-based activation motif-bearing (ITAM-bearing) receptor [16,17]. On ligand-induced cross linking of GPVI/FcRγ, this ITAM enables Lyn-dependent activation of Syk kinase and promulgation of downstream signaling cascade. Upon platelet activation, the platelet surface GPVI was cleaved off by protease, such as ADAM10, resulting in down regulating platelet reactivity and releasing of the soluble form GPVI (sGPVI) [18–20]. The sGPVI has recently received much attention as a platelet activation marker, because, i) it is specifically expressed on platelet, ii) the level of sGPVI in plasma are increased in patients with thrombosis, iii) the production mechanism is dependent on platelet activation. Indeed, several groups developed an immunoassay for sGPVI, and reported that the sGPVI would be a useful biomarker for disease caused by platelet activation such as acute coronary syndrome (ACS) and stroke [21–25].

This study, examined the activation of platelets by measuring of sGPVI level in 70 patients with TMA in comparison to 40 healthy

* Corresponding author at: Department of Laboratory Medicine, Mie University School of Medicine, 2-174 Edobashi, Tsu-city, Mie-ken 514-8507, Japan. Tel.: +81 59 232 1111; fax: +81 59 231 5204.
*E-mail address:* wadahide@clin.medic.mie-u.ac.jp (H. Wada).

0049-3848/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.thromres.2013.11.023

volunteers, 46 patients without thrombosis, 15 postoperative patients, 13 with disseminated intravascular coagulation (DIC).

## Materials and Methods

The plasma levels of sGPVI, ADAMTS13 activity, VWF and VWFpp were measured in 70 with TMA (37 females and 33 males, median age: 25-75%tile; 55.5 years old: 34-72 years old) from April 1, 1990 to March 31, 2012 in Mie University Hospital, in comparison to 40 healthy volunteers (14 females and 26 males, 22.0 years old: 22.0-26.0 years old), 46 patients without thrombosis (23 females and 23 males, 52.0 years old: 30.0-68.0 years old), including 18 patients with hematological diseases, nine with infections, seven with liver diseases, six with solid cancers, four with autoimmune diseases and two with renal diseases, 15 postoperative patients following orthopedic surgery (11 females and 4 males, 71.0 years old: 66.5-74.8 years old), 13 with DIC (2 females and 11 males, 59.0 years old: 39.0-69.5 years old). TMA was diagnosed according to the diagnostic criteria of TMA: (1) thrombocytopenia (less than 12 X $10^4$/μl), (2) hemolytic anemia (less than 11.0 g/dl of hemoglobin) due to the microangiopathy (presence of fragmented red cells, elevated total bilirubin, and LDH), (3) neurological dysfunction, (4) renal failure, and (5) fever [26]. The patients with (1) and (2) who had an ADAMTS 13 activity of less than 10%, who had an O-157 infection, and who had clinical symptoms, such as (3) or/and (4), were diagnosed with TMA. These TMA patients were classified into 4 groups; 6 patients with atypical HUS (HUSa), which has a frequent relapse and familial history for TMA, 5 patients with hematological TMA (TMA-H), which is due to bone mallow transplantation, 27 patients with ADAMTS13-related TMA (TMA-A), where the ADAMTS13 level was less than 10%; and 32 patients with TMA other (TMA-O), the cause of which was not known, which was associated with vascular endothelial cell damage. DIC was diagnosed using an International Society of Thrombosis Haemostasis overt-DIC diagnostic criteria [27]. The patients who had a complete remission and survived, were called survivors. The patients who did not have a complete remission and died within one year were called non-survivors.

The study protocol was approved by the Human Ethics Review ommittees of Mie University School of Medicine, and signed informed consent was obtained from each patient.

### *Measurement of ADAMTS13, VWF and VWFpp*

ADAMTS13 was measured using a FRETS-VWF73, which was chemically synthesized by the Peptide Institute, Inc. (Osaka, Japan) according to the method described by Kokame et al. [8,9]. TM was measured with a Thrombomodulin "MKI" EIA kit (Mitsubishi Chemical Medience Corporation, Tokyo, Japan). VWF and VWFpp levels were measured with a VWF&Propeptide assay kit (GTi DIAGNOSTiCs, Waukesha, USA)[12,15].

### *Measurement of sGPVI in plasma*

Recombinant sGPVI was used as calibrator in the assay. The extracellular region of human GPVI fused with histidine-tag at the C-terminus was transiently expressed by COS-1 cell, and the cultured supernatant was purified by Ni-column chromatography. The level of sGPVI in plasma was quantified by sandwich ELISA, which consists of two mouse anti-GPVI monoclonal antibodies, F1232-7-1 and F1232-10-2 which could recognize the extracellular domain I (D1) N-terminal loop and the extracellular domain D2 loop of GPVI, respectively. [28,29]. Briefly, a 96-well microtiter plate (Nunc-immuno Module Maxsorp, Thermo, Waltham, USA) was coated with F1232-7-1 diluted in phosphate buffer saline, pH7.4 (PBS), and then was blocked with 5% stabilguard (SurModics, Eden Prairie, USA) and 3.2% sucrose in PBS. The calibrators consisting of recombinant sGPVI and human plasma diluted in the sample dilution buffer (0.1% bovine serum albumin, 0.05% Tween20 and 0.3 mol/L NaCl in PBS) were added in duplicate to wells and the plate was incubated at room temperature for 2 h. After the plate was washed five times with wash buffer (0.05% Tween20 in saline), the captured sGPVI was detected by applying the peroxidase-labeled F1232-10-2 Fab in the dilution buffer (6% rat serum, 1% mouse serum, 0.3% BSA and 0.05% Tween 20 in PBS) for 1 h at room temperature. Then, the wells were washed five times with the wash buffer, and the level of sandwiched sGPVI was determined by adding tetramethyl benzidine for 20 min at room temperature, with the enzyme reaction being terminated by lowering the pH. The absorbance of each well at 450 nm was measured using a microplate reader (reference 620 nm or longer). Using this assay, quantitative measurements are available within 3 h. The standard curve was linear from 0.156-5.0 ng/mL and the intra-assay and inter-assay variation were less than 10%. The limit of detection and the limit of quantification in the assay were 0.067 and 0.156 ng/mL, respectively.

### *Identification of sGPVI in plasma*

sGPVI in plasma was confirmed by immunoprecipitation and western blotting. Briefly, each plasma was incubated with F1232-7-1 coupled bead, which is prepared with NHS-activated sepharose (GE healthcare, Buckinghamshire, UK), for 1 h at room temperature. The bead was washed twice with phosphate buffer (pH7.4), and treated with Tris-SDS buffer containing 2-mercaptoethanol. After centrifugation, the supernatant was subjected to SDS-PAGE using 10 % Bis-Tris Gel (Life Technologies, Carisbad, USA), and electrotransferred to nitrocellulose membrane. The membrane was immunoblotted with a rabbit anti-GPVI antibody [29]. The blots were probed with the HRP-conjugated goat anti-rabbit immunoglobulins antibody (Dako, Glostrup, Denmark), developed with ECL-Plus (GE healthcare), and imaged with CCD cameras.

### *Statistical analysis*

The data are expressed as the median (25% tile-75% tile). Differences between groups were examined for significance using the Mann-Whitney U test. A P-value of less than 0.05 was considered to indicate a significant difference. The correlations between sGPVI and hemostatic markers were examined using the Spearman's rank correlation coefficient.

All statistical analyses were performed using Stat flex, version 6, software package (Artec Co Ltd, Osaka, Japan,).

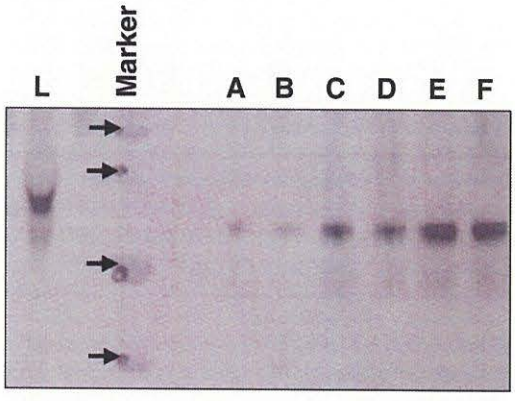


**Fig. 1.** The immunoprecipitation and Western blotting analyses of sGPVI in the plasma and washed platelet lysate. The immunoprecipited samples derived from plasma were analyzed by a Western blotting analysis using a rabbit anti-GPVI antibody. The samples in lanes A and B were supplied from healthy controls, while those examined in lanes C, D, E and F were supplied from TTP patients. The levels of sGPVI in the plasma were 7.47, 7.13, 20.0, 22.9, 40.4 and 44.4 ng/mL, respectively. The sample in lane L was washed platelet lysate, and the molecular weight marker was from Bio-Rad. The arrows from top to bottom indicate the 100, 75, 50 and 37 kDa, respectively.

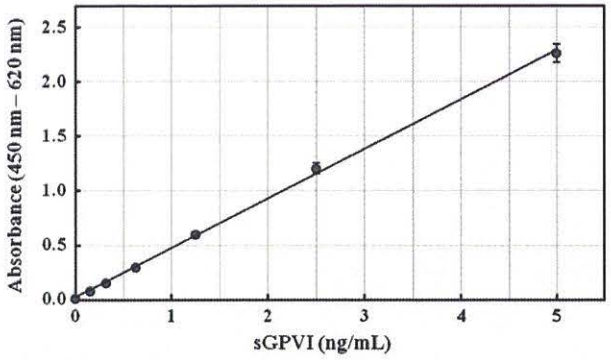


**Fig. 2.** A typical calibration curve for the assay. Various concentrations of recombinant sGPVI were used to generate as calibration curve. The values are expressed as the means ± SD (n = 4).

## Results

The immunoprecipitation and western blotting in healthy volunteers (Lane A and B) and TTP patients (Lane C ~ F) show a single band and molecular weight 55 KD. Each level of sGPVI in plasma was 7.47, 7.13, 20.0, 22.9, 40.4 and 44.4 ng/mL, respectively (Fig. 1). The standard curve was linear from 0.156-5.0 ng/mL and the intra-assay and inter-assay variation were less than 10%. The limit of detection and the limit of quantification in the assay were 0.067 ng/ml and 0.156 ng/mL, respectively (Fig. 2).

The plasma levels of sGPVI (median; 2.5-97.5%tile) were 11.4 ng/mL (5.9-19.5) ng/mL in healthy volunteers. Plasma levels of sGPVI (median; 25.0-75.0%tile) were significantly higher in patients without TH (16.2 ng/mL 12.6-22.5 ng/mL), post operation (31.6 ng/mL; 28.3-35.1 ng/mL), DIC (44.5 ng/mL; 36.6- 60.8 ng/mL), TMA (40.8 ng/mL; 32.9-56.7 ng/mL) than in healthy volunteers (11.4 ng/mL; 9.1-14.8 ng/mL)($p < 0.001$, respectively, Fig. 3). Those were significantly higher in patients with post operation than in patients without TH ($p < 0.001$), and significantly higher in those with TMA and in those with DIC than in those without TH ($p < 0.001$, respectively). Plasma levels of sGPVI were significantly higher in patients with TMA-A (36.8 ng/mL; 30.5-49.0 ng/mL) and TMA-O (51.9 ng/mL; 37.4-66.3 ng/mL) than in patients without TH ($p < 0.001$, respectively, Fig. 4). Those were significantly higher in patients with TMA-O than in patients with aHUS (33.2 ng/mL; 19.5-50.7 ng/mL, $p < 0.05$) and TMA-H (14.2 ng/mL; 6.1-21.4 ng/mL, $p < 0.001$). On one day after treatments such as plasma exchange, plasma levels of GPVI were significantly reduced in comparison to the onset of TMA ($p < 0.001$, Fig. 5).

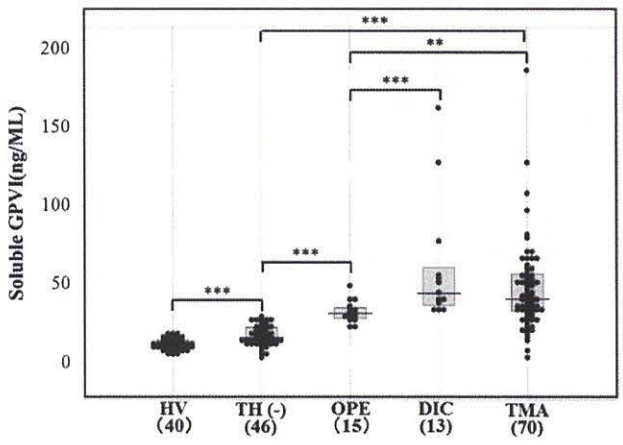


**Fig. 3.** The plasma levels of soluble GPVI in healthy volunteers, patients without thrombosis, postoperative patients, and patients with DIC or TMA HV; health volunteers, TH(-); patients without thrombosis, OPE; postoperative patients, DIC; patients with disseminated intravascular coagulation, TMA; patients with thrombotic microangiopathy. ***; $p < 0.001$, **; $p < 0.01$.

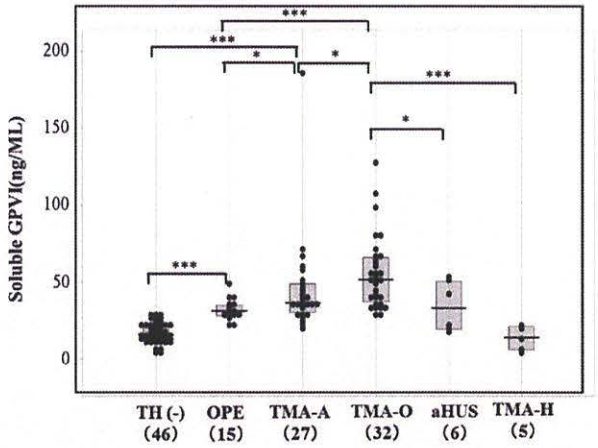


**Fig. 4.** The plasma levels of soluble GPVI in patients without thrombosis, postoperative patients and patients with TMA-A, TMA-O, aHUS or TMA-H. TH(-); patients without thrombosis, OPE; postoperative patients, TMA-A: TMA-ADAMTS13; patients with thrombotic microangiopathy (TMA) due to markedly reduced ADAMTS13, TMA-O: TMA-Other; patients with TMA due to other causes, aHUS; patients with atypical HUS, TMA-H; patients with TMA due to hematological malignancy. ***; $p < 0.001$, *; $p < 0.05$.

There was no significant difference in plasma levels of ADAMTS13, sGPVI, VWF and VWFpp/VWF ratio between TMA patients with survivor or non-survivor. Plasma VWFpp levels were significantly higher in the non-survivor (339 %; 263-441 %) than in the survivor (200 %; 165-241 %, $p < 0.001$, Table 1). Plasma levels of sGPVI were not well correlated with ADAMTS13, VWF, VWFpp, VWFpp/VWF ratio and TM in the patients with TMA (Table 2), but the correlation between ADAMTS13 and TM, between VWF and VWFpp, VWFpp and VWFpp/VWF ratio and VWFpp and TM was significant in the patients with TMA. The plasma sGPVI levels were not well correlated with the platelet counts ($Y = 50.0 - 1.40\,X$, $r = -0.5405$). The patients were divided into two groups; a normal platelet count group and a low platelet count group (Fig. 6).

## Discussion

Hypercoagulable and thrombotic states including platelet activation might exist in patients with TMA [11,26]. An activation of coagulation system can be examined by measuring soluble fibrin and thrombin-antithrombin complex [30], and an activations or injuries of vascular endothelial cells can be examined by measuring VWFpp and TM [12],

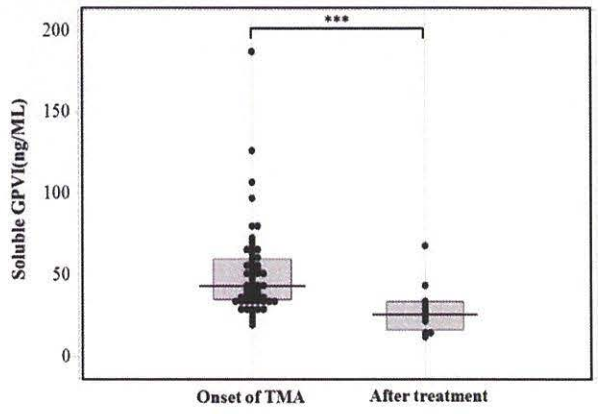


**Fig. 5.** The plasma levels of soluble GPVI in patients at the onset of TMA and one day after treatment of plasma exchange. ***; $p < 0.001$.

**Table 1**
The plasma levels of ADAMTS13 activity, sGPVI, VWF, VWFpp and the VWFpp/VWF ratio in surviving and non-surviving TMA patients.

| | Survivor | Non-survivor | |
|---|---|---|---|
| ADAMTS13 (%) | 65.0 (19.4 – 94.7) | 35.2 (17.5 – 61.3) | NS |
| sGPVI (ng/mL) | 56.6 (43.2 – 136.3) | 40.4 (32.7 – 58.8) | NS |
| VWF (%) | 197 (144 – 229) | 225 (193 - 299) | NS |
| VWFpp (%) | 200 (165 – 241) | 339 (263 – 441) | P < 0.001 |
| VWFpp/VWF ratio | 1.07 (0.98 – 1.42) | 1.30 (1.02 – 1.96) | NS |

NS; not significant.
Survivor: patients who had a complete remission and survived.
Non-survivor: patients who did not have a complete remission and died within one year.

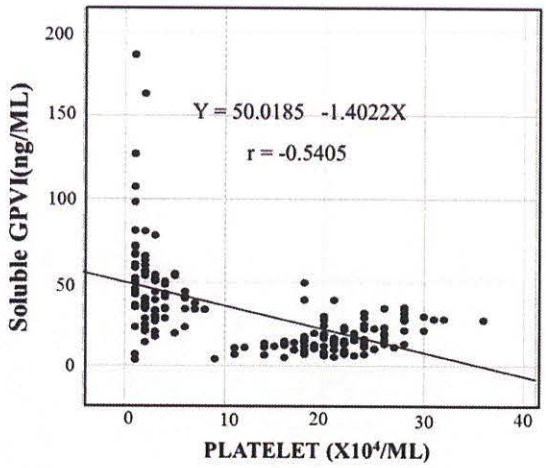


**Fig. 6.** The relationship between the plasma soluble GPVI level and the platelet count.

While an activation of platelet is difficult to be examined by routine tests. Although β-thromboglobin (β-TG) and platelet factor 4 (PF4) might be reflect to the platelet activation, those tests need to careful procedure and a low temperature centrifuge. This sGPVI assay is stable, easy and fast in comparison toβ-TG and PF4. A sGPVI exist in plasma and it is considered to be released from activated platelets. The normal range of plasma sGPVI was from 5.9 ng/mL to 19.5 ng/mL. Plasma sGPVI levels were significantly increased in patients with post operation, those with DIC and those with TMA, thus suggesting that plasma sGPVI levels increased in a thrombotic state which activates platelets. Several groups have already reported the development of an immunoassay for determination of sGPVI in plasma [31,32], but, we assumed that there are some discrepancy between each assay. The one group reported that the level of sGPVI was elevated in platelet activated disease [22,23,33] such as DIC, stroke and SAP which cause shear-dependent platelet activation. The other group represented that the levels of sGPVI was more negatively associated with the development of ACS, such as non-ST-elevation myocardial infarction and ST-elevation myocardial infarction, and they speculated the reason why the released sGPVI would bind to the atherosclerotic plaque and protect from atherothrombosis [32,34]. The cause for the difference is unclear, it might be due to a sample preparation, characteristic features of antibody or complexity of assay. These differences might reflect the different diseases and/or stages of disease studied in the different patient groups.

Plasma levels of sGPVI were the highest in those with TMA-O among TMA group. The one of causes might be activated platelet exception with activation of VWF/ADAMTS13 system. Markedly elevated VWFpp levels were also reported in the patients with TMA-O [13], indicating that severe vascular endothelial injuries existed in this state. Although TMA-H is also associated with severe vascular endothelial cell injuries, the patients with TMA-H had severe thrombocytopenia. Therefore, the consumption of platelets in TMA-H patients is not significant compared to that in patients with TMA-O, and subsequently, the plasma levels of sGPVI did not significantly increase in the patients with TMA-H. After treatments such as plasma exchange, plasma levels of GPVI were significantly reduced in comparison to the onset of TMA, suggesting that plasma levels of sGPVI might be useful to evaluate the efficacy of treatment for TMA. There was no significant difference in plasma levels of sGPVI between TMA patients with survivor or non-survivor, thus suggesting that the degree in the activation of platelets may not affect the outcome. However, the VWFpp levels were significantly high in the poor outcome patients with TMA [13]. As VWFpp is reflected to vascular endothelial injuries, sGPVI might not be a marker for vascular endothelial injuries. Plasma levels of sGPVI were not well correlated with ADAMTS13, VWF, VWFpp and TM in the patients with TMA, suggesting sGPVI might be new marker for reflecting the activation of platelet but not reflecting the activation or injury of vascular endothelial cells. As the plasma levels of ADAMTS13 are not closely correlated with the platelet counts, not only a reduced ADAMTS13 level, but also other factors, may activate platelets.

The measurement of sGPVI might be useful for assessing the activation of platelets in patients with acute myocardial infarction, cerebral infarction and DIC, and for the evaluating the efficacy of anti-platelet agents.

## Conflict of Interest Statement

The study was funded by Mochida Pharmaceutical CO., LTD and the sGPVI ELISA system was provided from the company. KN contributed to

**Table 2**
The correlations among the ADAMTS13, sGPVI, VWF and VWFpp levels, the VWFpp/VWF ratio and TM.

| | ADAMTS13 | sGPVI | VWF | VWFpp | VWFpp/ VWF ratio | TM |
|---|---|---|---|---|---|---|
| ADAMTS13 | | 0.129 (NS) | 0.194 (NS) | 0.126 (NS) | - 0.134 (NS) | 0.462 (p < 0.001) |
| sGPVI | 0.129 (NS) | | - 0.177 (NS) | - 0.144 (NS) | - 0.096 (NS) | 0.158 (NS) |
| VWF | 0.194 (NS) | - 0.177 (NS) | | 0.688 (p < 0.001) | - 0.199 (NS) | 0.524 (p < 0.001) |
| VWFpp | 0.126 (NS) | - 0.144 (NS) | 0.688 (p < 0.001) | | 0.492 (p < 0.001) | 0.602 (p < 0.001) |
| VWFpp/ VWF ratio | - 0.134 (NS) | - 0.096 (NS) | - 0.199 (NS) | 0.492 (p < 0.001) | | 0.114 (NS) |
| TM | 0.462 (p < 0.001) | 0.158 (NS) | 0.524 (p < 0.001) | 0.602 (p < 0.001) | 0.114 (NS) | |

NS; not significant.
The values indicate the Spearman's rank correlation coefficient.

the measurement and western blotting analysis of sGPVI, but was not involved in the interpretation of the results. KN and YH are employees of Mochida Pharmaceutical CO., LTD.

All authors have no othere COI.

## References


[1] Moake JL. Thrombotic microangiopathies. N Engl J Med 2002;347:589–600.
[2] Fujimura Y, Matsumoto M. Registry of 919 patients with thrombotic microangiopathies across Japan: database of Nara Medical University during 1998-2008. Intern Med 2010;49:7–15.
[3] Fujimura Y, Matsumoto M, Yagi H, Yoshioka A, Matsui T, Titani K. Von Willebrand factor-cleaving protease and Upshaw-Schulman syndrome. Int J Hematol 2002;75: 25–34.
[4] Moake JL, Rudy CK, Troll JH, Weinstein MJ, Colannino NM, Azocar J, et al. Unusually large plasma factor VIII:von Willebrand factor multimers in chronic relapsing thrombotic thrombocytopenic purpura. N Engl J Med 1982;307:1432–5.
[5] Zheng X, Chung D, Takayama TK, Majerus EM, Sadler JE, Fujikawa K. Structure of von Willebrand factor-cleaving protease (ADAMTS13), a metalloprotease involved in thrombotic thrombocytopenic purpura. J Biol Chem 2001;276:41059–63.
[6] Furlan M, Robles R, Lamie B. Partial purification and characterization of a protease from human plasma cleaving von Willebrand factor to fragments produced by in vivo proteolysis. Blood 1996;87:4223–34.
[7] Tsai H-M. Physiologic cleavage of von Willebrand factor by a plasma protease is depend on its conformation and requires calcium ion. Blood 1996;87:4235–44.
[8] Kokame K, Nobe Y, Kokubo Y, Okayama A, Miyata T. FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. Br J Haematol 2005;129:93–100.
[9] Kobayashi T, Wada H, Kamikura Y, Matsumoto T, Mori Y, Kaneko T, et al. Decreased ADAMTS13 activity in plasma from patients with thrombotic thrombocytopenic purpura. Thromb Res 2007;119:447–52.
[10] Wada H, Ohiwa M, Kaneko T, Tamaki S, Tanigawa M, Shirakawa S, et al. Plasma Thrombomodulin as a marker of vascular disorders in thrombotic thrombocytopenic purpura and disseminated intravascular coagulation. Am J Hematol 1992;39:20–4.
[11] Shimura M, Wada H, Hiyoyama K, Nakasaki T, Takagi M, Deguchi A, et al. Increased plasma soluble-adhesion molecules in patients with thrombotic thrombocytopenic purpura and those with disseminated intravascular coagulation. Clin Appl Thromb Hemost 1998;4:196–200.
[12] Ito-Habe N, Wada H, Matsumoto T, Ohishi K, Toyoda H, Ishikawa E, et al. Elevated Von Willebrand factor propeptide for the diagnosis of thrombotic microangiopathy and for predicting a poor outcome. Int J Hematol 2011;93:47–52.
[13] Borchiellini A, Fijnvandraat K, ten Cate JW, Pajkrt D, van Deventer SJ, Pasterkamp G, et al. Quantitative analysis of von Willebrand factor propeptide release in vivo: effect of experimental endotoxemia and administration of 1-deamino-8-D- vasopressin in humans. Blood 1996;88:2951–8.
[14] Federici Augusto B. VWF propeptide: a useful marker in VWD. Blood 2006;108: 3229–30.
[15] Habe K, Wada H, Ito-Habe N, Hatada T, Matsumoto T, Ohishi K, et al. Plasma ADAMTS13, von Willebrand Factor (VWF) and VWF Propeptide Profiles in Patients with DIC and Related Diseases. Thromb Res 2012;129:598–602.
[16] Tsuji M, Ezumi Y, Arai M, Takayama H. A novel association of Fc receptor gamma-chain with glycoprotein VI and their co-expression as a collagen receptor in human platelets. J Biol Chem 1997;272:23528–31.
[17] Leitinger B. Transmembrane collagen receptors. Annu Rev Cell Dev Biol 2011;27: 265–90.
[18] Gardiner EE, Arthur JF, Kahn ML, Berndt MC, Andrews RK. Regulation of platelet membrane levels of glycoprotein VI by a platelet-derived metalloproteinase. Blood 2004;104:3611–7.
[19] Gardiner EE, Karunakaran D, Shen Y, Arthur JF, Andrews RK, Berndt MC. Controlled shedding of platelet glycoprotein (GP)VI and GPIb-IX-V by ADAM family metalloproteinases. J Thromb Haemost 2007;5:1530–7.
[20] Gardiner EE, Al-Tamimi M, Andrews RK, Berndt MC. Platelet receptor shedding. Methods Mol Biol 2012;788:321–39.
[21] Al-Tamimi M, Arthur JF, Gardiner E, Andrews RK. Focusing on plasma glycoprotein VI. Thromb Haemost 2012;107:648–55.
[22] Al-Tamimi M, Gardiner EE, Thom JY, Shen Y, Cooper MN, Hankey GJ, et al. Soluble glycoprotein VI is raised in the plasma of patients with acute ischemic stroke. Stroke 2011;42:498–500.
[23] Al-Tamimi M, Grigoriadis G, Tran H, Paul E, Servadei P, Berndt MC, et al. Coagulation-induced shedding of platelet glycoprotein VI mediated by factor Xa. Blood 2011;117:3912–20.
[24] Nurden P, Tandon N, Takizawa H, Couzi L, Morel D, Fiore M, et al.,: An acquired inhibitor to the GPVI platelet collagen receptor in a patient with lupus nephritis. J Thromb Haemost; 7: 1541-1549.
[25] Bigalke B, Stellos K, Weig HJ, Geisler T, Seizer P, Kremmer E, et al. Regulation of platelet glycoprotein VI (GPVI) surface expression and of soluble GPVI in patients with atrial fibrillation (AF) and acute coronary syndrome (ACS). Basic Res Cardiol 2009;104:352–7.
[26] Mori Y, Wada H, Gabazza EC, Minami N, Nobori T, Shiku H, et al. Predicting response to plasma exchange in patients with thrombotic thrombocytopenic purpura with measurement of vWF-cleaving protease activity. Transfusion 2002;42:572–80.
[27] Taylor Jr FB, Toh CH, Hoots K, Wada H, Levi M. Towards a definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. Thromb Haemost 2001;86:1327–30.
[28] Hosoka Y, Naitoh K, Honda M: Novel platelet activation marker and method for determination thereof, European Patent Application publication No. EP2000802 A1, published on December 10, 2007.
[29] Takayama H, Hosaka Y, Nakayama K. A novel antiplatelet antibody therapy that induces cAMP-dependent endocytosis of the GPVI/Fc receptor gamma-chain complex. J Clin Invest 2008;118:1785–95.
[30] Wada H, Kobayashi T, Abe Y, Hatada T, Yamada N, Sudo A, et al. Elevated levels of soluble fibrin or D-dimer indicate high risk of thrombosis. J Thromb Haemost 2006;4:1253–12588.
[31] Al-Tamimi M, Mu FT, Moroi M, Gardiner EE, Berndt MC, Andrews RK. Measuring soluble platelet glycoprotein VI in human plasma by ELISA. Platelets 2009;20: 143–9.
[32] Bigalke B, Pötz O, Kremmer E, Geisler T, Seizer P, Puntmann VO, et al. Sandwich immunoassay for soluble glycoprotein VI in patients with symptomatic coronary artery disease. Clin Chem 2011;57:898–904.
[33] Al-Tamimi M, Tan CW, Qiao J, Pennings GJ, Javadzadegan A, Yong AS, et al. Pathologic shear triggers shedding of vascular receptors: a novel mechanism for down-regulation of platelet glycoprotein VI in stenosed coronary vessels. Blood 2012;119: 4311–20.
[34] Wurster T, Poetz O, Stellos K, Kremmer E, Melms A, Schuster A, et al. Plasma levels of soluble glycoprotein VI (sGPVI) are associated with ischemic stroke. Platelets 2013;24:560–5.