Supplementary information

Table I: Demographics: presentation TOAST classification

TOAST classification	**Ischaemic stroke**	**TIA**	**Total number of patients**
Large artery	19	11	30 (16.4%)
Embolism of cardiac origin	29	12	41 (22.4%)
Small blood vessel occlusion	5	4	9 (4.9%)
Other cause	3	1	4 (2.2%)
Undermined aetiology	47	52	99 (54.1%)
Total	103	80	183 (100%)

Table II: Modified Rankin score

Score	Characteristics
0	No symptoms at all
1	No significant disability despite symptoms, able to carry out all usual duties and activities;
2	Slight disability, unable to carry out all previous activities but able to look after own affairs without assistance
3	Moderate disability, requiring some help abut able to walk without assistance
4	Moderately severe disability, unable to walk without assistance and unable to attend to own bodily needs without assistance
5	Severe disability, bedridden, incontinent and requiring constant nursing care and attention
6	Dead

Table III: NIHSS score (National Institutes of Health Stroke Scale) 0-42

i) Level of consciousness Ability to answer questions Ability to follow commands,	0-3 0-3 0-3
ii) Gaze	0-2
iii) Visual	0-3 (based on hemianopia)
iii) Facial palsy	0-3
iv) Motor function: arm, leg (drift, timing of drift, effort against gravity)	0-4 for each limb
v) Limb ataxia	0-2
vi) Sensory	0-2
vii) Language (degree of aphasia)	0-3
viii) Articulation (degree of dysarthria)	0-2
ix) Extinction and inattention	0-2

Table IV: Demographics: comorbidities (total = 292 patients)

	Ischaemic stroke (n=103)	**TIA (n =80)**	**Controls (n=109)**
Hypertension (prevalence of risk factor in group)	*71 (68.9%)*	*47 (58.8%)*	*41 (38%)*
Hypercholesterolaemia	*41 (39.8%)*	*37 (46.3%)*	*38 (35.2%)*
Previous ischaemic stroke	*15 (14.6%)*	*12 (15%)*	*14 (13.6%)*
Previous haemorrhagic stroke	*2 (1.9%)*	*1 (1.3%)*	*1 (0.9%)*
Previous TIA	*13 (12.6%)*	*17 (21.3%)*	*9 (8.4%)*
Diabetes type 1	*0*	*2 (2.5%)*	*3 (2.9%)*
Diabetes type 2	*24 (23.3%)*	*13 (16.3%)*	*11 (10.7%)*
Previous venous thromboembolism	*4 (3.8%)*	*0*	*1 (0.9%)*
Atrial fibrillation	*25 (24.3%)*	*12 (15%)*	*10 (9.7%)*
Cardiac failure	*6 (5.8%)*	*1 (1.3%)*	*4 (3.9%)*
Previous myocardial infarction	*7 (6.8%)*	*3 (3.8%)*	*2 (1.9%)*
Peripheral arterial disease	*3 (2.9%)*	*0*	*0*
Previous PCI or CABG	*5 (4.9%)*	*5 (6.3%)*	*4 (3.9%)*
Smoker	*27 (26.2%)*	*22 (27.5%)*	*39 (37.9%)*

The most frequently occurring cardiovascular risk factor in the entire cohort was hypertension (n=166, 56.8%), followed by hypercholesterolaemia (n= 122, 41.7%), smoking (n=88, 30.1%), type 2 diabetes (n=49, 16.8%) and atrial fibrillation (n= 50, 17.1%; supplementary material table I). Baseline medications of the entire cohort included aspirin (15.8%), clopidogrel (10.3%), warfarin (6.8%) and a direct oral anticoagulant (1%). Baseline medications included aspirin (15.8%), clopidogrel (10.3%), warfarin (6.8%) and a direct oral anticoagulant (1%).

Table V: Demographics: presentation blood group

	Ischaemic stroke (n=103)	**TIA (n =80)**	**Controls (n=109)**	**TOTAL 292**
Blood group: O (total 149: 48% of total cohort)	52 (50%)	42 (53%)	45 (42%)	Chi squared =3.292 (p= 0.1928)
Blood group A/ B / AB (total 151: total 52% of total cohort)	51 (50%)	36 (45%)	64 (48%)	
Unknown (n=2	0	2 (3%)	0	

ABO blood group effect

Comparing blood group O versus non-O showed significant differences in VWFAg, Ac and FVIII in the overall cohort: median values in blood group O vs blood group A/B/AB (IU/dL); VWFAg 161.4 vs 193.6, p=0.0061, VWFAc 148.3 vs 170.6, p=0.0081 and FVIII 140 vs 164.5, p=0.0008. ADAMTS13Ac levels showed no differences according to blood group in the overall cohort: ADAMTS13 Ac 90.7 vs 92.0IU/dL, p=0.545. The same pattern was seen in the control group. There was, however, no difference in VWF levels according to blood group in ischaemic stroke and TIA. Adjusting for blood group in comparison of ischaemic stroke vs control patients maintained the same differences in VWFAg, VWFAc, FVIII and ADAMTS13Ac.

For the VWFAg- ADAMTS13Ac ratio, there was no difference in the ischaemic stroke group according to whether blood group O versus A/B/AB (2.25 vs 2.64, p=0.319). Adjustment for blood group did show a higher VWFAg-ADAMTS13 Ac in patients of blood group A/B/AB versus O in TIA and control groups (TIA blood groups A/B/AB 2.01 vs O 1.59, p=0.044; control blood groups A/B/AB 1.821 vs O 1.463, p=0.0086).

Anticoagulation

In the ischaemic stroke group, warfarin therapy did not influence presentation VWFAg: warfarinised (n=8) vs not (n=94); 238.8 vs 194.3IU/dL, p= 0.0677, ADAMTS13 (warfarinised vs not, 82.5 vs 86IU/dL, p=0.8144) or the overall VWFAg/ADAMTS13Ac ratio (warfarinised vs not, 3.351 vs 2.308, p=0.0576). Expected reduction in thrombin generation with anticoagulation was apparent: warfarinised vs not, ETP 790 vs 1730NM/min, p<0.0001; peak thrombin 85.06 vs 271.6nM, p<0.0001).

Anticoagulation with warfarin or full dose dalteparin did not influence follow up VWFAg or ADAMTS13Ac: anticoagulated (n=9) vs not (n=30): VWFAg 141 vs 140.8IU/dL, p=0.7996; ADAMTS13Ac 90.9 vs 96.95IU/dL, p=0.465).

Table VI: Multiple linear regression: CRP at presentation

Source	SS	df	MS		Number of obs	140
Model	4849.401	2	2424.7		F (2,137)	9.23
Residual	35999.975	137	262.77		Prob> F	0.0002
Total	40849.37	139	293.88		R-squared	0.1187
					Adj R-squared	0.1058
					Root MSE	16.21
ADAMTS13Ac presentation	Coefficient	Std error	t	P>t	95% confidence interval	
Bilirubin	-0.7345	0.2016	-3.64	0.000	-1.133197	-0.3358
CRP	-0.1767	0.0851	-2.07	0.040	-034514	-0.00830
_cons	96.31	2.3919	40.27	0.000	91.58229	101.0419

CRP and VWFAg- ADAMTS13Ac

There was a significant difference in CRP 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); KW 11.34, p=0.0035. There was correlation between the control group CRP and VWFAg: ADAMTS13Ac (r=0.324, p=0.0013), but not seen in ischaemic stroke (r= 0.175, p=0.107) or TIA (r= 0.175, p=0.136). Multivariate regression analysis identified CRP and bilirubin to be independent predictors of ADAMTS13 at presentation of ischaemic stroke and TIA, demonstrating a negative association (Table VI) not seen with VWFAg or VWFAg- ADAMTS13Ac. There was no correlation with presentation CRP and final follow-up VWFAg-ADAMTS13Ac.

Figure 1: ROC curve analysis: VWFAg-ADAMTS13Ac ratio as predictive for mortality

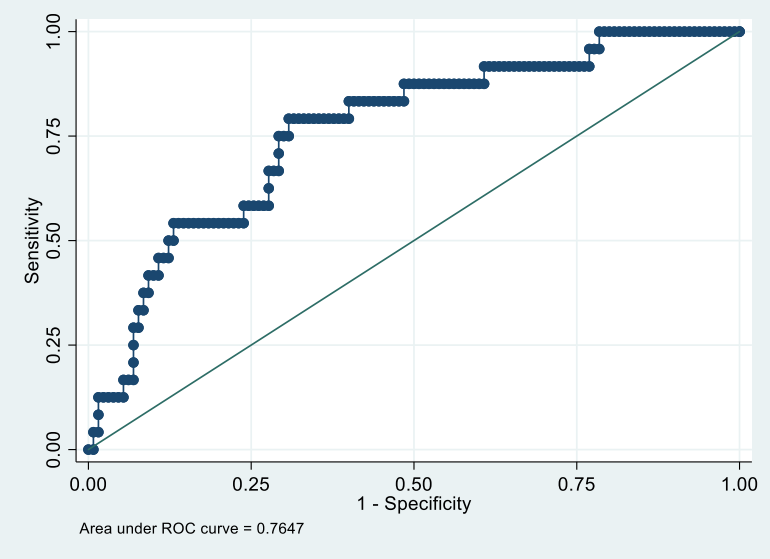


Figure 1 is a graphical representation of the receiver operator characteristic (ROC) curve analysis demonstrating sensitivity and specificity of the presenting VWFAg-ADAMTS13Ac ratio for mortality. The area under the curve (AUC) measures how well the presenting VWFAg-ADAMTS13Ac ratio can distinguish those patients who died and those who survived at follow up. The AUC of 0.765 (a perfect test being 1, a worthless test being 0.5) demonstrates predictive value of the ratio (95% confidence interval 0.664- 0.866).

Commented [AT1]: Additional statistical testing as recommended by most recent review, with explanation of purpose of ROC curve analysis.

Table VII: Survival in ischaemic stroke and TIA according to presentation VWFAg: ADAMTS13Ac: chi squared testing

Progressive increases in the baseline VWF-ADAMTS13 ratio, correlates significantly (by chi squared testing) with survival. Incremental ratios of VWFAg/ ADAMTS13Ac are shown, and with progressive ratio increase the significant correlation to mortality increases. Analysis at above and below a ratio of 2.6 is specifically added following the ROC curve analysis above highlighting this as an appropriate cut-off for sensitivity and specificity.

Baseline VWF-ADAMTS13 ratio	Alive	Deceased	Total	Chi squared
≤1	15	0	15	2.535 (p=0.1113, NS)
>1	140	24	164	
≤1.1	18	0	18	3.099 (P=0.0784, NS)
>1.1	137	24	161	
≤1.2	27	0	27	4.923 (p=0.0265)
>1.2	128	24	152	OR 10.5 (0.62-177.4)
≤1.3	36	0	36	6.977 (p=0.0083)
>1.3	119	24	143	OR 14.97 (0.89- 252.4)
≤1.4	38	0	38	7.47 (p=0.0063)
>1.4	117	24	141	OR 16.06 (0.953-270.5)
≤ 1.5	43	0	43	8.763 (p=0.0031)
> 1.5	112	24	136	OR 18.95 (1.127-318.6)
≤ 1.6	47	0	47	9.87 (p=0.0017)
> 1.6	108	24	132	OR 21.45 (1.28 -360.4)
≤ 1.7	54	1	55	9.19 (p=0.0024)
> 1.7	101	23	124	OR 12.3 (1.62-93.6)
≤ 1.8	61	2	63	8.77 (p=0.0031)
> 1.8	94	22	116	OR 7.14 (1.62- 31.2)
≤ 1.9	70	2	72	11.72 (p=0.0006)
> 1.9	85	22	107	OR 9.059 (2.06- 39.9)
≤ 2	77	3	80	11.62 (p=0.0007);
> 2	78	21	99	OR 6.9 (1.98 – 24.1)
≤ 2.6	80	5	85	14.23 (p=0.0002)
> 2.6	48	19	67	OR 6.33 (2.22-18.1)
≤ 3	10	121	131	14.03 (p=0.0002);
> 3	34	14	48	OR 4.98 (2.033-12.2)
≤ 4	143	15	158	17.7 (p< 0.0001)
> 4	12	9	21	OR 7.15 (2.59- 19.7)

Table VIII: Presentation VWFAg: ADAMTS13Ac correlation with stroke functional scores (Spearman rank non-parametric)

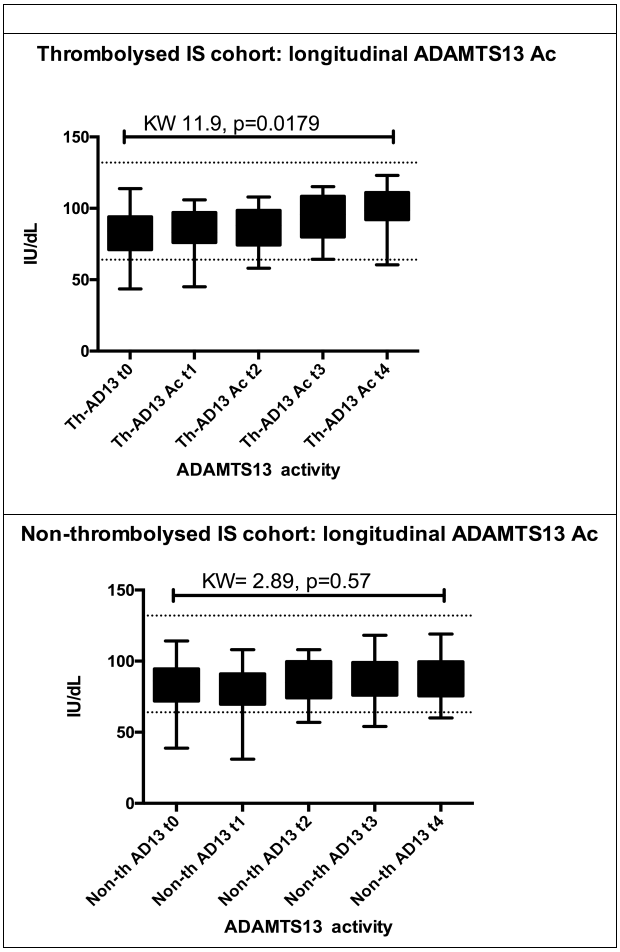
IU/dL. 95% C.I.	Age	mRS (Rankin)	NIHSS	GCS
VWFAg	0.531 (0.439- 0.611) p<0.0001	0.396 (0.289-0.493) p<0.0001	0.267 (0.152- 0.375) p<0.0001	-0.222 (-0.332- -0.105) p=0.0002
ADAMTS13	-0.328 (-0.430- -0.218) p<0.0001	-0.298 (-0.404- -0.185) p<0.0001	-0.283 (-0.389- -0.169) p<0.0001	0.194 (0.0769- 0.306) p=0.0009
VWFAg/ ADAMTS13	0.577 (0.491-0.651) p<0.0001	0.447 (0.345- 0.538) p<0.0001	0.337 (0.227- 0.439) p<0.0001	-0.255 (-0.363- -0.140) p<0.0001

Table VIII: There is a positive correlation (Spearman rank) between VWFAg with age, mRS and NIHSS and negative correlation with GCS. An inverse correlation was seen with ADAMTS13 and mRS, NIHSS and age; and a positive correlation with GCS. The VWFAg-ADAMTS13Ac ratio was positively correlated with age, mRS and NIHSS and negatively correlated with GCS: reflective of correlation with disability.

Thrombolysis and VWFAg-ADAMTS13Ac

Figure II below illustrates the resolution of ADAMTS13Ac over time in the thrombolysed IS cohort, not evident in the non-thrombolysed IS cohort.

Figure II: ADAMTS13Ac in thrombolysed IS



Longitudinal analysis shows increasing ADAMTS13Ac and decreasing vWFAg in the thrombolysed group but absent in the non- thrombolysed group.

Tables IX- XII: multiple linear regression in IS and TIA combined

Multiple linear regression was performed using Stata to more closely examine the potential relationships of clinical stroke scores with the VWFAg-ADAMTS13Ac axis in the ischaemic stroke/ TIA cohort. All the measured laboratory haemostatic markers (VWFAg, VWFAc, ADAMTS13, FVIII, peak thrombin, ETP) were entered into a multiple linear regression model to investigate impact on functional scores. Each variable was subtracted until significance. Presentation VWFAg and ADAMTS13Ac were included as independent variables in the model and reached significance for both presentation mRS (Table IX), and for presentation NIHSS score (Table X). Further longitudinal analysis showed significance of the presentation VWFAg-ADAMTS13Ac analysis for mRS at timepoint 2 (48 hours post presentation, Table XI) and final follow up (>6 weeks post presentation, Table XII).

Table IX: Multiple linear regression of VWFAg and ADAMTS13Ac combined as independent variables, with presentation mRS as dependent (outcome) variable. The model explains 15% of variability in presentation mRS score (R-squared= 15%). The coefficients show that VWFAg has a positive association with the model (VWFAg increases along with the presentation mRS), while ADAMTS13Ac is negatively associated (ADAMTS13Ac decreases, as the presentation mRS increases).

Table IX: Regression of presentation mRS with presentation VWFAg and ADAMTS13Ac

Source	SS	df	MS		Number of obs	174
Model	84.1246689	2	42.0623344		F (2,171)	15.2
Residual	473.214412	171	2.76733574		Prob> F	0.0000
Total	557.33908	173	3.22161318		R-squared	0.1509
					Adj R-squared	0.1410
					Root MSE	1.6635
mRS presentation	Coefficient	Std error	t	P>t	95% confidence interval	
VWFAg	0.0063287	0.0014144	4.47	0.000	0.0035368	0.0091205
ADAMTS13Ac	-0.0169759	0.0073922	-2.30	0.023	-0.315677	-0.0023841
_cons	2.293619	0.7661597	2.99	0.003	0.7812698	3.805967

Table X: Multiple linear regression of VWFAg and ADAMTS13 combined as independent variables, with presentation NIHSS as dependent (outcome) variable. The model explains 6.7% of variability in presentation NIHSS score (R-squared=0.67). Coefficients indicate that VWFAg has a positive

association with the model (as VWFAg increases, so does the presentation mRS), while ADAMTS13 is negatively associated (as ADAMTS13 decreases, the presentation mRS increases).

Table X: Regression of presentation NIHSS with presentation VWFAg and ADAMTS13Ac

Source	SS	df	MS		Number of obs	177
Model	406.169536	2	203.084768		F (2,174)	6.29
Residual	5618.67792	174	32.2912524		Prob> F	0.0023
Total	6024.84746	176	34.2320878		R-squared	0.0674
					Adj R-squared	0.0567
					Root MSE	5.6825
NIHSS presentation	Coefficient	Std error	t	P>t	95% confidence interval	
VWFAg	0.0095569	0.0048361	1.98	0.050	0.0000119	0.0191019
ADAMTS13Ac	-0.0615642	0.0248529	-2.48	0.014	-0.1106161	-0.0125123
_cons	7.635633	2.586321	2.95	0.004	2.531033	12.74023

Table XI: Univariate linear regression of VWFAg as an independent variable, with mRS at 48 hours post presentation as dependent (outcome) variable. The model explains 6% of variability in MRS score (R-squared= 0.596). The coefficient demonstrates that VWFAg has a positive association with the model (as VWFAg increases, so does mRS at 48 hours post presentation).

Table XI: Regression of mRS at 48 hours post presentation with presentation VWFAg

Source	SS	df	MS		Number of obs	67
Model	7.11846611	1	7.11846611		F (1, 65)	4.12
Residual	112.284519	65	1.72745414		Prob> F	0.0465
Total	119.402985	66	1.80913614		R-squared	0.0596
					Adj R-squared	0.0451
					Root MSE	1.3143
mRS t2	Coefficient	Std error	t	P>t	95% confidence interval	
VWFAg t0	0.0035811	0.0017641	2.03	0.046	0.0000579	0.0071043
_cons	2.533564	0.4368177	5.80	0.000	1.661179	3.405949

Table XII: Multiple linear regression of the VWFAg/ADAMTS13 ratio as an independent variable, with mRS at final follow-up as dependent (outcome) variable. The model explains almost 10% of the total variation in final mRS (R-squared= 0.0987). The coefficient indicates that the VWFAg-

ADAMTS13Ac ratio at presentation has a positive association with the model (as the ratio increases, so does the final follow up mRS).

Table XII: Regression of mRS at final follow up with presentation VWFAg-ADAMTS13Ac

Source	SS	df	MS		Number of obs	54
Model	14.208221	1	14.208221		F (1, 52)	5.69
Residual	129.791779	52	2.49599575		Prob> F	0.0207
Total	144	53	2.71698113		R-squared	0.0987
					Adj R-squared	0.0813
					Root MSE	1.5799
mRS t4	Coefficient	Std error	t	P>t	95% confidence interval	
VWFAg-ADAMTS13Ac t0	0.4466101	0.1871891	2.39	0.021	0.0709876	0.8222325
_cons	0.0058267	0.468855	0.01	0.990	-0.9350609	0.9467142