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Relationship between ADAMTS13 activity, von Willebrand factor antigen levels and platelet function in the early and late phases after TIA or ischaemic stroke

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Abstract

**Background:** Reduced ADAMTS13 activity is seen in thrombotic thrombocytopenic purpura (TTP), and may lead to accumulation of prothrombotic ultra-large von Willebrand factor (ULVWF) multimers *in vivo*. ADAMTS13 activity and its relationship with VWF antigen (VWF:Ag) levels and platelet function in ‘non-TTP related’ TIA or ischaemic stroke has not been comprehensively studied.

**Methods:** In this prospective pilot observational analytical case-control study, ADAMTS13 activity and VWF:Ag levels were quantified in platelet poor plasma in 53 patients in the early phase (≤4 weeks) and 34 of these patients in the late phase (≥3 months) after TIA or ischaemic stroke on aspirin. Data were compared with those from 22 controls not on aspirin. The impact of ADAMTS13 on platelet function in whole blood was quantified by measuring Collagen-ADP (C-ADP) and Collagen-Epinephrine closure times on a platelet function analyser (PFA-100®).

**Results:** Median ADAMTS13 activity was significantly reduced in the early phase (71.96% vs. 95.5%, P < 0.01) but not in the late phase after TIA or stroke compared with controls (86.3% vs. 95.5%, P = 0.19). There was a significant inverse relationship between ADAMTS13 activity and VWF:Ag levels in the early phase (r = -0.31; P = 0.024), but not in the late phase after TIA or stroke (P = 0.74). There was a positive correlation between ADAMTS13 activity and C-ADP closure times in early phase patients only, likely mediated via VWF:Ag levels.

**Discussion:** ADAMTS13 activity is reduced and VWF:Ag expression is increased within 4 weeks of TIA or ischaemic stroke onset, and can promote enhanced platelet adhesion and aggregation in response to stimulation with collagen and ADP via VWF-mediated pathways. These data improve our understanding of the dynamic haemostatic and thrombotic profiles of ischaemic cerebrovascular disease (CVD) patients, and are important in view of the potential future role that ADAMTS13 may have to play as an anti-thrombotic agent in CVD.
Introduction

Von Willebrand factor (VWF) is a multimeric plasma glycoprotein synthesised in vascular endothelial cells and megakaryocytes. (1, 2) VWF is stored as a mixture of multimers in the α-granules of platelets, and as ultra-large multimers in Weibel-Palade bodies of endothelial cells. (3) After release in response to injury or inflammation, VWF may bind to GP1b-IX-V or αIIbβ3 receptors on platelets, and promote platelet adhesion, aggregation and subsequent thrombus formation. If not consumed immediately, ultra-large VWF is cleaved by ADisintegrin-like And Metalloprotease with ThromboSpondin type I repeats 13 (ADAMTS13) into smaller, less adhesive multimers that circulate in plasma. (2) Deficiency of ADAMTS13 activity is observed in thrombotic thrombocytopenic purpura (TTP), (4) and is associated with accumulation of prothrombotic ultra-large von Willebrand factor (ULVWF) multimers. Murine stroke models have suggested that reduced ADAMTS13 activity significantly aggravates ischaemic brain injury. (5, 6) A number of studies have identified either low ADAMTS13 antigen levels or activity in coronary artery disease (7-9) or at a single timepoint after ischaemic stroke, (10-12) but other studies have not confirmed these findings. (1) Increased VWF levels have been reported in ischaemic cerebrovascular disease (CVD) patients, (1, 13-15) and may cause platelet hyper-reactivity and shortened ‘closure times’ on a high shear stress-dependent platelet function analyser called the PFA-100®. (16-18) ADAMTS13 activity has been shown to be inversely correlated with VWF antigen levels. (19) but to our knowledge, the impact of ADAMTS13 activity on platelet function on the PFA-100® in patients in both the early and late phases after TIA and stroke has not been studied.
The aims of this study were to assess ADAMTS13 activity in both the early and late phases after ‘non-TTP related’ TIA or ischaemic stroke, to assess the relationship between ADAMTS13 activity and VWF antigen (VWF:Ag) levels in CVD patients, and to determine whether ADAMTS13 activity independently influences platelet reactivity in CVD. We hypothesised that ADAMTS13 activity would be reduced in CVD patients versus controls, and that ADAMTS13 activity would correlate with VWF:Ag levels and platelet reactivity in ischaemic CVD.

Methods

CVD Patient Inclusion Criteria

Consecutive eligible patients referred by General Practitioners or Consultant colleagues to our secondary and tertiary referral stroke prevention outpatient clinic or inpatient stroke neurology or liaison neurology service at the National Hospital for Neurology and Neurosurgery, University College London Hospitals, UK were recruited to this prospective pilot observational analytical case-control study. Patients were included if they were older than 18 years of age, had experienced a TIA or ischaemic stroke within the preceding 4 weeks (early phase), had been commenced on aspirin, and were likely to be available for clinical and laboratory follow-up at least 3 months after symptom onset (late phase). The study was approved by the Local Research Ethics Committees of the participating Hospitals. Written informed consent (or written assent, where appropriate) was obtained in all cases.
CVD Patient Exclusion Criteria

We excluded patients who were on another antiplatelet or non-steroidal anti-inflammatory drug in combination with aspirin, or those receiving heparin or warfarin. Patients were also excluded if they had a history of primary intracerebral haemorrhage, myocardial infarction within the preceding 3 months, on-going unstable angina, unstable symptomatic peripheral vascular disease, major surgery or systemic haemorrhage within the preceding 3 months, or if they had systemic vasculitis, underlying neoplasia, or a known bleeding or clotting diathesis, including TTP.

One examiner (DJHM) clinically assessed all subjects at each timepoint, and information regarding vascular risk factors, smoking status, alcohol intake, and medication use was collected prospectively. Results of routine haematological, coagulation, biochemical and blood glucose testing were collected prospectively. All CVD patients had a brain CT or MRI, and colour Doppler ultrasound examination of carotid 18 and vertebral arteries. Some patients underwent extra- or intra-cranial magnetic resonance angiography or intra-arterial catheter angiography, if deemed appropriate by the treating physician. A chest radiograph and an electrocardiograph (ECG) were obtained in all patients. A 24-hour ECG recording was performed if paroxysmal atrial fibrillation or flutter was suspected clinically, and transthoracic ± transoesophageal echocardiography was also carried out if a cardioembolic cause for stroke or TIA was suspected, or when other investigations were uninformative, as previously described.(18) The underlying mechanism responsible for the TIA or ischaemic stroke was categorised according to slight modifications of the TOAST classification as large artery atherosclerotic, lacunar, cardio-
embolic, other determined, and undetermined aetiology (Table 2). (20) For the purpose of this study, large artery atherosclerotic TIA or stroke specifically referred to TIA or stroke in the vascular territory supplied by a severe (>70%) ipsilateral extracranial carotid stenosis or occlusion to comply with inclusion criteria for other ongoing collaborative studies in our laboratory. The late phase follow-up in the large artery atherosclerotic subgroup was performed ≥3 months after carotid surgery or endovascular treatment, unless this treatment had been delayed for at least 3 months after the initial event.

**Control Subjects Inclusion and Exclusion Criteria**

Control subjects were recruited from the staff at The Haemostasis Research Unit, University College London Hospitals, from the local population, and from amongst spouses of patients and control subjects. Subjects were excluded from the control group if they had a history of known vascular disease, or evidence of asymptomatic severe (>70%) carotid or vertebral artery stenosis on colour Doppler ultrasound imaging. Otherwise, the exclusion criteria were identical to those applied to the patient group. It was expected that control subjects would have a different vascular risk profile to the patient population.

**Blood Sampling and Laboratory Tests**

All subjects were rested for at least 20 minutes before venepuncture, and free-flowing blood was collected using a 21-G Butterfly needle (Venisystems™, Abbott, Ireland) and a Vacutainer® system with a luer adaptor (Becton-Dickinson Vacutainer Systems, UK). For all studies, the tourniquet was released during collection of the first 4ml of blood into a sterile Vacutainer tube.
containing 7.2mg of K$_2$ EDTA or 0.054ml of 15% K$_3$ EDTA. This sample was used for measurement of a full blood count (FBC). Four further 4.5ml samples were collected into sterile Vacutainer tubes containing 0.5ml of 3.2% buffered sodium citrate. The first 4.5ml citrate-anticoagulated whole blood sample was used for measurement of platelet reactivity and closure times on the PFA-100® in response to stimulation with Collagen-ADP (C-ADP) and Collagen-Epinephrine (C-EPI) between 2 and 2.5 hours after venepuncture, as previously described.(18) The maximum closure time recorded by the device is 300s, and we arbitrarily defined closure times >300s as 301s.(18)

The next two citrated samples were used to prepare double-centrifuged (2000g x 15 minutes) platelet-poor plasma (PPP) that was immediately stored at -70°C for later analysis. VWF antigen levels were measured in PPP with an automated latex agglutination assay (STA Liatest VWF, DiagnosticaStago, Asnsieres, France), modified for use on a Sysmex CA-1500™ fully-automated coagulometer (Sysmex UK Ltd, Milton Keynes, UK) using Coagulation Reference Plasma (Technoclone, Vienna, Austria) to prepare a standard curve.(18)

ADAMTS13 activity in double-centrifuged PPP was quantified using a collagen binding assay described by Yarranton _et al._, 2005, and modified from Gerritsen _et al._, 1999.(21, 22) Samples were analysed in batches at the end of the study period, with no sample stored for longer than 72 months. Freeze-thawing of samples was avoided by storing samples in multiple, separate aliquots. After dilution of the plasma, ADAMTS13 was activated by incubation at 37°C for 5 min with 10 mmol/L barium chloride and 0.9mmol/L Pefabloc SC® (Sigma-Aldrich, Poole, UK). Immediately after activation, VWF concentrate (final concentration = 0.561 IU/mL; French Laboratory of Fractionation and Biotechnology, Lille, France) was added and the samples
incubated overnight in the presence of urea (final concentration = 1.36 mol/L) at 37°C. The reaction was stopped by adding disodium-EDTA (final concentration = 0.1 mol/L). Samples were then diluted and added to a collagen-coated microtitre plate. The residual high molecular weight multimers of VWF bound to collagen on the plates and were detected with an anti-VWF antibody conjugated to HRP (Dako), followed by detection with 1,2-ortho-phenylenediamine substrate (Sigma-Aldrich). The results were expressed relative to the ADAMTS13 activity of pooled normal plasma. Although not performed under typical physiological conditions, this assay has been shown to be sensitive and reproducible, with a typical normal range of 101.6 ± 49.4%, and intra-and inter-assay coefficients of variation calculated as 3% and 7.2%, respectively.(23)

In an exploratory sub-study, all patients with **reduced ADAMTS13 activity** (ADAMTS13 activity at least 2 standard deviations below values obtained from PNP in our laboratory i.e. ADAMTS13 activity < 66%) had additional commercial assays performed to quantify plasma ADAMTS13 antigen levels, and to look for the presence of auto-antibodies to ADAMTS13. Antigen levels were measured with an Imubind® ADAMTS13 ELISA kit, and anti-ADAMTS13 IgG auto-antibodies were quantified with an Imubind® ADAMTS13 auto-antibody ELISA kit (American Diagnostica, Stamford, CT, USA), according to the manufacturer’s instructions.(24)

**Statistical Methodology**

The Mann–Whitney U-test was used for comparison of median values, an unpaired t-test for comparison of mean values, and one-way ANOVA for comparison of ADAMTS13 activity
between stroke subtypes, where appropriate. Analysis of covariance (ANCOVA) was employed to examine the relationship between ADAMTS13 and VWF:Ag levels, and to control for the effects of potential confounding variables, where appropriate. Linear regression analysis examined the relationship between plasma VWF:Ag levels and PFA-100® closure times. Stepwise regression assessed the impact of ADAMTS13 activity on PFA-100® closure times whilst controlling for VWF:Ag levels. P < 0.05 was considered statistically significant. All statistical calculations were performed with Minitab 6.0 for Windows.

Results

Between May 1999 and July 2001, 53 patients were assessed in the early phase (range: 0 – 27 days) after TIA (N = 3) or ischaemic stroke (N = 50) with complete clinical and laboratory follow-up data available in 34 of these patients in the late phase (≥3 months) after symptom onset (range: 90-725 days). The interval between symptom onset and venepuncture was lower than in other studies of ADAMTS13 in ischaemic stroke,(1, 10-12) with mean times to venepuncture of 7.85 (+/- 5.3) days in early phase CVD patients, and 160.3 (+/- 76.8) days in the late phase CVD group. The clinical details of the study subjects are outlined in tables 1 and 2.

CVD patients were older (P<0.001), and as expected, vascular risk factors were more common in CVD patients than controls (table 1). In particular, hypertension and a definite diagnosis of hyperlipidaemia were more common in early and late phase CVD patients, and the proportion of smokers was higher in late phase patients than controls. No study subject had a platelet count < 150 x 10⁹/L, and none had any other clinical features of TTP.
Table 1: Demographic Data and Vascular Risk Factor Profile of Study Subjects at Enrolment

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Early (n = 53)</th>
<th>P Value</th>
<th>Late (n=34)</th>
<th>P Value</th>
<th>Controls (n=22)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>66.7 ±[12.71]</td>
<td>0.001</td>
<td>66.29 ±[11.04]</td>
<td>0.0052</td>
<td>58.14 ± [10.64]</td>
</tr>
<tr>
<td>Sex (% Female)</td>
<td>47%</td>
<td>0.059</td>
<td>44%</td>
<td>0.06</td>
<td>68%</td>
</tr>
<tr>
<td>Stroke at presentation</td>
<td>50 (94.3%)</td>
<td>0.966</td>
<td>49 (92.45%)</td>
<td>N/A</td>
<td>0</td>
</tr>
<tr>
<td>Prior Stroke/TIA</td>
<td>14(26.4%)</td>
<td>&lt;0.001</td>
<td>3(8.8%)</td>
<td>0.271</td>
<td>0</td>
</tr>
<tr>
<td>Ischaemic Heart Disease Hypertension</td>
<td>8(15.1%)</td>
<td>0.869</td>
<td>4(11.8%)</td>
<td>0.838</td>
<td>3(13.6%)</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>11(20.8%)</td>
<td>0.027</td>
<td>1(2.9%)</td>
<td>0.310</td>
<td>0</td>
</tr>
<tr>
<td>Atrial Fibrillation /Flutter Prior DVT/PE</td>
<td>5(9.4%)</td>
<td>0.313</td>
<td>2(5.9%)</td>
<td>0.514</td>
<td>2(5.9%)</td>
</tr>
<tr>
<td>Peripheral Vascular Disease Hyperlipidaemia*</td>
<td>14(20.8%)</td>
<td>0.027</td>
<td>10(29.4%)</td>
<td>0.004</td>
<td>0</td>
</tr>
<tr>
<td>Migraine</td>
<td>6(11.3%)</td>
<td>0.171</td>
<td>3(8.8%)</td>
<td>0.271</td>
<td>0</td>
</tr>
<tr>
<td>MRS†</td>
<td>3</td>
<td>0.048</td>
<td>2</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Smoking at Enrolment</td>
<td>15(28.3%)</td>
<td>0.807</td>
<td>10(29.4%)</td>
<td>0.849</td>
<td>7 (31.8%)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>19(35.85%)</td>
<td>0.128</td>
<td>14(41.2%)</td>
<td>0.248</td>
<td>5 (22.7%)</td>
</tr>
<tr>
<td>Never smoker</td>
<td>19(35.85%)</td>
<td>0.807</td>
<td>10(29.4%)</td>
<td>0.262</td>
<td>10 (45.45%)</td>
</tr>
<tr>
<td>Statin Therapy</td>
<td>21(39.6%)</td>
<td>&lt;0.001</td>
<td>17(50%)</td>
<td>&lt;0.001</td>
<td>0</td>
</tr>
<tr>
<td>Family History Stroke</td>
<td>19(35.9%)</td>
<td>0.620</td>
<td>15(44.1%)</td>
<td>0.922</td>
<td>10(45.5%)</td>
</tr>
<tr>
<td>Stroke on Aspirin</td>
<td>20(37.7%)</td>
<td>N/A</td>
<td>10(29.4%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Median Daily Aspirin Dose (Range)</td>
<td>150mg (75 – 300)</td>
<td>N/A</td>
<td>75mg (75 – 300)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>
Legend for Table 1: Values are means [±SD] or absolute values (percentages in parentheses where appropriate), unless otherwise stated; *Hyperlipidaemia = total cholesterol > 5.0 mmol/L or LDL > 3.5 mmol/L. P values refer to differences between early or late phase patients and controls. Significant P values are highlighted in bold. †Median Modified Rankin Scale scores;

Table 2: TOAST subtyping in early and late phase CVD patients

<table>
<thead>
<tr>
<th>Stroke Subtype</th>
<th>Early (n = 53)</th>
<th>Late (n = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large Artery Atherosclerotic</td>
<td>11 (20.75%)</td>
<td>6 (17.6%)</td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>11 (20.75%)</td>
<td>9 (26.5%)</td>
</tr>
<tr>
<td>Lacunar</td>
<td>16 (30.2%)</td>
<td>12 (35.3%)</td>
</tr>
<tr>
<td>Other determined or undetermined aetiology</td>
<td>15 (28.3%)</td>
<td>7 (20.6%)</td>
</tr>
</tbody>
</table>

Table 2: TOAST subtyping in early and late phase CVD patients. Values are absolute numbers with percentages in parentheses.

Median ADAMTS13 activity was significantly reduced in the early phase after TIA or stroke compared with controls (71.96% vs. 95.5%, P < 0.01; figure 1). After controlling for differences in hypertension, hyperlipidemia, smoking status, and statin use between groups, significant differences in ADAMTS13 activity between early phase CVD patients and controls persisted (P < 0.02). There was no significant difference in ADAMTS13 activity between late phase CVD patients and controls (86.03% vs. 95.5%, P = 0.19; figure 1), even after controlling
for the above factors (P = 0.561). Post-hoc analysis indicated that there was no significant difference in ADAMTS13 activity between TIA (N = 3) and ischaemic stroke patients (N = 50; P = 0.658), but the number of subjects in the TIA subgroup was far too small to make any definitive conclusions, and this study was not designed to address such a subgroup analysis.

![Graph showing comparison of median ADAMTS13 levels between controls and early and late phase CVD patients.](image)

**Figure 1:** Comparison of median ADAMTS13 levels between controls and early and late phase CVD patients. P values refer to comparisons between patients and controls.

There was no significant correlation between ADAMTS13 activity and age in our patient population in the early phase (r = -0.22, P = 0.12), nor was there any significant difference in ADAMTS13 activity between different stroke subtypes (Tukey ANOVA, P = 0.50).
As reported previously, (18) VWF:Ag levels were higher in the early (201.83 IU/dl, \( P < 0.001 \); figure 2) and late phases after TIA or stroke (175.38 IU/dl; \( P < 0.001 \)) than in controls (123.87 IU/dl; figure 2).

**Figure 2:** Comparison of mean VWF antigen levels (IU/dl) between controls and early and late phase CVD patients. \( P \) values refer to comparisons between controls and patients.

In our CVD population, there was a distinct inverse relationship between ADAMTS13 activity and VWF:Ag levels in the early phase (\( r = -0.31, P = 0.024 \); figure 3) but not in the late phase after symptom onset (\( r = -0.2, P = 0.738 \)).
Figure 3: Relationship between VWF:Ag levels (IU/dl) and ADAMTS13 activity in the early phase after TIA or ischaemic stroke.

There was a positive correlation between ADAMTS13 activity and C-ADP closure times ($r = 0.35$, $P = 0.012$) but not C-EPI closure times ($r = 0.24$; $P = 0.092$) on the PFA-100® in the early CVD group, suggesting that reduced ADAMTS13 activity might be associated with shorter C-ADP closure times, and hence platelet hyper-reactivity to collagen-ADP early after TIA or stroke. However, when stepwise logistic regression analysis was performed to control for the influence of VWF:Ag levels on the potential relationship between ADAMTS13 activity and C-ADP closure times, the relationship became non significant ($P = 0.066$).

Of the 53 early phase patients, 23 had reduced ADAMTS13 activity < 66% of PNP in our laboratory, but none of these patients had reduced ADAMTS13 antigen levels in the early or late...
phase after symptom onset. Three of these 23 patients had elevated anti-ADAMTS13 IgG auto-antibody titres (defined as > 9.6 AU/dl) in the early phase, but only one of these patients had persistent anti-ADAMTS13 IgG auto-antibodies in the late phase.

Discussion

This novel pilot study has shown that ADAMTS13 activity is significantly reduced in the early but not in the late phase after TIA or ischaemic stroke. Our early phase samples were collected sooner after symptom onset than in prior published studies in CVD patients (mean time to blood sampling was just over 1 week; (range: 0-27 days), (1,10-12) and these novel data provide unique, important insights into the dynamic profile of ADAMTS13 and its relationship with VWF:Ag early after TIA or ischaemic stroke. One must accept that the lack of significant differences between late phase patients and controls could represent a type II error due to the smaller number of subjects studied ≥ 3 months after symptom onset. However, the lack of significantly reduced ADAMTS13 activity in late phase CVD patients suggests that this is a dynamic protease whose activity may decrease as an ‘acute phase response’ to TIA or ischaemic stroke, but returns to levels similar to those seen in controls during late-phase follow up.

Prior studies by our group (18) and others (1, 13, 14) have clearly demonstrated that circulating VWF:Ag levels are increased in the early and late phases after TIA and stroke, especially associated with large artery atherosclerosis. Population-based studies have also shown that ADAMTS13 activity is inversely correlated with VWF:Ag titres,(1, 19) is reduced in renal failure, in patients on dialysis and in acute inflammatory states, but is elevated in patients with
cirrhosis. (19) Our pilot hospital-based study is in agreement with these population-based data (19) because we also found that there was an inverse relationship between ADAMTS13 activity and VWF:Ag levels in early phase CVD patients. However, one cannot comment on the relationship between ADAMTS13 activity and levels of ‘individual fractions’ of circulating VWF multimers following TIA or stroke because our VWF assay measured overall VWF:Ag levels and did not differentiate between smaller, larger and ultra-large VWF multimers.

As stated above, previous studies have investigated whether low ADAMTS13 activity could be a risk factor for CVD or ischaemic heart disease. (7-12) Desmopressin infusion induces the secretion of very large VWF multimers into the plasma, (25) producing plasma ULVWF levels similar to those seen with acute tissue hypoxia in vivo. (15) In vivo testing of healthy volunteers revealed a steep decline in ADAMTS13 activity after desmopressin infusion suggesting that the protease is consumed as it cleaves very large VWF multimers. (25) These desmopressin-induced effects also occur in systemic inflammation (26) which has been noted in ischaemic stroke. (27) Our study revealed initial low ADAMTS13 activity and a distinct inverse relationship between VWF:Ag levels and ADAMTS13 activity only in the early phase after TIA or ischaemic stroke. Based on our longitudinal data, the early phase findings are consistent with the hypothesis that ADAMTS13 may be consumed or inactivated as it degrades ultra-large VWF multimers (25) associated with acute cerebral or ocular ischaemia rather than reflecting a ‘pre-existing deficiency’ of ADAMTS13 antigen in our CVD population.

Because the protease targets the largest, most thrombogenic multimers of VWF which have the greatest influence on platelet adhesion and aggregation, (28) lower ADAMTS13 activity, which would be expected to be associated with increased VWF:Ag levels, was associated with
‘apparent platelet hyper-reactivity’ and shorter C-ADP closure times. Stepwise logistic regression analysis revealed that it is highly likely that the relationship between ADAMTS13 activity and PFA-100 C-ADP closure times is mediated via VWF:Ag, and that ADAMTS13 itself does not directly influence closure times. By the late phase, ADAMTS13 activity was not lower than in controls, and the clear inverse relationship between ADAMTS13 and VWF:Ag levels seen in the early phase was no longer evident. This lack of a significant relationship between these two variables may also reflect a type II error due to the smaller number of subjects with late phase data, but could represent a shift towards normal VWF multimers in plasma over time, for which ADAMTS13 has less affinity, thus resulting in normalisation of ADAMTS13 activity during late phase follow up.

The exploratory laboratory assays that were performed in the patient subgroups with low ADAMTS13 activity indicate that the findings in our early phase CVD patients cannot not explained by a primary deficiency of ADAMTS13 antigen. Because only 3 of the 23 early phase CVD patients, and only one late phase CVD patient with low ADAMTS13 activity had positive anti-ADAMTS13 IgG auto-antibodies, one can conclude that the initial reduction in ADAMTS13 activity in non-TTP-related TIA or stroke is more likely to be due to an acute phase response, and cannot predominantly be attributed to the presence of the measured auto-antibodies to ADAMTS13.(29)

This study had some limitations. The study was designed to focus on CVD patients on aspirin monotherapy who also had simultaneous PFA-100® testing, so one cannot comment on the potential impact of other commonly-prescribed antiplatelet regimens, such as aspirin and dipyridamole or clopidogrel monotherapy on ADAMTS13 activity in TIA and ischaemic stroke.
This issue deserves future study. Patient data were compared with controls who were not on Aspirin; however, there is no evidence from longitudinal studies that Aspirin therapy influences either circulating VWF antigen levels (30) or ADAMTS13 activity. (31) One of the more important conclusions to draw from this pilot study is that early phase CVD patients had lower levels of ADAMTS13 activity despite treatment with aspirin therapy at standard therapeutic doses used for secondary prevention of recurrent vascular events. Aspirin has been clearly shown to prolong C-EPI closure times in patients following TIA or stroke,(18, 32) and may well have influenced the analysis of the relationship between ADAMTS13 activity and C-EPI closure times. However, because we have demonstrated that it is highly likely that any apparent relationship between ADAMTS13 activity and C-ADP closure times is mediated via VWF:Ag levels, it is unlikely that any relationship between ADAMTS13 and C-EPI closure times would be of clinical relevance. This study was also performed when the evidence for statin therapy in TIA or ischaemic stroke was emerging,(33) so it will be of interest to see whether these data can be replicated in a larger cohort of patients on secondary preventative therapy with statins.

**Conclusions**

ADAMTS13 is a dynamic protease whose activity initially decreases and then returns towards baseline levels over time following TIA or stroke. VWF:Ag levels are increased in both the early and late phases after TIA or stroke onset, and can promote enhanced platelet adhesion or aggregation in response to stimulation with collagen-ADP at moderately high shear stress. Further longitudinal studies in larger patient populations on other secondary preventive therapies are warranted to assess the profile of ADAMTS13 activity and VWF sub-fractions following TIA or ischaemic stroke, and its impact on platelet reactivity at different shear rates. This is
especially important in view of the role that ADAMTS13 may have as a potential anti-thrombotic agent in CVD.(34)

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References:


11. Andersson HM, Siegerink B, Luken BM, Crawley JT, Algra A, Lane DA, Rosendaal FR. High VWF, low ADAMTS13, and oral contraceptives increase the


22. Gerritsen HE, Turecek PL, Schwarz HP, Lammle B, Furlan M. Assay of von Willebrand factor (vWF)-cleaving protease based on decreased collagen binding


Highlights:

- ADAMTS13 activity is significantly reduced in the early phase after TIA/ischaemic stroke
- ADAMTS13 activity has a distinct inverse relationship with VWF in the early phase
- Positive correlation between ADAMTS13 activity and C-ADP closure times in early phase patients
- ADAMTS13 has future potential as an antithrombotic agent