

## Original Article

# The changes of von willebrand factor/a disintegrin-like and metalloprotease with thrombospondin type I repeats-13 balance in aneurysmal subarachnoid hemorrhage

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**Abstract:** The aim of this study was to investigate the role of Von Willebrand Factor/thrombospondin type I repeats-13 (VWF/ADAMTS13) balance in aSAH. Fifty eight patients with aSAH at the First Affiliated hospital of Soochow University, Suzhou, China, between January 2012 and January 2014 were eligible for the study. They were divided into delayed cerebral ischemia group (DCI group) and non-delayed cerebral ischemia group (no DCI group), or cerebral vasospasm group (CVS group) and no spasm group (no CVS group), or good outcome group and poor outcome group. The control group consisted of twenty healthy people. All patients underwent CT, DSA, or (and) CTA diagnosed with intracranial subarachnoid hemorrhage which is caused by aneurysm rupture. Venous blood was drawn in tubes at 3 time points: 1 day after SAH (T1), (4±1) days after SAH (T2), and (9±1) days after SAH (T3) to determine plasma concentrations of ADAMTS13, VWF, P-selectin and IL-6 via enzyme-linked immunosorbent assay (ELISA). Transcranial doppler sonography (TCD) was used to measure mean blood flow velocity of the middle cerebral artery (VMCA). Glasgow Outcome Scale (GOS) was measured before discharge. Among 58 patients, 12 (20.7%) had DCI, 40 (68.9%) had TCD evidence of CVS, and 20 (34.5%) had poor outcome. The concentrations of VWF, P-selectin and IL-6 on T1, T2 and T3 after SAH were significantly higher in DCI, CVS and poor outcome groups compared with those of the control group ( $P < 0.05$ ). The concentrations of VWF, P-selectin and IL-6 were significantly higher in DCI, CVS and poor outcome groups compared with those of the no DCI, no CVS and good outcome groups. The activity of ADAMTS13 was lower in DCI and poor outcome groups compared with those of the no DCI and good outcome groups ( $P < 0.05$ ). The activity of ADAMTS13 showed no difference in CVS group and no CVS group ( $P > 0.05$ ). The results of our study suggest that the increased VWF and decreased ADAMTS13 activity were associated with DCI and poor outcome. The balance of VWF/ADAMTS13 could be used to predict the clinical outcome. The deficiency of ADAMTS13 can not only induce DCI but also accelerate inflammatory reaction. Our results reported in this paper may provide new insights into the possible use of ADAMTS13 as a therapeutic agent in aneurysmal subarachnoid hemorrhage.

**Keywords:** Aneurysmal subarachnoid hemorrhage, von willebrand factor (VWF), ADAMTS13, p-selectin, interleukin-6

## Introduction

Although the aneurysmal subarachnoid hemorrhage (aSAH) diagnostic methods, treatment means and perioperative management has made considerable progress, but the death rate and disability rate is still high. Delayed cerebral ischemia (DCI) is a common complication after aneurysmal subarachnoid hemorrhage, which occurs in approximately 30% of patients [1-4]. Although DCI is common, the pathogenesis of it has not been elucidated yet. Some theories were proposed to explain the relationship between DCI and outcome of aSAH patients [1, 5-10]. Von Willebrand Factor (VWF) is a large adhesive glycoprotein, participating in the adhesion of platelets [11]. It is produced and released by vascular endothelial cells and,

rhage, which occurs in approximately 30% of patients [1-4]. Although DCI is common, the pathogenesis of it has not been elucidated yet. Some theories were proposed to explain the relationship between DCI and outcome of aSAH patients [1, 5-10]. Von Willebrand Factor (VWF) is a large adhesive glycoprotein, participating in the adhesion of platelets [11]. It is produced and released by vascular endothelial cells and,

**Table 1.** Demographic data and clinical characteristics (Mean  $\pm$  SD)

Characteristic	control group (n=20)	patients with aSAH
Age (means $\pm$ SD, years)	48.6 $\pm$ 6.4	53.0 $\pm$ 11.1
Sex (male/female)	8/12	24/34
conscious disturbance	-	32
Hunt-Hess scale		
I	-	10
II	-	18
III	-	22
IV	-	8
V	-	0
modified Fisher grade		
0	-	0
1	-	12
2	-	24
3	-	16
4	-	6
Size of aneurysm(cm)		
< 1	-	42
1-2.5	-	12
$\geq$ 2.5	-	4

in much smaller amounts, by platelets [12]. VWF is a marker of both acute and chronic endothelial cell activation. A disintegrin-like and metalloprotease with thrombospondin type I repeats-13 (ADAMTS13) inhibits platelet adhesion by quickly cleaving hyperactive VWF multimers which are released from the Weibel-Palade bodies under conditions of fluid shear stress [13-15].

We hypothesized that the balance of VWF/ADAMTS13 played a role in thrombosis because reduced ADAMTS13 activity would result in less degradation of VWF multimers and thereby increased VWF activity. The aim of our study was to investigate the role of VWF/ADAMTS13 balance in DCI after aSAH.

## Patients and methods

### Patients and healthy donors

The Ethics Committee of the First Affiliated hospital of Soochow University approved the protocol of the present study, and informed written consents were obtained from all patients or their family members. Fifty eight patients with aneurysmal subarachnoid hemorrhage at the First Affiliated hospital of Soochow University,

Suzhou, China, between January 2012 and January 2014 were eligible for the study. Patients were excluded from the study if they were admitted for more than 72 hours or they had undergone surgery, interventional or conservative treatment outside the hospital; or were using of antiplatelet drugs such as aspirin, clopidogrel, or other anticoagulants such as warfarin, etc. Patients were also excluded if they had blood system diseases, or had impaired kidney or liver function, pregnant or breast-feeding, imminent death or with infections within the previous 2 weeks, treatment with radiotherapy, chemotherapy or immunodepressant drugs, blood transfusion before or during hospital stay. Patient characteristics are detailed in **Table 1**. Fifty eight patients were divided into delayed cerebral ischemia group (DCI group) and non-delayed cerebral ischemia group (no DCI group), or cerebral vasospasm group (CVS

group) and no vasospasm group (no CVS group), or good outcome group and poor outcome group. The control group consisted of twenty healthy people.

### Study design

Patients were admitted to the Intensive Care Unit. All patients were under continuous observation, with continuous monitoring of ECG, pulse rate, blood pressure, oxygen saturation, and respiratory frequency. Every hour, pupillary reflexes and level of consciousness were assessed by means of the Glasgow Coma Scale (GCS) score [16]. Blood pressure was measured at least every 4 hours. Medical treatment consisted of administration of oral nimodipine and intravenous fluids, which aimed at the maintenance of a neutral fluid balance. Non-steroidal anti-analgesic drugs were not used. Low molecular weight heparin was given in a prophylactic dose. Two observers independently assessed the occurrence of DCI. Clinical features of DCI were defined as a deterioration in consciousness (a decrease in the GCS Score by  $> 1$  point) or the appearance of focal signs that lasted for at least 1 hour and could not be explained by recurrent bleeding, epileptic seizures, hydrocephalus or metabolic disturbanc-

es [17]. Transcranial doppler sonography (TCD) criteria for vasospasm were defined by mean FVs > 120 cm/s and  $\geq 3$ -fold that of the internal carotid artery for anterior vessels and > 80 cm/s for the vertebrobasilar system. In all groups, TCD recordings were performed within the first 72 hours after admission and thereafter every day until the patients' discharge or TCD stabilization at normal FV values [18-21]. Outcome was classified according to the Glasgow Outcome Scale as good (grade 4 and 5) and poor (grade 1-3) [22].

#### *Blood samples*

Peripheral blood samples for measurement of VWF, ADAMTS13, P-selectin and IL-6 were taken 1 day after SAH (T1), (4 $\pm$ 1) days after SAH (T2), and (9 $\pm$ 1) days after SAH (T3). Sampled blood was collected into EDTA tubes and centrifuged at 3000 rpm for 10 min at 4°C immediately after sampling. Thereafter, plasma was stored at -70°C until all the samples were collected. Peripheral blood samples of 20 healthy volunteers were taken in control group too.

#### *ADAMTS13 activity measurement*

Monoclonal anti-VWFA1 antibody SZ-129, 5  $\mu$ g/ml in TBS was used to coat 96-well microtiter plates overnight at 4°C. After blocking and washing, 100  $\mu$ l of the diluted plasma samples were added in triplicate to the wells of the plates and incubated at 37°C for 2 hours. Binding antigen was detected using biotinylated-anti VWFA3 antibody SZ-125 at 1:2000 in PBS with 0.3% milk as previously described. Followed by HRP-labeled streptavidin diluted 1:200 in PBS with 0.3% milk. Wells were washed with PBS, 0.05% (v/v) Tween 20 (PBST) six times and developed with TMB. Plates were then read on an ELISA plate scanner at 450 nm, and data were analyzed as the fraction VWF antigen level remaining after denaturing compared to the VWF antigen level in the undenatured control plasma samples. One hundred percent minus residual VWF antigen level was arbitrarily regarded as the ADAMTS13 activity. At the same time the ADAMTS13 activity was assayed by the residual-collagen binding assay.

#### *The concentrations of VWF, P-selectin and IL-6*

In plasma we measured VWF antigen and P-selectin (ELISA using antibodies from He-

matology Center of Soochow University, China). The levels of IL-6 in plasma were measured with commercial quantitative sandwich enzyme-linked immunosorbent assay kits (Xitang of biotechnology, Corp., Shanghai, China). Standards were prepared, and the appropriate volume of sample or standard was added to a 96-well polystyrene microtiter plate precoated with monoclonal antibody to the VWF, P-selectin or IL-6. All samples and standards were run in duplicate. The plate was incubated for the recommended time. Each well was then aspirated, and the plates were washed with the buffered surfactant provided. An enzyme-linked polyclonal antibody against the VWF, P-selectin or IL-6 was then added, and again the plates were incubated and washed. Substrate solution was added to each well, and the optical density was read at the appropriate wavelength for each assay period. All values are reported as picogram or nanogram per milliliter.

#### *Statistical analysis*

Analyses were performed to use the statistical software package SPSS 13.0 (SPSS, Chicago, IL, USA). Data were expressed as mean  $\pm$  SD. Patient characteristics such as gender and age were evaluated by ANOVA with repeated measures. We used the Kruskal-Wallis rank sum test to compare Glasgow Outcome Scale among groups. The levels of VWF, P-selectin or IL-6 and the activity of ADAMTS13 were constructed by two-way repeated measures by ANOVA.  $P < 0.05$  was considered statistically significant

## **Results**

### *Overview*

Among 58 patients, 12 (20.7%) had DCI, 40 (68.9%) had TCD evidence of CVS, and 20 (34.5%) had poor outcome.

### *The activity of ADAMTS13 and the concentrations of VWF, P-selectin and IL-6 in patients with and without DCI*

The concentrations of VWF, P-selectin and IL-6 on T1, T2, T3 after SAH were significantly higher in DCI group as compared with those of the control group ( $P < 0.05$ ). The concentration of VWF on T3 and P-selectin on T1 were significantly higher in no DCI group as compared with those of the control group. The activity of ADAMTS13 on T1 was lower in DCI group as

## VWF/ADAMTS13 balance in aSAH

**Table 2.** The activity of ADAMTS13 and the concentrations of VWF, P-selectin and IL-6 in patients with and without DCI

	control group	DCI group (n=12)			no DCI group (n=46)		
	(n=20)	T1	T2	T3	T1	T2	T3
VWF (u/ml)	1.3±0.5	4.8±2.7 <sup>a,b</sup>	5.1±4.5 <sup>a,b</sup>	3.6±3.0 <sup>a</sup>	2.4±2.4	1.7±1.1	3.1±1.6 <sup>a</sup>
ADAMTS13 (%)	56.3±15.5	29.8±14.6 <sup>a,b</sup>	65.9±19.6	48.2±15.6	63.1±17.4	59.6±22.0	60.0±19.0
P-selectin (ng/ml)	17.4±8.1	58.1±24.0 <sup>a</sup>	39.3±14.4 <sup>a,b</sup>	25.6±14.9	41.1±24.7 <sup>a</sup>	27.2±12.4 <sup>a</sup>	23.2±12.9
IL-6 (pg/ml)	42.3±3.3	77.0±19.0 <sup>a,b</sup>	58.2±23.3 <sup>a</sup>	57.9±22.5 <sup>a</sup>	57.0±17.2 <sup>a</sup>	47.5±23.2	56.5±20.7 <sup>a</sup>

Note: Data presented as means ± SD. <sup>a</sup>P < 0.05 vs. controls, <sup>b</sup>P < 0.05 vs. no DCI group.

**Table 3.** The activity of ADAMTS13 and the concentrations of VWF, P-selectin and IL-6 in patients with and without CVS

	control group	CVS group (n=40)			no CVS group (n=18)		
	(n=20)	T1	T2	T3	T1	T2	T3
VWF (u/ml)	1.3±0.5	3.0±2.7 <sup>a</sup>	3.9±1.3 <sup>a,b</sup>	4.0±2.0 <sup>a,b</sup>	2.0±1.2 <sup>a</sup>	2.7±1.7 <sup>a</sup>	1.5±0.8
ADAMTS13 (%)	56.3±15.5	53.6±24.5	60.8±22.1	55.6±18.5	61.9±11.9	61.0±19.6	61.8±19.7
P-selectin (ng/ml)	17.4±8.1	49.5±23.0 <sup>a</sup>	42.6±15.6 <sup>a,b</sup>	47.1±14.1 <sup>a,b</sup>	33.7±17.7 <sup>a</sup>	23.3±11.4	21.6±11.3
IL-6 (pg/ml)	42.3±3.3	72.7±30.3 <sup>a</sup>	59.6±26.2 <sup>a,b</sup>	63.1±26.6 <sup>a</sup>	61.0±18.1 <sup>a</sup>	47.9±12.4	53.9±13.3 <sup>a</sup>

Note: Data presented as means ± SD. <sup>a</sup>P < 0.05 vs. controls, <sup>b</sup>P < 0.05 vs. no CVS group.

**Table 4.** The activity of ADAMTS13 and the concentrations of VWF, P-selectin and IL-6 in patients with good and poor outcome

	control group	good outcome group (n=38)			poor outcome group (n=20)		
	(n=20)	T1	T2	T3	T1	T2	T3
VWF (u/ml)	1.3±0.5	2.0±1.5 <sup>b</sup>	2.0±0.8 <sup>a,b</sup>	2.1±1.0 <sup>a,b</sup>	4.5±3.6 <sup>a</sup>	3.4±1.1 <sup>a</sup>	3.6±1.4 <sup>a</sup>
ADAMTS13 (%)	56.3±15.5	62.0±16.1 <sup>b</sup>	58.2±21.3	63.1±19.5 <sup>b</sup>	41.1±22.7 <sup>a</sup>	65.9±21.7	47.2±12.3
P-selectin (ng/ml)	17.4±8.1	32.9±10.0 <sup>a,b</sup>	28.5±14.6 <sup>a</sup>	22.5±12.1 <sup>b</sup>	66.9±23.0 <sup>a</sup>	31.9±18.6 <sup>a</sup>	44.8±16.1 <sup>a</sup>
IL-6 (pg/ml)	42.3±3.3	49.2±23.3 <sup>b</sup>	54.8±20.4 <sup>a,b</sup>	52.5±20.4 <sup>a</sup>	69.0±25.3 <sup>a</sup>	74.3±29.9 <sup>a</sup>	56.1±17.1 <sup>a</sup>

Note: Data presented as means ± SD. <sup>a</sup>P < 0.05 vs. controls, <sup>b</sup>P < 0.05 vs. poor outcome group.

compared with those of the control group ( $P < 0.05$ ). The concentrations of VWF on T1 and T2, P-selectin on T2 and IL-6 on T1 were significantly higher in DCI group as compared with those of the no DCI group ( $P < 0.05$ ). The activity of ADAMTS13 on T1 was lower in DCI group as compared with those of the no DCI group ( $P < 0.05$ , **Table 2**).

*The activity of ADAMTS13 and the concentrations of VWF, P-selectin and IL-6 in patients with and without CVS*

The concentrations of VWF, P-selectin and IL-6 on T1, T2, T3 after SAH were significantly higher in CVS group as compared with those of the control group ( $P < 0.05$ ). The concentrations of VWF on T1 and T2, P-selectin and IL-6 on T1 were significantly higher in no CVS group as compared with those of the control group ( $P <$

0.05). The concentrations of VWF and P-selectin on T2, T3 and IL-6 on T2 were significantly higher in CVS group as compared with those of the no CVS group ( $P < 0.05$ ). The activity of ADAMTS13 showed no significant difference between CVS group and no CVS group ( $P > 0.05$ , **Table 3**).

*The activity of ADAMTS13 and the concentrations of VWF, P-selectin and IL-6 in patients with good and poor outcome*

The concentrations of VWF, P-selectin and IL-6 on T1, T2 and T3 after SAH were significantly higher in good outcome group and poor outcome group compared with those of the control group ( $P < 0.05$ ). The activity of ADAMTS13 on T1 was lower in poor outcome group as compared with that of the control group ( $P < 0.05$ ). The concentration of VWF on T1, T2 and T3,

P-selectin on T1 and T3 and IL-6 on T1 and T2 were significantly lower in good outcome group as compared with those of the poor outcome group ( $P < 0.05$ ). The activity of ADAMTS13 on T1 and T3 was higher in good outcome group as compared with that of the poor outcome group ( $P < 0.05$ , **Table 4**).

## Discussion

The present study demonstrated that the balance of VWF/ADAMTS13 was broken as the activity of VWF increased and activity of ADAMTS13 decreased in plasma which associated with DCI, and the decreased ADAMTS13 activity can predict the outcome. The endothelium dysfunction and the unbalanced VWF/ADAMTS13 were correlated with the occurrence of DCI and poor clinical outcome.

VWF is a plasma glycoprotein which is a mediator of platelet adhesion, becoming available when the endothelium is damaged [11, 23]. VWF is released as ultralarge (UL) multimers which results in platelet aggregation and thrombus formation [12]. The concentration and activity of VWF are influenced by several factors, including inflammation and proteolysis by ADAMTS13 [12, 14, 23, 24]. Thus we considered the balance of VWF/ADAMTS13 would be a meaningful indicator. To our knowledge, this is the first study to determine the balance of VWF/ADAMTS13 in patients with DCI and clinical outcome. We observed that levels of ADAMTS13 activity were somewhat lower and the levels of VWF were significantly higher in DCI, CVS and poor outcome groups. Proteolytic cleavage of VWF by ADAMTS13 into less active, smaller multimers determines the activity levels of VWF in plasma. We therefore hypothesized that the lower ADAMTS13 activity might be associated with the higher level of VWF and the risk of DCI, CVS and poor outcome. Some studies indicate that inflammation may play a role in determining levels of VWF. But it should not be the only important regulatory mechanism since VWF levels are independent of inflammation. It is also associated with the relative risk of ischemic stroke [12, 25]. ADAMTS13 could clean hyperactive VWF, and down-regulates not only thrombosis but also inflammation [13, 14]. ADAMTS13 plays a key role in down-regulating inflammation by preventing excessive leukocyte rolling in unstimulated veins, and leukocyte adhesion and extravasation

under inflammatory conditions and ADAMTS13 forms a link between thrombosis and inflammation [13, 14]. The reason of lower ADAMTS13 activity was still unclear, so we could only speculate about the underlying mechanism: low levels of ADAMTS13 resulting in higher levels of VWF, or increased VWF levels excesses consumption of ADAMTS13, which resulted in lower levels of ADAMTS13. The disbalance of VWF/ADAMTS13 resulted in poor outcome in our study.

P-selectin is a transmembrane glycoprotein expressed on activated vascular endothelium, activated platelets and leucocytes, and is involved in rolling and activation of leucocytes [26-28]. P-selectin concentration was significantly higher in those patients with DCI, CVS and poor outcome in our study. It is unknown in our study whether the origin of P-selectin is platelet-derived or endothelial-derived. Further research is needed to ascertain the origin of the P-selectin. On the basis of serum concentrations of P-selectin, we concluded that P-selectin may be involved in the pathophysiology of DCI after aSAH [29].

IL-6 is a proinflammatory cytokines and is released during inflammation [30, 31]. IL-6 has been shown to have distinct effects on endothelial release of VWF and its processing [32]. Recently, it has been shown in vitro that VWF promotes leukocyte adhesion and platelets bound to VWF can support leukocyte tethering and rolling under high shear stress [33]. We found that the concentrations of IL-6 were significantly higher in DCI, CVS and poor outcome groups. The higher concentration of IL-6 could also promote the endothelial release of VWF. This reaction also broke the balance of VWF/ADAMTS13.

There were two major limitations relevant to the interpretation of the results in the present study. First, there were only 58 patients in our study. The number of patients in each group was small. Second, we only observed three time point but did not find the dynamic change of VWF/ADAMTS13, P-selectin and IL-6.

## Conclusion

The results of our study suggested that the increased VWF and decreased ADAMTS13 activity were associated with DCI and poor outcome. The balance of VWF/ADAMTS13 could



be used to predict the clinical outcome. The endothelium dysfunction and platelet activation were correlated with the occurrence of DCI and clinical outcome. The deficiency of ADAMTS13 could not only induce DCI but also accelerate inflammatory reaction. Our results reported in this paper might provide new insights into the possible uses of ADAMTS13 as a therapeutic agent in aneurysmal subarachnoid hemorrhage.

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### Disclosure of conflict of interest

None.

### Abbreviations

DCI, delayed cerebral ischemia; aSAH, aneurysmal subarachnoid hemorrhage; CVS, cerebral vasospasm; VWF, von Willebrand factor; ADAMTS13, disintegrin-like and metalloprotease with thrombospondin type I repeats-13; ELISA, enzyme-linked immunosorbent assay; TCD, transcranial doppler sonography; VMCA, mean blood flow velocity of the middle cerebral artery; IL-6, Interleukin-6.

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