Delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage: the role of coagulation and fibrinolysis
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Serial changes in von Willebrand factor and ADAMTS13 in patients with and without delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage

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Submitted
Abstract

Background and purpose
The pathogenesis of delayed cerebral ischemia (DCI) after aneurysmal subarachnoid hemorrhage (SAH) has not yet been elucidated. Recent studies suggest that besides vasospasm microthrombosis plays an important role. Since in patients with thrombotic thrombocytopenic purpura an ADAMTS13 deficiency leads to higher concentrations of large von Willebrand Factor (vWF) multimers which results in microthrombosis, our purpose was to compare vWF and ADAMTS13 in patients with and without DCI after aneurysmal SAH.

Methods
Blood samples were obtained at standard intervals after SAH. DCI was defined as the gradual onset of new focal neurological impairment and/or a decreased level of consciousness of at least 2 points as recorded on the Glasgow Coma Scale. In plasma we measured vWF antigen, vWF propeptide, vWF ristocetin cofactor activity, and ADAMTS13 activity.

Results
Thirty-one patients were included. Eleven patients (35%) developed DCI. No differences were observed in baseline characteristics between patients with and without DCI. Patients with DCI had a more profound increase of vWF antigen, vWF propeptide and vWF activity in the first few days after the hemorrhage, and a stronger decrease of ADAMTS13 activity (p-values for difference in polynomial time trend 0.020, 0.004, 0.188, and 0.0001, respectively). VWF antigen was strongly correlated to both vWF propeptide ($r=0.765$, $p<0.001$) and vWF activity ($r=0.88$, $p<0.001$). The decrease in ADAMTS13 activity was independent of the increase in vWF ($r=-0.027$, $p=0.736$).

Conclusions
Our results suggest that microthrombosis plays an important role in the pathogenesis of DCI, as a result of endothelium dysfunction and decreased ADAMTS13 activity.
Introduction

Delayed cerebral ischemia (DCI) is a common complication after aneurysmal subarachnoid hemorrhage (SAH), which occurs in approximately 30% of patients. Although DCI is common, the pathogenesis of DCI has not yet been elucidated. Clinical signs and symptoms of DCI are often attributed to vasospasm shown by angiography. However, DCI can occur in the absence of vasospasm, and, conversely, severe vasospasm can occur in the absence of symptoms of DCI. Therefore, several alternative explanations for DCI have been postulated, such as microthrombosis, microvascular spasm, and cortical spreading ischemia.

Von Willebrand factor (vWF) is a glycoprotein which induces platelet adhesion and aggregation at sites of vascular injury or under stress conditions. VWF is secreted as UltraLarge von Willebrand Factor (ULvWF) multimers from Weibel-Palade bodies in the vascular endothelium and from α-granules of platelets. Under normal circumstances ULvWF is cleaved by a protease called A Disintegrin And Metalloprotease with ThromboSpondin repeats-13 (ADAMTS13). Since large vWF multimers are more potent mediators of platelet thrombus formation than small vWF multimers, cleavage of ULvWF by ADAMTS13 results in lower-molecular weight vWF forms with reduced adhesive and aggregation potential. In contrast, an ADAMTS13 deficiency, such as in patients with thrombotic thrombocytopenic purpura (TTP), leads to higher concentrations of ULvWF, which results in microthrombosis followed by ischemic complications, such as cerebral infarction.

Since in patients with aneurysmal SAH microthrombosis might play a role in the pathogenesis of DCI, the purpose of the present study was to investigate whether a similar pathogenesis of microthrombi, as has been demonstrated in TTP, is also present in SAH. Therefore, we compared serial measurements of vWF and ADAMTS13 in patients with and without DCI after aneurysmal SAH.

Methods

We used plasma samples from an exploratory single-center, prospective, randomized, double-blind, placebo-controlled trial investigating the effects of simvastatin on endothelial function, coagulation, fibrinolysis, and inflammation in patients with aneurysmal subarachnoid hemorrhage. This study was registered in the International Standard Randomised Controlled Trial registry (ISRCTN45662651) and approved by the local Institutional Review Board. In this study, use of simvastatin did not influence any parameters of endothelial function (including von Willebrand factor antigen), coagulation, fibrinolysis, and inflammation, and no effect was observed on vasospasm as detected with transcranial Doppler (TCD) or clinical signs and symptoms of DCI. Therefore, all patients of the study, except one patient who had no aneurysm on angiography, were included in the present study.
Patients

Patients with signs and symptoms of aneurysmal SAH admitted to the Academic Medical Center, Amsterdam, The Netherlands, were included if a computed tomography (CT) scan showed an aneurysmal or perimesencephalic bleeding pattern, in combination with the presence of an appropriate aneurysm at angiography, and if written informed consent was obtained. Exclusion criteria were: 1) under 18 years of age; 2) if death appeared imminent; 3) patients using aspirin, warfarin, or statins; 4) more than 72 hours after SAH; 5) contra-indication for simvastatin (active liver disease, liver alanine aminotransferase or aspartate aminotransferase more than 3 times the normal upper limit, myopathy); 6) kidney insufficiency; 7) pregnancy or lactation. All patients received standard care including treatment with nimodipine 360 mg a day orally (60 mg every 4 hours). Initiation of hypertension and hypervolemia therapy was at the discretion of the treating neurosurgeon, according to the Academic Medical Center Subarachnoid Hemorrhage Treatment-guideline to which all neurologists, neurosurgeons, and intensive care physicians adhere.

At baseline, several baseline characteristics were recorded such as age, sex, Glasgow Coma Scale (GCS), presence of focal neurological deficits, loss of consciousness during ictus, warning leak, blood pressure at admission, smoking, history of hypertension, and location of aneurysm.

DCI was defined as the gradual onset of new focal neurological impairment and/or a decreased level of consciousness of at least 2 points as recorded on the Glasgow Coma Scale, either with cerebral infarction on CT scan compatible with clinical presentation or proven at autopsy, or in case no CT scan or autopsy was obtained, suspect for infarction with exclusion of other causes by appropriate laboratory studies. Events were scored by one investigator (MDIV), and in case of uncertainty discussed with another investigator (YBWEMR).

Ancillary investigations

The amount of blood on admission CT scan was calculated using the Hijdra score. Blood withdrawals were performed at six standardized moments during hospitalization: 2±1, 4±1, 7±1, 10±1, 14±1, and 17±1 days after SAH, between 8 AM and 9.30 AM avoiding the effect of diurnal fluctuations in blood parameters. During the first blood withdrawal at day 2, and the last at day 17 after SAH, patients did not use study medication. All blood withdrawals were performed by the same investigator (MDIV). In case of hospital discharge before day 17 after SAH, patients did not return to the hospital for the remaining blood withdrawals. After withdrawal, blood was directly processed and stored in a -80 degrees Celsius freezer, until laboratory analyses were performed. In citrate plasma we measured von Willebrand factor antigen (ELISA using antibodies from DAKO, Glostrup, Denmark), von Willebrand factor propeptide (ELISA from Sanquin, Amsterdam, the Netherlands), von Willebrand factor ristocetin cofactor activity (BC von Willebrand, Dade Behring, Marburg, Germany), and ADAMTS13 activity. Measurements of prothrombin fragment F1+2 (Dade Behring, Marburg, Germany) were performed by ELISA. Interleukin (IL)-6 and TNF-α were measured
ADAMTS13 activity is associated with DCI

Statistics and analyses

To investigate differences in baseline characteristics between patients with and without DCI we used the unpaired *t*-test, Mann-Whitney test, *χ*², or Fisher’s exact test where appropriate.

The possible differences in vWF antigen, vWF propeptide, vWF cofactor activity, and ADAMTS13 activity over time between the groups of patients with and without DCI were investigated with a linear random effects model, using the nlme package in the R statistical program. Since in the analyses of vWF antigen, vWF propeptide, and vWF cofactor activity levels first increased and than decreased over time, and in the analysis of ADAMTS13 activity first decreased and then increased, a polynomial trend over time was assumed.
which was allowed to differ by treatment group. Furthermore, since ADAMTS13 activity might be influenced by IL-6, TNF-α, and thrombin, the associations of these parameters (for thrombin the related parameter prothrombin fragment 1+2 was used) with ADAMTS13 were investigated by calculation of correlation coefficients, using the measurements at all time points simultaneously. We used Pearson or Spearman’s correlation coefficients depending on the observed distribution of the markers. In the same way, the associations between vWF antigen and vWF cofactor activity, vWF antigen and vWF propeptide, and vWF antigen and ADAMTS13 activity were investigated.

**Figure 1.** Boxplots showing plasma levels of patients with and without DCI: A) vWF antigen; B) vWF propeptide; C) vWF cofactor activity; D) ADAMTS13 activity.

Legends: On X-axis consecutive blood withdrawals. Symptoms of DCI started at a median of 6 days (range 4-10 days).
Results

After written informed consent was obtained, 31 patients were included in the present study. Baseline characteristics are listed in the Table. In twenty-four patients (77%) the aneurysms were coiled and in 7 patients (23%) clipped. Median day of aneurysm treatment after SAH was day one (range 0-30).

Eleven patients (35%) developed DCI. Signs of DCI started at a median of 6 days (range 4-10 days). No differences were observed in baseline characteristics between patients with and without DCI occurrence, although patients with DCI had, as expected, a higher Hijdra score on admission CT scan (p=0.06).

In patients who developed DCI vWF antigen, vWF propeptide, and vWF activity showed a more profound increase in the first few days, however for vWF activity this difference was not significant (p-values for difference in polynomial time trend 0.020, 0.004 and 0.188 respectively) (Figure 1A-C). Patients with DCI showed a stronger decrease of ADAMTS13 in the first few days after SAH (p-value for difference in polynomial time trend 0.0001) (Figure 1D).

Several correlations were investigated. No correlation was found between ADAMTS13 and IL-6 (Spearman correlation coefficient r=−0.033, p=0.676), or between ADAMTS13 and prothrombin fragment 1+2 (Spearman correlation coefficient r=−0.38, p=0.634). Since in all plasma samples TNF-α levels were below the detection level, no correlation between ADAMTS13 and TNF-α was calculated. VWF antigen was strongly correlated to both vWF propeptide (Spearman correlation coefficient r=0.765, p<0.001) and vWF activity (Pearson correlation coefficient r=0.88, p<0.001) (Figures 2A and 2B). No indication of correlation between vWF antigen and ADAMTS13 was found (r=−0.027, p=0.736) (Figure 2C).

Discussion

The results of our study show that patients with DCI after aneurysmal SAH have a significantly different development of vWF and ADAMTS13 over time compared to patients without DCI. Patients with DCI had a more profound increase of vWF antigen, vWF propeptide, and vWF activity in the first few days after the hemorrhage, although for vWF activity this difference was not significant. However, vWF antigen was strongly correlated to both vWF propeptide and vWF activity. Furthermore, the results of the present study show that patients with DCI had a stronger decrease of ADAMTS13. Interestingly, the decrease in ADAMTS13 activity could not be explained by an increase of vWF antigen, since there was no correlation between vWF antigen and ADAMTS13 activity. In addition, the decrease in ADAMTS13 activity could not be explained as a result of suppression by IL-6, TNF-α, or thrombin (investigated using the substitute parameter prothrombin fragment 1+2).
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**Figure 2A.** Relation between vWF antigen and vWF propeptide

**Figure 2B.** Relation between vWF antigen and vWF ristocetin cofactor activity

**Figure 2C.** Relation between vWF antigen and ADAMTS13 activity
ADAMTS13 activity is associated with DCI

ADAMTS13 has never been studied before in patients with aneurysmal SAH. However, some studies investigated ADAMTS13 in ischemic stroke. In a case-control study of 124 first-ever ischemic stroke patients and 125 age- and sex-matched controls, vWF antigen and vWF activity were associated with the occurrence of acute ischemic stroke. This association was not affected by ADAMTS13 activity. Recently, it was observed mice with an ADAMTS13 deficiency had approximately 20% larger infarcts after induction of cerebral ischemia. Interestingly, infusion of recombinant human ADAMTS13 immediately before reperfusion two hours after occlusion significantly reduced infarct volume with approximately 30%. These data support our current findings of a temporary dysbalance between vWF and ADAMTS13.

The results of the present study add to an accumulating body of evidence that DCI cannot be fully explained by vasospasm. This study shows that microthrombosis plays a more important role in the pathogenesis of DCI than is generally accepted. Since von Willebrand factor is a parameter of endothelium function and an important contributor of thrombus formation, the results of the present study suggest that endothelium dysfunction and the hemostatic system are involved in the development of DCI. The mechanism by which ADAMTS13 decreases in patients with DCI remains to be elucidated. A plausible explanation might be that an increased use of ADAMTS13 results from higher vWF antigen levels, however in our study no correlation was found between ADAMTS13 activity and vWF antigen. Previously it has been shown that thrombin and plasmin potentiate the proteolytic inactivation of ADAMTS13, and therefore another explanation might be that ADAMTS13 activity is suppressed by thrombin and plasmin present in the subarachnoid space after the hemorrhage. However, in our study no correlation was found between ADAMTS13 activity and prothrombin fragment 1+2, and therefore it is unlikely that the decrease of ADAMTS13 activity in DCI is caused by thrombin. ADAMTS13 activity might also be suppressed by IL-6, since IL-6 inhibits the cleavage of ULvWF by ADAMTS13 under flow conditions. In patients with aneurysmsal SAH increased levels of IL-6 are associated with DCI. However, in our study no correlation was found between ADAMTS13 activity and IL-6. Finally, decreased ADAMTS13 activity might be caused by locally present neutralizing antibodies against ADAMTS13, however ADAMTS13 antibodies have never been studied in patients with SAH.

A possible limitation of our study is that only systemic levels of biomarkers were assessed instead of local levels from the cerebral circulation. Therefore, the results might not be a good representation of pathophysiological processes in the cerebral circulation. On the other hand, our results may also be interpreted as diluted values of locally increased levels, and therefore an underestimation of the local pathophysiological processes. Another limitation is that our study was not designed to measure vWF and ADAMTS13 levels at the days of clinically manifest DCI. Therefore, many blood samples were not taken on the day of first signs of DCI, and our data only indicate that vWF antigen level increases and ADAMTS13 decreases in the time period that DCI develops.
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We conclude that patients with DCI after aneurysmal SAH show a more profound increase of vWF antigen and vWF propeptide levels, and a stronger decrease in ADAMTS13 activity within the first days after the hemorrhage compared to patients without DCI. Although the results of the present study do not prove a causal relationship between decreased ADAMTS13 activity and the pathogenesis of DCI, our results suggest that microthrombosis plays an important role in the pathogenesis of DCI, as a result of endothelial dysfunction.
ADAMTS13 activity is associated with DCI

References


