Von Willebrand factor- ADAMTS13 ratio at presentation of acute ischaemic brain injury is predictive of outcome

(Running title: "VWF-ADAMTS13 in acute ischaemic brain injury')

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Background and purpose: Acute ischaemic stroke (IS) and transient ischaemic attack (TIA) are associated with raised von Willebrand factor (VWF) and decreased ADAMTS13 activity (Ac). Their impact on mortality and morbidity is unclear.

Methods: Prospective investigation of the VWF-ADAMTS 13 axis in 292 adults with acute IS (n=103), TIA (n=80), or controls (n=109), serially, from presentation until after 6 weeks. Assessment of stroke severity used the National Institute of Health Stroke Score (NIHSS) and modified Rankin scale (mRS).

Results: Presenting median VWFAg: ADAMTS13Ac ratios were: IS-2.42 (0.78-9.53), TIA 1.89 (0.41-8.14) and controls 1.69 (0.25-15.63). Longitudinally, the median VWFAg-ADAMTS13Ac ratio decreased (IS: 2.42 to 1.66, p=0.0008, TIA: 1.89 to 0.65, p<0.0001). VWFAg-ADAMTS13Ac ratio was higher at presentation in IS cases that died (3.683 vs 2.014, p<0.0001). Presenting VWFAg-ADAMTS13Ac ratio > 2.6 predicted mortality (OR 6.33; range 2.22- 18.1). VWFAg-ADAMTS13Ac ratio in the highest quartile (>3.091) had 31% increased risk mortality. The VWFAg-ADAMTS13Ac ratio at presentation of ischaemic brain injury was associated with higher mRS (p=0.021) and NIHSS scores (p=0.029) at follow up. Thrombolysis resulted in prompt reduction of VWFAg-ADAMTS13Ac ratio, and significant improvement in mRS on follow up.

Conclusion: Raised VWFAg-ADAMTS13Ac ratio at presentation of acute IS and TIA is associated with increased mortality and poorer functional outcome. A ratio of 2.6 appears to differentiate outcome. Prompt reduction in the ratio, in thrombolysed patients, was associated with decreased mortality and morbidity. The VWFAg-ADAMTS13Ac ratio is a biomarker for the acute impact of an ischaemic event and longer term outcomes.

Key points:

1) The VWFAg-ADAMTS13Ac ratio in acute ischaemic stroke is associated with clinical outcome, independent of age. A ratio of greater than 2.6 predicts mortality.
2) The VWFAg-ADAMTS13Ac ratio resolves over longitudinal follow up. Normalisation is faster following thrombolysis.

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Introduction

Von Willebrand factor (VWF) together with ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) play a key role in arterial thrombosis. VWF is integral for platelet adhesion to collagen fibres and atherosclerotic plaques, and platelet aggregation under high shear conditions.[1,2] ADAMTS13 is responsible for the breakdown and ultimate control of ultra-large VWF multimers (ULVWFM) secreted from the endothelium.[3]

Prospective studies identified that raised VWF and decreased ADAMTS13 predispose to increased risk of stroke, including the well-characterised Rotterdam cohort of more than 6000 patients stroke-free at baseline. [4–10]

Animal models have shown that extent of thrombosis in stroke is influenced by VWF and ADAMTS13 in an interdependent manner in genetically manipulated mice.[11–13] In these models, ADAMTS13 deficiency in a VWF-dependent manner was associated with increased infarct volume, with consequential functional outcome. More recent mouse work supports ADAMTS13 in thrombus dissolution and restoration of vessel patency. [14,15]

In a smaller cohort of acute stroke and TIA patients, ADAMTS13 activity was reduced and VWFAg increased within 4 weeks of onset of ischaemic stroke or TIA. Examining patients at more than 3 months post presentation showed that VWFAg levels remained high. ADAMTS13, in contrast, showed significant reduction in ischaemic stroke compared to controls in the early phase, but not later, potentially suggesting recovery of ADAMTS13 following consumption in ischaemic stroke.[16]

Clinical case-control studies suggest a role for increased VWF and decreased ADAMTS13 at presentation and later follow up suggesting a link with severity and recurrence risk. [17–21] There is no clear consensus about whether any marker of haemostasis can be used to predict clinical outcome post stroke.[22] The functional outcome of patients with ischaemic stroke related to baseline ADAMTS13, and comparison of the VWF-ADAMTS13 axis in TIA or those patients with a more ‘stuttering’ type of stroke phenotype, has not been studied.

We hypothesized that increased levels and activity of VWF, low levels of ADAMTS13 activity and the balance of these haemostatic markers in individual patients, would be predictive of functional recovery after acute ischaemic stroke and TIA.

This unique, prospective study investigated VWF antigen (Ag), VWF activity (Ac), and ADAMTS13 activity (Ac) in patients presenting with acute ischaemic stroke and TIA, compared to controls and examined sequentially. This is the largest cohort examined over the hyperacute and convalescent phases, with correlation of these haemostatic markers with clinical outcome, as measured by functional stroke scores and follow-up mortality. Deepening our understanding of the role of the VWF-ADAMTS13 axis in ischaemic brain injury could herald the use of novel therapies such as recombinant ADAMTS13: to reduce infarct size in an acute cerebral ischaemic presentation, prevent deterioration of cerebral perfusion in patients presenting with TIA, reduce the risk of recurrence and improve clinical outcome.

Methods

We prospectively investigated the VWF-ADAMTS 13 axis in acute ischaemic brain injury over a 30-month period. The National Stroke Strategy has revolutionized stroke services in the UK, with eight hyperacute stroke units (HASU) commissioned in London.[23] Each services a population of approximately one million people, with streamlining of services allowing for emergency brain scans and improved delivery of thrombolysis.[24] In line with this, our institution has a daily TIA clinic, for assessment of symptoms suggestive of a TIA, in patients well enough for outpatient scans and investigations.

Patients aged 18 years or older presenting with symptoms suggestive of stroke with onset less than 48 hours were eligible, and recruited from our institution, a regional hyperacute stroke unit and daily TIA clinic. Patients routinely underwent a full investigative pathway of clinical, laboratory and imaging assessment. Imaging consisted either of CT or MRI head, reported by a neuroradiologist. Senior stroke physicians would base categorization of patients (ie stroke or TIA) following this clinical diagnostic pathway. Control patients

were those who presented with symptoms suggestive of stroke or TIA, but the diagnostic pathway found no evidence of acute ischaemic brain injury, such as those with subsequent diagnoses of migraine or seizure.

Exclusion criteria included secondary precipitating causes such as known active malignancy and autoimmune disease. Written consent was obtained from patients or from their nearest relative, in the event of lack of capacity or with stroke- specific disabilities. The study was approved by an institutional review board (research ethics committee reference 14/EE/0169). Patients were categorized as ischaemic stroke, TIA or control after neuroimaging and clinical assessment by senior stroke physicians. Control cases consisted of patients referred to the stroke service with acute symptoms suggestive of stroke or TIA but in whom subsequent investigations identified other medical causes for presentation, such as seizure or migraine. Clinical data was collected concerning vascular risk factors, admission medications, and ischaemic stroke subtype as defined by the TOAST classification.[25] Functional and neurological impairments were documented at each time point using the modified Rankin Scale (mRS), the National Institute of Health Stroke Score (NIHSS) and the Glasgow Coma Score (GCS) [26–29] (see supplementary information for TOAST classification, mRS and NIHSS scoring, Tables I-III).

Blood was sampled at presentation, sequentially during admission at (i) 24 hours, (ii) 48 hours and (iii) at 7-14 days post presentation if feasible) and at the final follow up (iv) minimum 6 weeks post presentation. Measures at each time point were not achieved for every patient, such as those who attended the TIA clinic as out-patients, but particular focus was given to measuring bloods at follow up.

At each time point, blood collection consisted of 2 citrated plasma samples (each x 4.5ml). Venepuncture was performed via standard procedures. Samples were double centrifuged, at 1500g for 10 minutes for each run. Samples were then stored at -80°C until further processing.

Laboratory processing

Laboratory measurements of VWFAg, VWFAc, factor VIII (FVIII), ADAMTS13Ac and thrombin generation were conducted. VWFAg and VWFAc were measured using a standard automated immunoturbimetric assay, in a Sysmex CS-2000i analyser, with Siemens kit (VWF Ag® and INNOVANCE® VWF Ac. Siemens Healthcare Diagnostics, Marburg, Germany). FVIII analysis was also performed using a Sysmex CS-2000i analyser, with Siemens kit (Coagulation factor VIII deficient plasma, reference OTXW). ADAMTS13Ac was performed by FRETS- ADAMTS13 activity assay (Fluorescence Resonance Energy Transfer), based on previously published work. [30,31]

Statistics

Statistical packages used were Stata: multiple regression and receiver-operator curve (ROC) analysis, and Graphpad PRISM: correlation, t-tests, Mann-Whitney, Kruskal Wallis and chi-square testing. The VWFAg/ ADAMTS13Ac ratio was calculated for each set of results for each patient. Comparison of presenting haemostatic markers between 2 groups was performed using the Mann Whitney (if non- parametric) or unpaired t-test (if parametric) for unmatched data sets; and Wilcoxon matched pairs signed rank test for non-parametric matched data sets. Kruskal Wallis testing was used to compare the medians of 3 or more groups (non-parametric): ie whether VWFAg varied significantly at each time point from t0 to t4. Multiple linear regression and receiver-operator characteristic (ROC) curve analysis was used to determine the independent influence of VWF and ADAMTS13 on stroke scores and mortality.

Results

Patient characteristics

A total of 292 patients were recruited, with 141 males and 151 females, aged from 23 to 100 years (median 71.5 years). Patients were categorized into subgroups according to discharge diagnosis: ischaemic stroke (n=103), TIA (n=80), or control (n=109).
31% of the whole cohort had a previous history of ischaemic stroke or TIA. Cardiovascular risk factors were documented (see supplementary material for further demographic information including co-morbidities, ABO blood group and anticoagulation, Tables IV-V).

Differences between IS, TIA and control groups at presentation

Comparing VWFAg, VWFAc and Factor VIII between groups showed significant differences, the highest levels seen in ischaemic stroke. Conversely, ADAMTS13Ac was significantly lower in ischaemic stroke compared to the other groups (Figure 1).
Calculation of the VWFAg-to-ADAMTS13Ac ratio for each patient was then performed to capture the intra-individual balance between these haemostatic markers, and examine associations between the groups. A significant difference was seen between group medians as follows (median, range): ischaemic stroke 2.42 (0.78-9.53), TIA 1.89 (0.41-8.14) and control 1.69 (0.25-15.63); KW 24.7, $p<0.0001$.

C Reactive Protein (CRP) and VWFAg- ADAMTS13Ac

There was a significant difference in CRP (normal range 0-5 mg/l) between groups at presentation (median, range): ischaemic stroke 2.7 (0.6-105), TIA 1.8 (0-74.1) and control 1.5 (0.6-120.9) mg/l; KW 11.34, $p=0.0035$.
There was correlation between the control group CRP and VWFAg: ADAMTS13Ac ratio ($r=0.324$, $p=0.0013$), not seen in ischaemic stroke ($r= 0.175$, $p=0.107$) or TIA ($r= 0.175$, $p=0.136$). Multiple linear regression analysis identified CRP and bilirubin to be independent predictors of ADAMTS13 at presentation of ischaemic stroke and TIA,

demonstrating a negative association (see supplementary information, Table VI) not seen with VWFAg or VWFAg- ADAMTS13Ac.

Longitudinal follow up

Follow up blood samples from 6 weeks to over 2 years post presentation, with a median of 6 months, were obtained in 96 patients (ischaemic stroke =34, TIA=35, Controls=27; median follow up 188 days, range 41 to 889 days).

Both VWFAg and FVIII significantly decreased in ischaemic stroke (Figure 2a, 2c), with the reverse seen with ADAMTS13Ac, increasing from presentation to final follow up (Figure 2d), demonstrating normalisation. The VWFAg-ADAMTS13Ac ratio correspondingly decreased over time (2.42 to 1.66, p=0.0008; Wilcoxon matched pairs signed rank test median difference 0.278, p=0.005). Significant changes in ischaemic stroke were demonstrated for each marker in comparison of presentation versus final follow up (Table 2). Paired testing of 36 TIA cases demonstrated increasing ADAMTS13Ac from presentation to final follow up (by 3.6IU/dL, p=0.05), with a corresponding decrease in the VWFAg-ADAMTS13Ac ratio (p<0.0001).

Follow up blood samples in 27 control patients (median follow up 204 days, range 44-566) demonstrated a downward trend in VWFAg, VWFAc and FVIII that did not reach significance, consistent with resolution of an acute phase response. There was no notable change in ADAMTS13 (95.6 to 98.9IU/dL, p=0.144) over follow up. There was consequently no significant longitudinal change seen in the VWFAg-ADAMTS13 ratio in the control group ((1.69 to 1.21, p=0.074; Wilcoxon matched pairs signed rank test median difference 0.0362, p=0.562), suggesting that acute phase elevation of the VWFAg-ADAMTS13 ratio with normalization over time is specific to ischaemic brain injury.

Recurrence and mortality

There were 5 patients with recurrent ischaemic stroke (all occurring in patients whom had initially presented with ischaemic stroke) and 3 patients with recurrent TIA (all occurring in patients whom had initially presented with TIA). Numbers are too small for meaningful interpretation, so we cannot comment further on whether there was a difference in VWFAg-ADAMTS13Ac ratio in these patients at presentation or follow up compared to the larger stroke/TIA cohort. Within ischaemic stroke and TIA, the mortality rate was 13% (21 ischaemic stroke patients and 3 TIA), with median time 152 days from recruitment until death. Median age at presentation of those patients who survived was 75 years (range 25-99) and 84 years in those who died (61-97). Deceased patients (n=24) versus patients alive at final follow up (n=156) showed significant differences in all presentation haemostatic markers (Figure 3). The VWFAg-ADAMTS13Ac ratio was significantly higher in those who died (median 3.68, range 1.70-8.81) compared to ratios for patients who survived (median 2.01, range 0.41-9.53).

ROC curve analysis was used to determine sensitivity of the presenting VWFAg-ADAMTS13Ac ratio for mortality, demonstrating clear predictive value (area under curve of 0.765, 95% confidence interval 0.664- 0.866, see Figure 1, supplementary information). In comparison, ROC analysis for sensitivity of presenting age for mortality, was slightly less (area under curve 0.75, 95% confidence interval 0.665- 0.845).

Using ROC curve analysis to determine sensitivity and specificity of the VWFAg-ADAMTS13Ac ratio for mortality, a ratio of 2.6 was considered a suitable cut-off as it provides a good balance of sensitivity (79.2%) and specificity (62.3%). This cut-off ratio was then applied to chi -square testing of the ischaemic stroke and TIA cohort split according to presentation VWFAg-ADAMTS13Ac ratio. Those patients with VWFAg-ADAMTS13Ac greater than 2.6 were significantly more likely to die (chi-square 14.2, p=0.002; odds ratio 6.33, range 2.22-18.1), see supplementary information, Table VII).

In patients presenting with VWFAg-ADAMTS13Ac less than 2.6, 80 were alive and 5 dead at follow up (mortality 6%). In those presenting with VWFAg-ADAMTS13Ac greater than or equal to 2.6, 48 were alive at follow up with 19 deceased (mortality = 28%).

Logistic regression demonstrated that the association of the presenting VWFAg-ADAMTS13Ac ratio with mortality was independent of age. In an unadjusted logistic regression model, the ratio was associated with mortality (odds ratio 1.67, 95% CI 1.25-2.22, p=0.00). After adjusting for age, there was still an association between the ratio and mortality, albeit slightly reduced in magnitude (odds ratio 1.50, 95% CI 1.11-2.02, p=0.008).

Difference in mortality was also illustrated by splitting the ischaemic stroke and TIA cohort into quartiles according to presentation VWFAg-ADAMTS13 ratio. Those patients in in the highest quartile (VWFAg-ADAMTS13Ac ratio 3.09- 9.53) had a 31% mortality rate (n=14) compared to those in the lowest quartile (VWFAg-ADAMTS13Ac ratio 0.41-1.55) where no deaths occurred (p<0.0001, Table 3).

VWFAg-ADAMTS13Ac ratio is associated with functional scores

At presentation, ischaemic stroke demonstrated a median higher mRS score and NIHSS score compared to TIA and control groups (Table 1). Spearman rank testing showed correlation of the VWFAg-ADAMTS13Ac ratio with age (0.577, p<0.0001), mRS (0.477, p<0.0001) and NIHSS (0.337, p<0.0001). A negative correlation was seen with GCS (-0.255, p<0.0001), corresponding with disability (supplementary information, Table VIII).

Division of the ischaemic stroke and TIA cohorts according to mRS at both presentation and final follow up showed prognostic differences in the VWFAg-ADAMTS13 ratio. Patients presenting with mRS 3-5 (n=78) compared to patients with mRS 0-2 (n=98) demonstrated a higher VWFAg-ADAMTS13Ac ratio (mRS 3-5 median 2.722, range 0.85-8.81 vs mRS 0-2 median 1.858, range 0.415-9.53; p<0.0001). Despite an overall longitudinal reduction of the VWFAg-ADAMTS13Ac ratio, this significant difference

persisted at final follow up. Patients with more functional impairment at follow up (n=18), as reflected by mRS 3-5, maintained a higher VWFAg-ADAMTS13Ac ratio of 1.521 (0.32-3.42) compared to patients at follow up with mRS 0-2 (n=48), median 0.845, range 0.42-3.29; p=0.0102). Although the ranges overlap, this higher VWFAg-ADAMTS13Ac ratio was closely related to increased morbidity.

Defining the VWFAg-ADAMTS13Ac ratio of 2.6 as predictive of mortality was then extrapolated to morbidity. Patients with a presenting ratio of less than 2.6 (n=109) had a significantly lower mRS (1, 0-5) compared to those with a presenting ratio of greater than or equal to 2.6 (n=65, mRS 3, 0-5; p<0.0001). This was also reproduced with NIHSS scoring: patients presenting with a ratio of less than 2.6 (n=111) had a significantly lower NIHSS (1, 0-28) compared to those with a presenting ratio of greater than or equal to 2.6 (n=66, NIHSS 4, 0-22; p<0.0001).

Regression modeling similarly reflected higher mRS and NIHSS scores, reflective of more disability, to be positively associated with presentation VWFAg and negatively associated with ADAMTS13Ac in combined modelling (supplementary material, Tables IX- XI).

In follow up, the presentation VWFAg-ADAMTS13Ac was associated with the NIHSS score at 7-14 days post presentation (p= 0.029), and the mRS at more than 6 weeks post presentation (p=0.021) on regression (supplementary material, Table XII). GCS at 7-14 days post presentation was significantly negatively associated with the VWFAg-ADAMTS13Ac ratio (coefficient -4.425, p=0.011) in keeping with the hypothesis- ie as VWF increases and ADAMTS13 decreases, GCS decreases- synonymous with worsening clinical state. Multiple linear regression associations of presentation VWFAg-ADAMTS13Ac axis scores with final follow up functional scores were specific to ischaemic brain injury. Controls demonstrated no significant change in median functional scores from presentation to final follow up, in contrast to the improved functional status seen in the ischaemic stroke and TIA groups.

Impact of thrombolysis on VWFAg-ADAMTS13Ac parameters

Thirty-eight patients within the ischaemic stroke group received thrombolysis within 4 hours of onset of symptoms. Presentation ADAMTS13Ac was comparable between the groups but the thrombolysed group showed increased ADAMTS13Ac at follow up (84 to 100.8IU/dL, p= 0.0025, median follow up 221 days) with no change in the non-thrombolysed group (86 to 90.9IU/dL, p=0.285, median follow up n= 286 days).

Presentation VWFAg was lower in the thrombolysis group compared to the non-thrombolysed group at presentation (median 187.9 vs 227.2IU.dL, p=0.0352), with no differences between the groups at any point in later follow up. It is likely that the VWF was higher in the group who was not subsequently thrombolysed because these patients had reached their ischaemic peak, reflected in the VWF levels- but timing of symptom onset mean they were excluded from being safely thrombolysed (ie more than 4.5 hours from symptom onset).

The thrombolysed group showed a significantly reduced VWFAg at follow up (187.9IU/dL to 141.1IU/dL, p=0.0187) which was not evident on follow up in the non-thrombolysed group (227.2 to 190.9IU/dL, p=0.0944).

The difference in VWFAg-ADAMTS13Ac ratio from presentation to final follow-up was therefore far more marked in the thrombolysed group compared to the non-thrombolysed (2.024 to 1.355 in thrombolysed group, p=0.0052; 2.672 to 2.091 in non-thrombolysed group, p=0.0339). This suggests that normalisation of the VWFAg-ADAMTS13Ac balance is better achieved with thrombolysis.

Similarly, there was a significant difference in the mRS from presentation to final follow up in the thrombolysed ischaemic stroke group (3.5 to 0, p=0.0020), not seen in the non-thrombolysed group (3 to 2, p=0.0622, see supplementary information, Figure II).

Discussion

We have, for the first time, shown a strong association between the levels of VWF and ADAMTS13 on admission with respect to the severity, functional outcome and mortality of acute ischaemic stroke. We have summarized the relationship by focusing on the VWFAg-ADAMTS13Ac ratio as a biological measure of the disturbed haemostatic balance following ischaemic stroke and TIA, incorporating both the elevation of VWF and reduction of ADAMTS13. Longitudinal changes in those presenting with acute cerebral ischaemia supports incremental ADAMTS13Ac recovery in the aftermath of acute ischaemic stroke and TIA in the largest cohort of patients yet published, with no such longitudinal changes seen in controls. Our data suggests the corresponding VWFAg-ADAMTS13Ac ratio can be used as biomarker for clinical outcome in acute ischaemic stroke and TIA. The presentation VWFAg- ADAMTS13Ac ratio was significantly higher in patients who subsequently died (deceased 3.683 vs alive 2.014). A ratio of greater than or equal to 2.6 at presentation of ischaemic stroke or TIA was associated with increased mortality (26%) vs those with a ratio of less than 2.6 (8%), based on ROC curve analysis accounting for sensitivity and specificity of the VWFAg-ADAMTS13Ac ratio as discriminatory for mortality (area under curve 0.765, 95% C.I 0.65-0.87). This also fits with the median value in the control group VWFAg-ADAMTS13Ac ratio of 1.69 at presentation, and 1.21 in convalescence; significantly different to the ischaemic stroke ratio of 2.42 at presentation and 1.66 in convalescence. There was an escalating risk of death according to presentation VWFAg-ADAMTS13Ac, as reflected by comparison of the highest to the lowest quartile showing a 31% increase in mortality.

As well as mortality, regression modeling showed association of presentation VWFAg-ADAMTS13Ac ratio to later functional outcome, specific to ischaemic brain injury. A higher ratio was associated with higher functional and neurological impairment, as defined by mRS and NIHSS scoring systems, both of which have been extensively validated within

stroke medicine. In the era of anti-VWF and recombinant ADAMTS13 treatments for TTP, therapeutic implications of limiting raised VWF levels and normalising ADAMTS13 activity could be critical in optimizing outcomes from ischaemic stroke or TIA.

Increased VWF and reduced ADAMTS13 in acute stroke has been previously described, and it is known that ischaemic brain injury and the damaged vascular endothelium is associated with increased VWF. [4,5,9,16,19,32,33] It is unclear whether reduced baseline ADAMTS13 predisposes to stroke; or is consumed as it meets demand of the acute outpouring of ULVWF multimers in ischaemic insult. We found a significant reduction in ADAMTS13Ac in ischaemic stroke compared to both TIA and control groups. Conversely, VWFAg was significantly higher in ischaemic stroke compared to both TIA and control groups, with similar trends seen with VWFAc. Normalisation of both markers was seen in convalescence, therefore consistent with consumption of ADAMTS13 in the ischaemic insult.

Earlier work demonstrated reduction of ADAMS13Ac in the early phase post stroke compared to late.[16] However, an inverse relationship between VWFAg and ADAMTS13 levels was only seen in the early phase. This supports the hypothesis that ADAMTS13 is consumed in the action of degradation of ultra-large VWF multimers released in the ischaemic insult. The Rotterdam prospective study suggests that reduced ADAMTS13 predisposes to stroke.[6–8] The nature of our study design means we cannot comment further on whether reduction of ADAMTS13 in acute ischaemic stroke is causative or a consumptive phenomenon in the ischaemic insult. We determine that although presenting VWFAg-ADAMTS13Ac perturbations are witnessed in general sickness and linked with consequent disability, resolution of VWFAg-ADAMTS13Ac is specifically associated with convalescence post ischaemic brain injury.

The overall trend indicated by regression modeling is an increased morbidity according to presentation VWFAg-ADAMTS13Ac, in both the acute phase and convalescence. Firstly, higher mRS and NIHSS scores at presentation, reflective of more impairment, were associated with a higher VWFAg-ADAMTS13Ac ratio. Secondly, the presentation VWFAg-ADAMTS13Ac was associated with longitudinal clinical scoring in ischaemic stroke, as reflected by significant association with the final mRS score. Altogether this supports the hypothesis that the acute-phase VWFAg-ADAMTS13Ac axis is predictive of poorer functional outcome in patients presenting with acute ischaemic brain injury, extending to mortality.

Impact of thrombolysis on the VWFAg-ADAMTS13Ac axis has not been previously investigated, and we demonstrate normalisation of the VWFAg-ADAMTS13Ac axis to be more effectively achieved with thrombolysis. This further reinforces a role for the VWFAg-ADAMTS13Ac axis in the pathophysiology of ischaemic stroke, with thrombolysis demonstrating a significant reduction in ratio and impact on clinical scores in follow up. Animal model studies showed recombinant ADAMTS13 treatment in cerebrovascular occlusion versus thrombolysis with tPA, reduces infarct volume and improves blood flow, without risk of massive intracerebral haemorrhage associated with tPA.[34] The postulated mechanism was the specificity of ADAMTS13 for ULVWF in conditions of high shear stress and thus pathological thrombi, rather than targeting the VWF-platelet primary haemostatic thrombi and inducing haemorrhage following the dissolution of thrombi post ischaemia.[34,35] More recent clinical work suggests ADAMTS13 levels predict response to reperfusion attempts in patients with acute stroke, via endovascular treatment or thrombolysis, with higher levels associated with arterial recanalization and lower levels predicting an unfavourable clinical outcome post thrombolysis.[36–38] Potential mechanisms include a role for ADAMTS13 in post-ischaemic angiogenesis, with recombinant ADAMTS13 markedly

boosting neovascularization, vascular repair and functional recovery at 2 weeks post ischaemic insult in recent murine data.[39]

Limitations of our study include firstly the 48-hour allowance of symptom onset before recruitment. This may have affected biomarkers in this period. Secondly, those patients whom were thrombolysed will have presented within 4.5 hours of symptom onset, so will arguably have received more prompt medical attention in relation to their symptoms, so clinical recovery may have been independent of thrombolytic therapy. Thirdly, the cause of death in our cohort was not documented so we cannot comment on how aberrations in the VWFAg- ADAMTS13Ac axis may have contributed nor can we comment on whether related to stroke or other causes. Patients may have re-presented to other hospitals, so recurrence may not be fully captured. Our recurrence data was limited, and further work is merited to investigate whether persistently depleted ADAMTS13Ac heightens recurrence risk of acute ischaemic brain injury; e.g. via increased thrombus propensity and increased cardiovascular mortality. We acknowledge that although we have demonstrated significance between the VWF-ADAMTS13 balance and mortality, there is overlapping between patient groups and no defining VWFAg-ADAMTS13Ac ratio predictive of functional outcome. Finally, further work is warranted into how inflammation interacts with the VWF-ADAMTS13 axis. We demonstrate a significant difference in CRP between groups at presentation, and negative association with presentation CRP and ADAMTS13 on multivariate analysis as seen by other groups.[10] This would be in keeping with an escalating CRP in conjunction with inflammation, with a correspondingly low ADAMTS13. This certainly supports the inflammatory stimulus of ischaemic stroke contributing to a disordered VWFAg- ADAMTS13Ac axis, but does not clarify whether causative or reactive. In conclusion, presentation VWFAg-ADAMTS13Ac ratio is a surrogate biomarker for extent of brain injury, consequential clinical recovery, and may be used for future prognostication and guidance of therapy. VWFAg-ADAMTS13Ac ratio in acute ischaemic brain injury has

potential as a haemostatic parameter for cerebral ischaemia. The ratio reflects not just severity of functional impairment, but also eventual mortality, independent of age. A presenting ratio of greater than 2.6 defines a significantly poorer prognosis relating to functional outcome and mortality in patients with ischaemic brain injury, with a mortality rate of 26% compared to 8% in those presenting with a ratio of less than or equal to 2.6. We also demonstrate worsening impact as the ratio increases. Over a ratio of 2, the mortality rate is 21%; rising to 29% over a ratio of 3 and 43% over a ratio of 4. Resolution of the VWFAg-ADAMTS13Ac axis over convalescence links with clinical recovery. Given the limited acute treatments available for ischaemic stroke and TIA, there may be potential for the VWFAg-ADAMTSA13Ac ratio beyond a biomarker. Manipulation of the axis via reducing raised VWF levels and normalising ADAMTS13 activity in acute ischaemic stroke and TIA offers important therapeutic potential.

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Authorship Contributions

A Taylor recruited patients, designed and performed research, performed laboratory analysis, analysed data and wrote the paper.

C Vendramin and D Singh performed laboratory analysis.

M Brown designed the research and reviewed the paper

M Scully designed the research, reviewed and revised the paper.

Disclosure of Conflicts of Interest

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