Alterations in the Von Willebrand Factor/ADAMTS13 axis in preeclampsia

Short title: **VWF/ADAMTS13 in preeclampsia**

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Essentials

- Preeclampsia is a common gestational hypertensive disorder with significant morbidity/mortality.
- We assessed von Willebrand Factor (VWF)/ADAMTS13 axis in preeclampsia by case-control study.
- VWF antigen and VWF/ADAMTS13 ratio are both elevated in preeclampsia.
- Thrombocytopenia in preeclampsia correlates with VWF activity, and angiogenic imbalance.

Abstract

Background:

Preeclampsia is a gestational hypertensive disorder, characterised by maternal endothelial activation, and increased ratio of soluble fms-like tyrosine kinase inhibitor-1 (sFlt-1) to placental growth factor (PlGF). The von Willebrand Factor (VWF)/ADAMTS13 axis is of interest because of the underlying endothelial activation, and clinical overlap with pregnancy-associated thrombotic thrombocytopenic purpura.

Objectives:

To assess VWF, ADAMTS13, and VWF/ADAMTS13 ratio in preeclampsia, and look for associations with sFlt-1/PlGF ratio, and clinical features.

Patients/Methods:

34 preeclampsia cases and 48 normal pregnancies were assessed in a case-control study. 12 normal pregnancies in women with a history of preeclampsia formed an additional comparator group. VWF antigen (VWF:Ag) and activity (VWF:Ac (VWF:GPIbM)) were measured via automated immunoturbidimetric assay; ADAMTS13 activity via FRETS-VWF73 assay; and sFlt-1 and PlGF via ELISA.

Results:

VWF:Ag was higher in preeclampsia than normal pregnancy (median 3.07 vs 1.87 iu/ml, P<0.0001). ADAMTS13 activity was slightly lower (89.6 vs 94.4 iu/dl, P=0.02), with no severe deficiencies. Significant elevations in VWF:Ac were not observed in preeclampsia, resulting

in reduced VWF:Ac/VWF:Ag ratios (0.77 vs 0.97, P <0.0001). VWF:Ag/ADAMTS13 ratios were significantly higher in preeclampsia (3.42 vs 2.06, P <0.0001), with adjusted odds ratio of 19.2 for ratio>2.7 (>75th centile of normal pregnancy). Those with a history of preeclampsia had similar ratios to normal pregnant controls. VWF:Ag/ADAMTS13 and sFlt-1/PlGF were not correlated. However, % fall in platelets correlated positively with VWF:Ac (P=0.01); VWF:Ac/VWF:Ag ratio (P=0.004), and sFlt-1/PlGF ratio (P=0.01).
Conclusions: The VWF/ADAMTS13 axis is significantly altered in preeclampsia. Further investigation of potential clinical utility is warranted.

Keywords

Preeclampsia, von Willebrand factor, ADAMTS13, angiogenic imbalance, thrombocytopenia

1. Introduction

Preeclampsia is a common but heterogeneous obstetric disorder, affecting 3-5% of pregnancies[1], with significant associated fetomaternal morbidity and mortality[2]. Pathogenesis is incompletely understood, but reduced placental perfusion is considered the underlying driver. This results in release of soluble factors into the circulation which cause maternal endothelial injury, with hypertension and organ injury, as well as varying degrees of placental insufficiency, with fetal growth restriction, prematurity and stillbirth[3]. Haematological complications include thrombocytopenia, and rarely, thrombotic microangiopathy (TMA)[4]. There is an associated increased risk of venous thrombosis, and an increased lifetime risk of both venous and arterial thrombosis[5-7]. Haemolysis, Elevated Liver Enzymes, Low Platelets (HELLP) syndrome, which is generally recognised as a severe form of preeclampsia[8], is poorly understood but is thought to be a form of TMA mainly involving the liver[9].

'Angiogenic imbalance', including increased levels of soluble fms-like tyrosine kinase-1 (sFlt-1), and reduced circulating levels of placental growth factor (PlGF), is recognized as a key driver of maternal endothelial activation/dysfunction[3, 10, 11], and the markers have clinical utility [12, 13]. sFlt-1 is the soluble form of vascular endothelial growth factor

receptor 1 (VEGFR-1)[10]. PlGF, which is structurally similar to the potent vasoactive agent VEGF-A, is primarily produced by the placenta and appears to have a regulatory role in angiogenic and inflammatory processes[14]. Both VEGF-A and PlGF bind to sFlt-1 and this is thought to decrease their availability to signal through endothelial VEGF receptors to protect the endothelium.

Von Willebrand Factor (VWF) is a large multimeric glycoprotein secreted by endothelial cells and platelets in response to activation. In conditions of high shear stress it mediates platelet adhesion and aggregation. It is also a carrier protein for FVIII. Importantly, individuals with blood group O tend to have lower VWF levels[15]. Newly secreted highly thrombogenic ultra-large (UL) multimers are normally rapidly cleaved to smaller, less reactive multimers by the metalloproteinase ADAMTS13, which is mainly produced in hepatic stellate cells [16]. Severe deficiency (<10 iu/dL) of ADAMTS13 causes thrombotic thrombocytopenic purpura (TTP), a rare thrombotic microangiopathy, as a result of abnormal persistence of UL VWF multimers which leads to microvascular thrombosis[17, 18]. In pregnancy, VWF rises [19], and ADAMTS13 falls [20, 21], as a normal physiological response (although the physiological fall in ADAMTS13 is not to TTP levels). Pregnancy is a well-recognised trigger for TTP[9], which can be difficult to distinguish clinically from preeclampsia or HELLP[9], so it is important to exclude severe ADAMTS13 deficiency in cases of pregnancy TMA where the diagnosis is unclear. Conversely, preeclampsia can accompany pregnancy-associated TTP[22]. Even in the absence of severe ADAMTS13 deficiency, a high VWF/ADAMTS13 ratio has been shown to be a useful marker of disease severity/prognosis in other conditions involving endothelial activation/dysfunction including acute COVID19 infection[23, 24], post-COVID syndrome[25], stroke[26] and sepsis[27], and also a predictor of cardiovascular disease in later life[28, 29].

The VWF/ADAMTS13 axis is therefore of potential interest in preeclampsia: firstly because of the endothelial basis of its pathogenesis; secondly because of the associated thrombotic risk; and thirdly because of clinical overlap with TTP. Previous studies demonstrate VWF is elevated in preeclampsia[30-36] and HELLP syndrome[35, 37, 38], compared to normal pregnancy, likely secondary to endothelial activation. ADAMTS13 activity has been studied

in preeclampsia and HELLP to a lesser extent and with discrepant results[31-38]. The VWF/ADAMTS13 ratio has not to our knowledge previously been assessed.

The aim of the present study was to further investigate the VWF/ADAMTS13 axis in preeclampsia compared to normal pregnancy, and to look for correlations with (i) the degree of angiogenic imbalance (as measured by sFlt-1 and PlGF); (ii) clinical features. VWF/ADAMTS13 parameters were also assessed in women with a current normal pregnancy, but a history of preeclampsia.

2. Methods

2.1 Ethical aspects

The study was conducted in accordance with the declaration of Helsinki. The protocol was approved by a Regional Ethics Committee (REC reference 18/NW/0552) and all participants gave written informed consent.

2.2 Patients

A case-control design was used. 39 pregnant women with preeclampsia and 50 women with uncomplicated pregnancies ('normal pregnant controls') were recruited from a single large regional referral unit, between October 2018 and January 2022. In addition, 15 women with a history of preeclampsia in previous pregnancies but with a currently normal pregnancy ('history of preeclampsia group') were recruited as an additional comparator group. Study size was predicted to be sufficient based on the results of previously published similar studies[31, 32, 34, 36].

Participants were followed up until 6 weeks postpartum. Of those recruited, 2 normal pregnant controls were excluded due to subsequent development of hypertension; and 5 preeclampsia cases were excluded as they were twin pregnancies. The remaining 34 preeclampsia cases and 48 normal pregnant controls were analysed. Of the history of

preeclampsia group, 1 developed preeclampsia, 1 was lost to follow up and 1 developed significant thrombocytopenia, so 12 were included in the final analysis.

2.3 Inclusion criteria

Preeclampsia was defined according to 2018 International Society of Hypertension in Pregnancy (ISSHP) criteria[4]. Early onset preeclampsia was defined as onset before 34 weeks' gestation. Since the ISHHP no longer classify preeclampsia as severe or non-severe, severe features were classified as per American College of Obstetricians and Gynaecologists (ACOG)[8]. HELLP was also defined as per ACOG[8]. Normal pregnant controls were normotensive singleton pregnancies, with no proteinuria, no significant abnormal findings on routine antenatal bloods or scans, no history of hypertension, and delivered at term (≥37 weeks) with normal birthweight. The history of preeclampsia group met the criteria for the normal pregnant control group but had suffered preeclampsia in ≥1 previous pregnancy. All women were recruited ≥20 weeks' gestation, prior to delivery, and none were in active labour. IVF conceptions were included for all groups.

2.4 Exclusion criteria

Exclusion criteria for all groups included a known haematological/coagulation abnormality, chronic hypertension, diabetes mellitus, active autoimmune disease or chronic inflammatory disease, current infection, cancer, cardiovascular, renal or hepatic disease.

2.5 Blood Sampling

Blood samples were obtained on one occasion at the time of recruitment (at ≥20 weeks' gestation, prior to onset of labour/delivery), from an antecubital vein into sodium citrate and serum separator (SST) tubes, centrifuged at 2000g for 15 minutes, aliquoted and frozen at -80C within 1 hour of sample collection.

2.6 Laboratory Assays

ADAMTS13 activity was measured in citrated plasma using the fluorescence resonance energy transfer-VWF73 (FRETS-VWF73) assay[39]. VWF antigen and VWF activity were measured in citrated plasma with a standard automated immunoturbidimetric assay in a Sysmex CS-2500 analyzer with a Siemens kit (vWF Ag (VWF:Ag) and INNOVANCE VWF Ac kit (VWF:GPIbM), Siemens Healthcare Diagnostics, Marburg, Germany). Non-pregnant normal ranges were: 60-146 IU/dl for ADAMTS13 activity; 0.50-1.60 IU/ml for VWF:Ag; 0.50-1.87 IU/ml for VWF:GpIbM). sFlt-1 and PlGF were measured in serum using commercially available ELISA kits (Human VEGFR1/Flt-1 Quantikine ELISA kit (R&D Systems, MN); Human PlGF Quantikine ELISA kit (R&D Systems, MN)) according to the manufacturer's instructions. VWF multimer analysis was performed in 2 cases, using gradient agarose gel electrophoresis and overnight blotting to nitrocellulose, followed by labelling with biotinylated rabbit antihuman VWF and visualisation with HRP alkaline phosphatase. Triplet structure of the bands was compared visually with normal control plasma (densitometric analysis was not performed).

2.7 Statistical analysis

Statistical analysis was performed using GraphPad Prism 9 (GraphPad software, San Diego, CA). Continuous data were summarized as the median and interquartile range (IQR). The number and percentage was used to summarize categorical data. The VWF:Ag/ADAMTS13 ratio was calculated by (VWF:Ag (IU/ml)/ADAMTS13 activity (IU/dl)) × 100. Non-parametric statistical methods were used, as variables showed signs of non-normality using the Shapiro-Wilk test. Mann-Whitney U-test was used to compare continuous variables between two groups. Fisher's exact test was used to compare categorical variables between groups. Spearman's rank was used to assess correlation between variables. The association between VWF:Ag/ADAMTS13 ratio the occurrence of preeclampsia was assessed using multivariate logistic regression using the 75^{th} centile value of the normal control group as the reference for VWF:Ag/ADAMTS13 ratio. All statistical tests were 2-sided and significance was set at $P < 0.05$.

3. Results

3.1 Patient characteristics and routine laboratory parameters

Table 1 shows clinical characteristics and selected laboratory parameters of the study participants. Comparing the preeclampsia and normal pregnancy groups, maternal age and gestational age (GA) at blood sampling were well matched, but blood group O (associated with lower VWF levels) was more common in the preeclampsia group, who also had higher body mass indexes (BMI).

Comparing the history of preeclampsia group with the normal pregnancy group (Table 1), maternal age, BMI and proportion of blood group O were not significantly different, but GA at blood sampling was significantly lower in the history of preeclampsia group, and there were significantly fewer nulliparous women.

The preeclampsia group tended to be delivered earlier, with lower birthweights than the normal pregnancy group (Table 1). The history of preeclampsia group also delivered earlier than the normal pregnancy group, but it was departmental policy to aim for delivery by 38 weeks in this group, and there was no significant difference in birthweight (Table 1).

Of the preeclampsia group, 47.0% (16/34) were early onset; 67.6% (23/34) met the criteria for severe preeclampsia; 44.1% (15/34) delivered before 37 weeks' gestation; and 39.3% (13/33) resulted in a small for gestational age (SGA) infant (defined as birthweight <10^{th} centile on customized growth chart). Median peak creatinine was 78μmol (IQR 66-87). Median peak alanine aminotransferase (ALT) was 43 IU/l (IQR 28-144), with peak ALT above the upper limit of normal in 18/34 (52.9%). Thrombocytopenia was more common and more severe (in terms of platelet nadir and % fall to nadir) in the preeclampsia group compared to the other two groups (Table 1). Four women in the preeclampsia group displayed the combination of low platelets, elevated liver enzymes and features of haemolysis but only 1/4 met full laboratory criteria for HELLP syndrome at the time of the study bloods.

Table 1. Clinical characteristics of the study groups.

	Normal pregnancy	Preeclampsia	History of preeclampsia	P value (Preeclampsia vs normal pregnancy)	P value (History of preeclampsia vs normal pregnancy)
Number analysed	48	34	12	-	-
Maternal age at bloods	32.5 (29-36)	33 (30-35)	35.5 (32.3-36)	0.46	0.11
GA at bloods (weeks)	35.5 (29-38)	36 (33-37)	27.5 (24-32)	0.38	0.0004*
BMI (first trimester) (kg/m^2)	23 (21-25)	25 (23-27)	26.3 (21-29)	0.02*	0.18
Blood group O	14/48 (29.1%)	20/34 (58.8%)	2/12 (16.7%)	0.01*	0.48
IVF conception	6/48 (12.5%)	7/34 (20.6%)	0/12 (0%)	0.37	0.33
Nulliparous	30/48 (62.5%)	24/34 (70.6%)	0/12 (0%)	0.49	0.0001*
GA at delivery (weeks)	40 (39-41)	37 (34-38)	38 (37.3-39)	<0.0001*	<0.0001*
Birthweight (g)	3500 (3070-3840)	2510 (1815-3125)	3130 (2800-3585)	<0.0001*	0.06
Small for gestational age[$]	0/48 (0%)	13/33 (39.4%)	0/12 (0%)	<0.0001*	>0.9999
Nadir platelet count ($x10^9/L$)**	203 (170-234)	164 (118-199)	218.5 (171-269.5)	0.002*	0.22
% fall in platelets to nadir	15.2 (4.0-23.0)	39.6 (20.0-53.0))	21.2 (13.3-26.4)	<0.0001*	0.18
Platelets <150 $x10^9/L$	6/48 (12.5%)	14/34 (41.2%)	1/12 (8.3%)	0.004*	>0.9999
Platelets <100 $x10^9/L$	0/48 (0%)	8/34 (23.5%)	0/12 (0%)	0.0005*	>0.9999

Continuous data are given as median (interquartile range); categorical data given as proportion (%). P values derived using Mann-Whitney test for continuous data and Fisher Exact test for categorical data. *Denotes statistical significance at P <0.05 level.
GA, gestational age; BMI, body mass index; IVF, in vitro fertilization. [$]Small for gestational age defined as birthweight less than 10^{th} centile on customised growth charts. **Nadir platelet count refers to the lowest platelet count, either pre- or post- delivery. First trimester platelet counts (and therefore % fall in platelets to nadir) and BMI were unavailable for 3 preeclampsia cases. GA at delivery and birthweight was unavailable for 1 normal pregnant control who had an uncomplicated home birth at term.

3.2 ADAMTS13 activity, VWF antigen (VWF:Ag) and activity (VWF:Ac), VWF:Ag/ADAMTS13 ratio and VWF multimeric pattern.

There were no cases of severe ADAMTS13 deficiency (<10 iu/dl) in any group (excluding TTP in all cases) . All ADAMTS13 activities fell within the non-pregnant normal laboratory range except for one preeclampsia case (54.1 iu/dl).

Comparing preeclampsia cases to normal pregnant controls, ADAMTS13 activity was lower in preeclampsia (89.6 (80.0-101.4) vs 97.4 (91.7-104.5) iu/dl, P= 0.02, Fig 1(a)). VWF:Ag was markedly higher in preeclampsia (3.07 (2.17-3.58) vs 1.87 (1.57-2.67) iu/ml, P< 0.0001, Fig 1(b)). VWF:Ag/ADAMTS13 ratio was also markedly elevated in preeclampsia (3.42 (2.28-4.07) vs 2.06 (1.59-2.67), P <0.0001, Fig 1(d)). Interestingly VWF:Ac was not significantly elevated in preeclampsia cases compared to normal pregnancy (2.37 (1.93-2.65 vs 2.02 (1.50-2.54) iu/ml, P= 0.10, Fig 1(c)).

In contrast, there was no difference between the history of preeclampsia and normal pregnancy groups, in ADAMTS13, either VWF parameter, or VWF:Ag/ADAMTS13 ratio (for the history of preeclampsia group: ADAMTS13 activity 95.6 (85.6-107.2) iu/dl, P= 0.69, Fig 1(a); VWF:Ag 1.57 (1.34-2.17) iu/ml, P= 0.05, Fig 1(b); VWF:Ag/ADAMTS13 ratio 2.00 (1.41-2.42), P= 0.46, Fig 1(d), VWF:Ac 1.89 (1.38-2.32) iu/ml, P= 0.27, Fig 1(c)).

VWF multimer analysis performed on two samples with the highest VWF:Ag/ADAMTS13 ratios did not demonstrate the presence of ultra large VWF multimers.

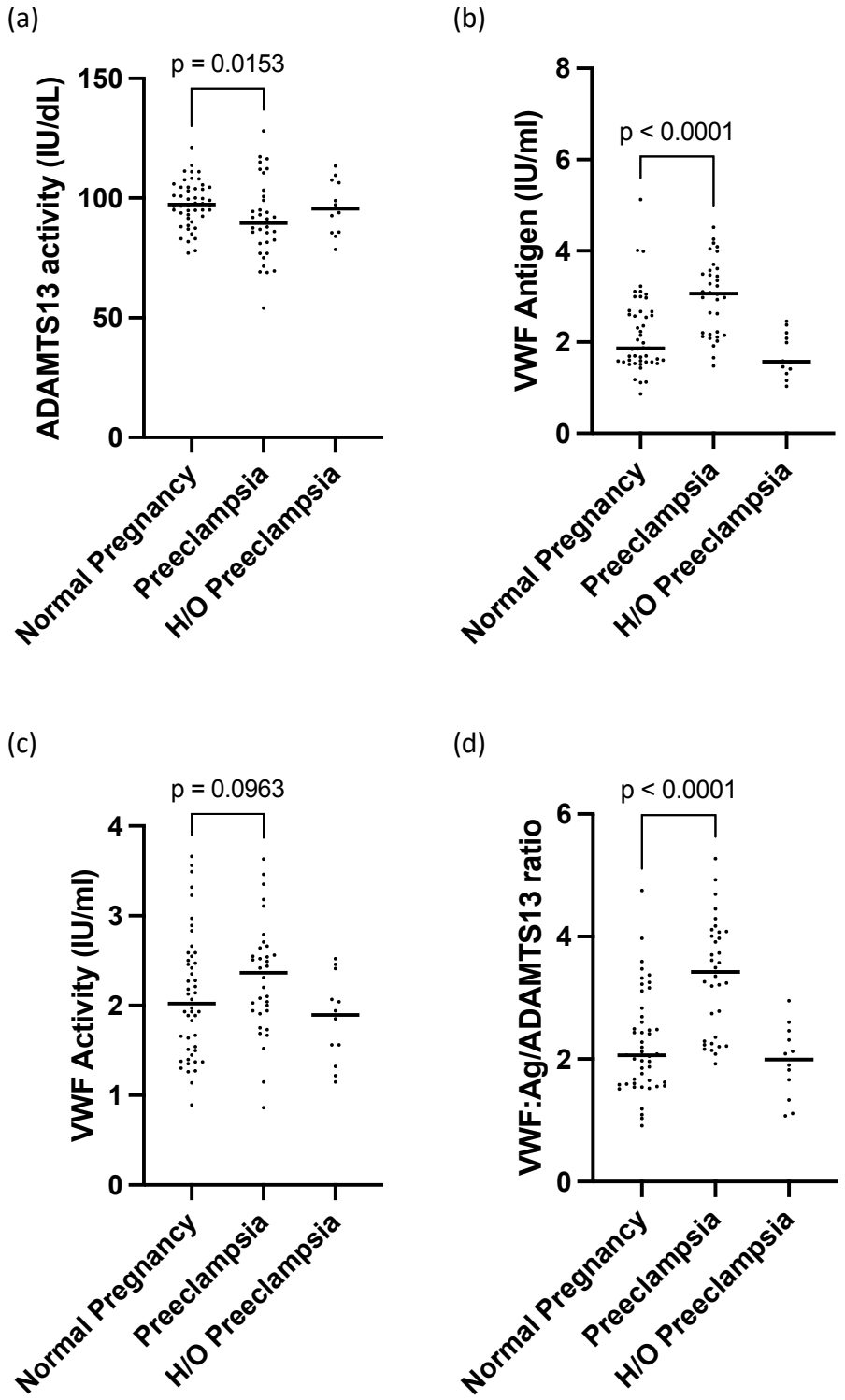

Figure 1. **Changes in von Willebrand Factor (VWF), ADAMTS13, and VWF/ADAMTS13 ratio in preeclampsia (n=34) compared to normal pregnancy (n=48) and normal pregnancy with a history of preeclampsia (H/O Preeclampsia) (n=12).** Scatter plots for (a) ADAMTS13 activity (via FRETS-VWF73 assay); (b) VWF antigen (VWF:Ag) (via automated immunoturbidometric assay); (c) VWF activity (VWF:GP1bM) (via automated immunoturbidometric assay); (d) VWF:Ag/ADAMTS13 ratio ((VWF:Ag (IU/ml)x100)/ADAMTS13 activity (IU/dl), for the three study groups. The solid line represents the median value for each group. P- values derived using the Mann-Whitney test.

For the normal pregnant control group, there was no significant correlation between either VWF:Ag and ADAMTS13 activity, or VWF:Ac and ADAMTS13 activity (r =0.16, P =0.27; and r =0.01, P =0.95 respectively, Fig 2).

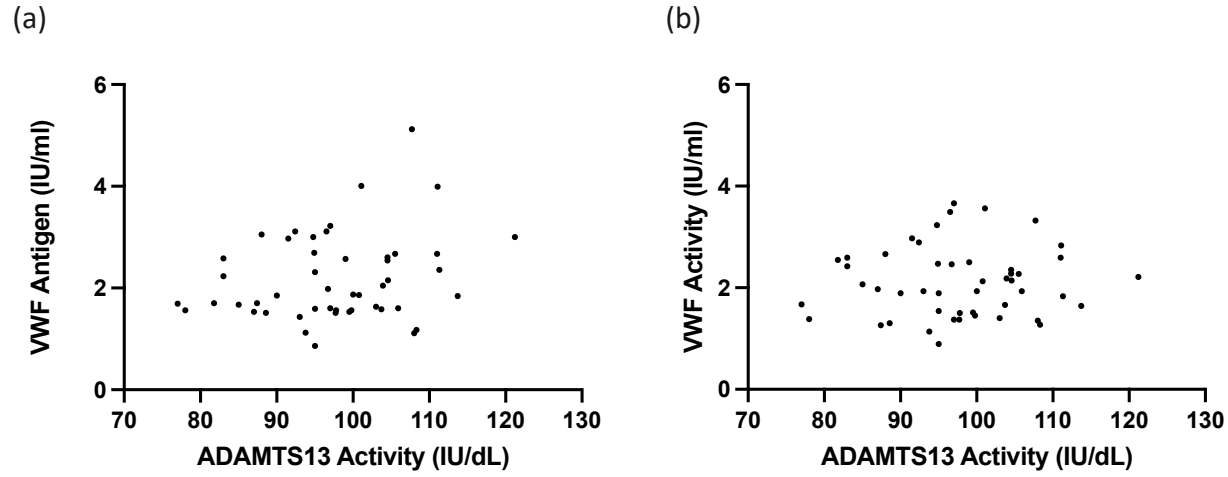

Figure 2. Scatter plots of VWF parameters against ADAMTS activity for normal pregnant controls (n=48). (a) VWF antigen against ADAMTS13 activity, (b) VWF activity against ADAMTS13 activity. There was no significant correlation between either VWF parameter and ADAMTS13 activity, as assessed by Spearman's rank correlation coefficient (r= 0.16, P= 0.27, for VWF antigen; r= 0.01, P= 0.95 for VWF activity)

3.3 Consideration of potential confounding factors

For the normal pregnant controls, there was no significant correlation between gestational age (GA) and ADAMTS13 activity (p= 0.44) but there were positive correlations between GA and (i) VWF activity (r= 0.32, P= 0.03) (ii) VWF:Ag (r= 0.52, P = 0.0001) and (iii) VWF:Ag/ADAMTS13 ratio (r= 0.49, P = 0.0004). As expected, normal pregnancies with blood group O had lower VWF:Ag, VWF:Ac and VWF:Ag/ADAMTS13 ratios than non-group O (median VWF:Ag, 1.58 vs 2.42 iu/ml (P= 0.0006); median VWF:Ac, 1.44 vs 2.20 iu/ml (P=0.0001); median VWF:Ag/ADAMTS13 ratio, 1.6 vs 2.3 (P= 0.008)). There was no difference in ADAMTS13 according to group O status (97.8 (O) vs 96.9 (non-O) iu/dl, P= 0.68).

Multivariate logistic regression analysis was performed to estimate the odds ratio (OR) of a high VWF:Ag or high VWF:Ag/ADAMTS13 being associated with preeclampsia, taking into account the effect of potential confounders of (i) gestational age (GA) (ii) blood group O status, (iii) nulliparity vs non nulliparity, (iv) BMI, (v) IVF vs spontaneous conception. A 'high' VWF:Ag or VWF:Ag/ADAMTS13 ratio were both defined as >2.7, based on the 75th centile values for the normal pregnant controls for both parameters both coincidentally being 2.67. As shown in Table 2, there was a significant association between both 'high' VWF:Ag iu/ml and also 'high' VWF:Ag/ADAMTS13 ratio and preeclampsia, independent of GA, blood group O status, parity, BMI and IVF status. The adjusted ORs were in fact higher than the crude ORs probably because of the overrepresentation of blood group O (with lower VWF levels than non-O) in the preeclampsia group.

Table 2. Odds ratios for Risk of Preeclampsia, for VWF antigen (VWF:Ag) and for VWF:Ag/ADAMTS13 ratio

	Preeclampsia cases	Normal pregnant controls	Odds ratio (95% CI)	Adjusted odds ratio (95% CI)
VWF:Ag >2.7 iu/ml	21/34	10/48	6.14 (2.32-15.9)	16.8 (4.2-93.5)
VWF:Ag/ADAMTS13 >2.7	24/34	11/48	8.07 (2.80-22.1)	19.2 (4.8-111.9)

Adjusted odds ratio adjusted for gestational age, blood group O status, BMI, nulliparity and IVF status. VWF:Ag and VWF:AG/ADAMTS13 cut offs chosen according to 75th percentile values for normal pregnant controls. Values given for preeclampsia cases and normal pregnant controls are proportion of subjects.

3.4 Post hoc analysis of VWF activity/ VWF antigen ratio

Following the observation that whilst VWF antigen was markedly increased in preeclampsia, VWF activity was not, VWF activity to VWF antigen ratios (VWF:Ac/VWF:Ag) were calculated. VWF:Ac/VWF:Ag was significantly lower in preeclampsia cases than normal pregnant controls (0.77 (0.68-0.92) vs 0.97 (0.88-1.08), P <0.0001).

3.5 Correlation of VWF:Ag/ADAMTS13 ratio with sFlt-1 and PlGF

For the preeclampsia cases, median sFlt-1 was 8960 pg/ml (IQR 6412-15626); median PlGF was 73.0 pg/ml (IQR 47.2-101.9; and median sFlt-1/PlGF ratio was 140.9 (IQR 77.8-249.1) respectively. No significant correlation was seen with VWF:Ag or VWF:Ag/ADAMTS13 ratio for any of the 3 parameters (For VWF:Ag, sFlt-1: r =0.23, P =0.19; PlGF: r =0.10, P =0.58; sFlt-1/PlGF ratio: r =0.07, P =0.70; for VWF:Ag/ADAMTS13 ratio, sFlt-1: r =0.11, P =0.55; PlGF: r =-0.11, P =0.57; sFlt-1/PlGF ratio: r =0.05, P =0.80).

3.6 Relationship between VWF:Ag and VWF:Ag/ADAMTS13 ratio and clinical features in preeclampsia

There were no significant differences in either VWF:Ag, or VWF:Ag/ADAMTS13 ratio, depending on severity of preeclampsia, SGA neonate or not, or presence/absence of thrombocytopenia <100 x10^9/L (the threshold defined by ACOG as a severe feature of preeclampsia) (Table 3).

Table 3. VWF:Ag/ADAMTS13 values according to selected clinical features of preeclampsia.

	VWF:Ag (iu/ml)	P value	VWF:Ag/ADAMTS13 ratio	P value
Severity of preeclampsia	Severe: 2.98 (2.15-3.70) Non-severe: 3.10 (2.20-3.47)	0.86	Severe: 3.35 (2.25-4.01) Non-severe: 3.57 (2.74-4.17)	0.45
Small for gestational age (SGA) [$]	SGA: 3.05 (2.00-3.59) Non-SGA: 3.09 (2.20-3.64)	0.51	SGA: 3.19 (2.22-4.05) Non-SGA: 3.66 (2.87-4.08)	0.19
Nadir platelet count**	<100 x10^9/L: 2.96 (2.27-3.40) ≥100 x10^9/L: 3.09 (2.16-3.63)	0.80	<100 x10^9/L: 3.53 (2.41-3.96) ≥100 x10^9/L: 3.38 (2.25-4.09)	0.95

VWF:Ag, von Willebrand Factor antigen. VWF:Ag and VWF:Ag/ADAMTS13 ratios given as median (interquartile range). P values obtained using Mann-Whitney test, with significance level defined as P <0.05. Severe preeclampsia defined according to ACOG criteria[8]. [$]Small for gestational age defined as birthweight less than 10th centile on customised growth charts. **Nadir platelet count refers to the lowest platelet count, either pre- or post- delivery.

3.7 Post hoc analysis of thrombocytopenia in preeclampsia

Given the increased frequency and severity of thrombocytopenia in the preeclampsia cases compared to normal pregnant controls (Table 1), additional associations between thrombocytopenia and the measured laboratory parameters were investigated. Since VWF is involved in platelet activation/aggregation, it was postulated that VWF activity might be more relevant than VWF antigen in this regard. As illustrated in Figure 3, preeclampsia cases with a platelet nadir <100 x10^9/L (n=8) had higher VWF activities and VWF:Ac/VWF:Ag ratios, than those with nadirs ≥100 x10^9/L (n=26) (median VWF:Ac: 2.61 vs 2.14, P =0.03; median VWF:Ac/VWF:Ag ratio: 0.94 vs 0.76, P =0.008). This was despite a higher frequency of blood group O (with an expected lower VWF activity) in the group with a platelet nadir <100 x10^9/L (6/8 (75%) vs 15/26 (58%)). There was no difference between GA at bloods in the two groups, (median GA, 34 vs 36 weeks, P = 0.88), so this was not considered a confounder.

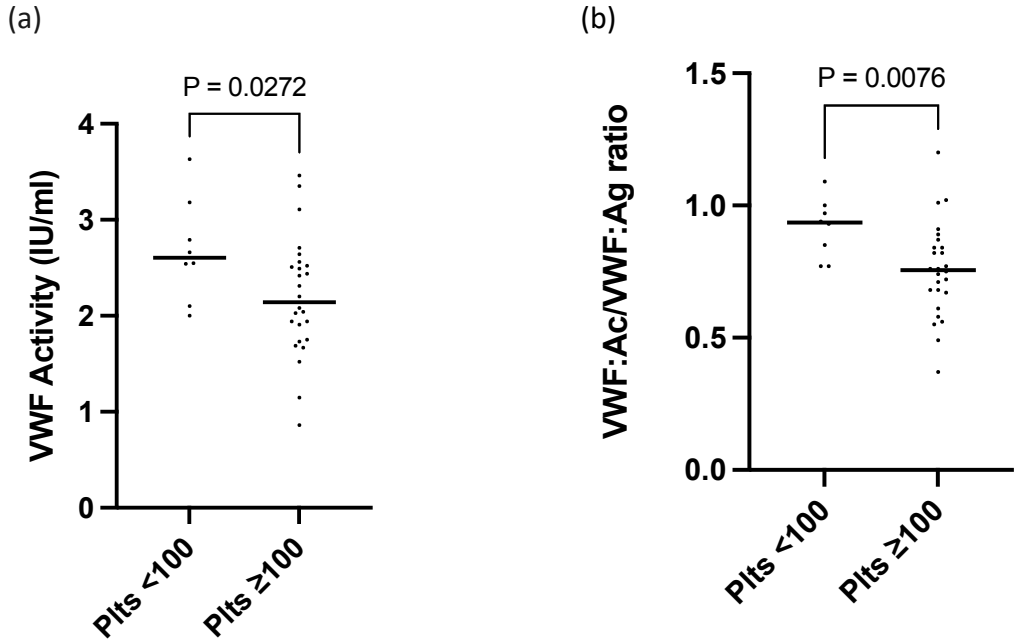

Figure 3: VWF activity and VWF activity/VWF antigen ratio (VWF:Ac/VWF:Ag) for preeclampsia cases (n=34) according to nadir platelet count. Scatter plots of (a) VWF activity and (b) VWF:Ac/VWF:Ag ratio for preeclampsia cases with nadir platelet counts < 100 x10^9/L (n=8) compared to those with nadir platelet counts ≥100 x10^9/L (n=26). Plts, nadir platelet count (pre- or

post-delivery) x10^9/L. The solid line represents the median value for each group. P- value derived using the Mann-Whitney test

In addition, positive correlations were observed between: (i) % fall in platelet count to nadir, and VWF activity (r =0.45, P =0.01, Fig 5(a)); (ii) % fall in platelet count to nadir and VWF:Ac/VWF:Ag ratio (r= 0.50, P =0.004, Fig 5(b)); (iii) % fall in platelet count to nadir and sFlt-1/PlGF ratio (r =0.45, P =0.01, Fig 5(d)); but not between % fall in platelet count to nadir and VWF:Ag/ADAMTS13 ratio (r =0.14, P =0.46, Fig 5(c)).

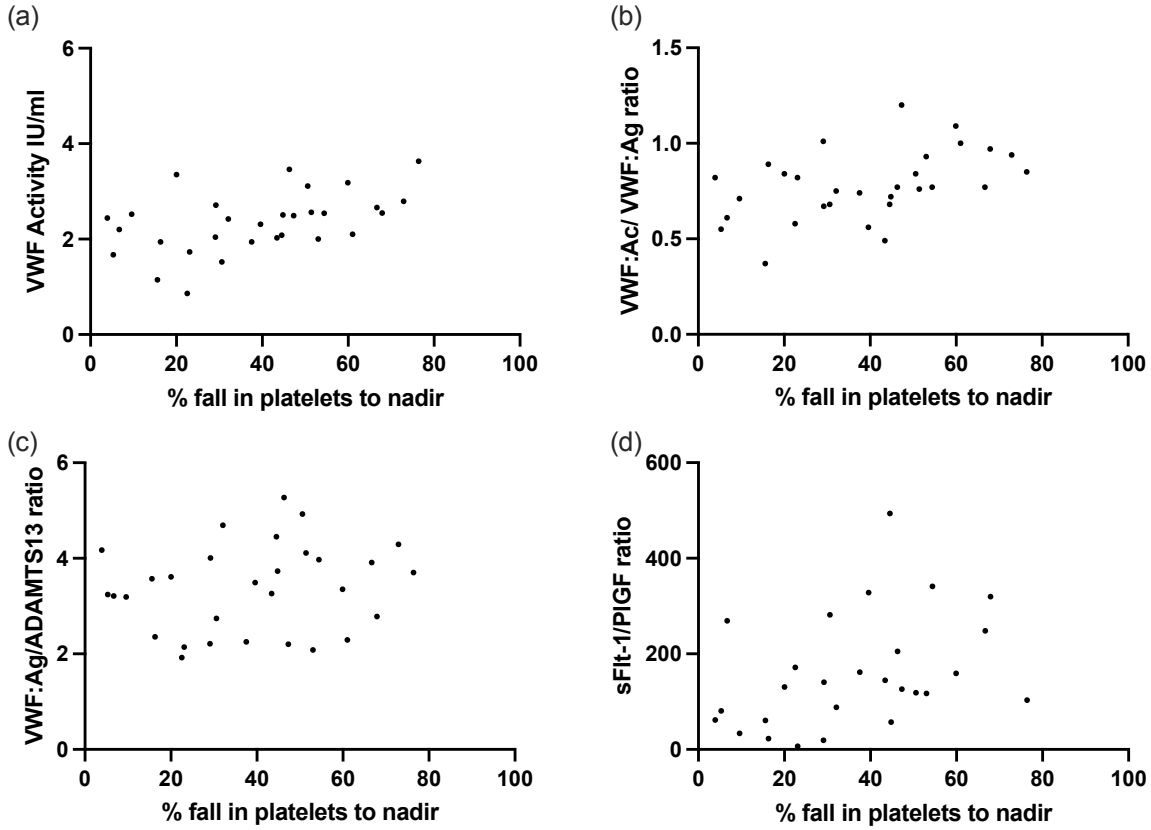

Figure 4. Scatter plots of (a) VWF activity, (b) VWF activity/VWF antigen ratio (VWF:Ac/VWF:Ag ratio), (c) VWF antigen/ADAMTS13 ratio (VWF:Ag/ADAMTS13) and (c) sFlt-1/PlGF ratio, against % fall in platelet count to nadir. Significant positive correlations were observed via Spearman's rank correlation coefficient for three data sets: VWF activity and % fall in platelets to nadir (r =0.45, P =0.01); VWF:Ac/VWF:Ag ratio and % fall in platelets to nadir (r= 0.50, P =0.004); sFlt-1/PlGF ratio and % fall in platelets to nadir (r =0.45, P =0.01), but not for VWF:Ag/ADAMTS13 ratio and % fall in platelets (r =0.14, P =0.46)

4. Discussion

In summary, we have demonstrated marked increases in both VWF:Ag and VWF:Ag/ADAMTS13 ratio in preeclampsia compared to normal pregnancy, with median levels 1.7 times higher for both parameter. ADAMTS13 activity was slightly reduced, but still within laboratory normal range in all but one case (with a slightly low value). Interestingly however, VWF:Ac was not shown to be significantly higher, and the VWF:Ac/VWF:Ag ratio was significantly lower in preeclampsia than normal pregnancy.

Previous studies have demonstrated similar elevations in VWF:Ag in preeclampsia [31-34, 36], and it is likely that endothelial activation with increased Weibel-Palade body release of VWF is the underlying cause [40]. Regarding ADAMTS13, whilst previous small studies have shown mild-to-moderately reduced ADAMTS13 activity in HELLP syndrome [35, 37, 41], the existing literature on preeclampsia is conflicting, with 3 studies showing mildly reduced ADAMTS13 activity compared to normal pregnancy ((ranging from 74% to 89% of the normal pregnant values)[33, 34, 36], but 2 studies showing no difference[31, 32]. The variance is likely explained by clinical heterogeneity of preeclampsia cases, variation in baseline ADAMTS13 levels between individuals, and study size and design (including method of ADAMTS13 analysis and GA at bloods).

Low ADAMTS13 activity in the face of significantly elevated VWF may be expected due to increased consumption of the protease by increased substrate, and a reciprocal relationship has been shown in other settings, such as inflammation [42, 43]. However, we did not find a negative correlation between ADAMTS13 activity and VWF:Ag, mirroring the findings of Molvarec et al [31]. Meanwhile, Stepanian et al [33], Aref et al [36] and Alpoim at al [34] all demonstrated that high VWF and low ADAMTS13 are independently associated with preeclampsia, implying a more complex relationship. Alternative reasons for reduced ADAMTS13 activity in preeclampsia include: increased proteolytic inactivation by thrombin and plasmin[44] [7, 45]; reduced synthesis [46] and inhibition of activity[47] due to inflammatory cytokines [48]; as well effects on synthesis/secretion due to liver dysfunction if present. Also of note, expression of *ADAMTS13* mRNA and protein has been identified in

placental tissue, with evidence for a role in promoting angiogenesis and trophoblastic cell development, and with reduced expression in preeclamptic placentas[49].

VWF:Ag/ADAMTS13 ratios in preeclampsia, or indeed normal pregnancy, have not to our knowledge been previously reported. The median ratios for both preeclampsia and normal pregnancy (3.57 and 2.06 respectively), were both elevated compared to the non-pregnant state, in which median values have been previously reported in the region of 1 [24, 25]). For comparison, median ratios of 2.4 in acute ischaemic stroke[26], and 3.2[23] and 6.1[24] in acute COVID19 infection are reported in the literature, with demonstrated prognostic utility/clinical correlations. Although VWF:Ag/ADAMTS13 ratios in preeclampsia were significantly elevated compared to normal pregnancy, multimer analysis suggested that the imbalance was not severe enough to cause systemic persistence of ultralarge (UL) VWF multimers (as are present in TTP). This has also been demonstrated by others previously [31, 50]. However, even in the absence of systemically circulating UL VWF multimers, the raised VWF:Ag/ADAMTS13 ratio could still theoretically contribute to microthrombotic risk in the preeclamptic placenta and maternal organs, where VWF is subject to high shear forces which promote ADAMTS13 binding, Under such conditions, VWF/ADAMTS13 imbalance could become functionally relevant and could predispose to placental and end organ thrombosis/ischaemia. Our data did not demonstrate an association between VWF:Ag/ADAMTS13 (or VWF:Ag) and preeclampsia severity or risk of fetal growth restriction, but this may have been because of the small sample size, which is a limitation of our study.

VWF:Ag/ADAMTS13 ratios in women with a current normal pregnancy, but a history of preeclampsia, were not significantly different to those of normal pregnant controls, although this group was small and earlier median GA at bloods was a significant confounder. Whilst acknowledging the limitations, this finding suggests that the elevated ratios observed in preeclampsia are due to the acute disease process, rather than an underlying disposition, and do not confer similar changes in future pregnancies. Similarly, no increased risk of preeclampsia was demonstrated in women carrying three functional genetic variants of ADAMTS13 associated with reduced activity, or in women with a lower baseline (non-pregnant) ADAMTS13 activity, in a Norwegian study[51]. Nevertheless, it would be

interesting to investigate a potential role for the VWF/ADAMTS13 axis in mediating the long term thrombotic risks following preeclampsia[5, 6].

We had postulated that if VWF level or VWF/ADAMTS13 ratio is a marker of the degree of endothelial activation in preeclampsia, there might be positive correlation with the sFlt-1/PlGF ratio, given that an excess of sFlt-1 relative to PlGF is believed to be a key mediator of endothelial dysfunction[10, 11]. However, the data did not support this hypothesis, with no significant correlation observed between with either VWF:Ag or VWF:Ag/ADAMTS13 ratio for any of sFlt-1, PlGF or sFlt-1/PlGF ratio (although sample size is again a limitation).

The finding of a reduced VWF:Ac/VWF:Ag ratio in preeclampsia was intriguing. Most previous studies of VWF in preeclampsia measured VWF:Ag levels only, although Hulstein et al[35] also measured VWF:RCo, which was more modestly elevated than VWF:Ag in a small group of preeclampsia patients, in agreement with our findings. It is conceivable that preeclamptic vascular changes produce conditions of increased shear stress which promote VWF proteolysis by ADAMTS13, and a reduced proportion of larger more active multimers (in a situation analogous to severe aortic stenosis[52], and as recently reported in severe COVID19[53]). In other words, although VWF levels are high, there is a skew to smaller less active multimers. The limited multimeric analysis performed in this study was primarily intended to rule in/out the presence of ultra-large multimers and did not use densitometry or normal pregnant plasma controls. More extensive and quantitative multimeric analysis would be needed to investigate for a relative loss of higher molecular weight multimers in preeclampsia.

Thrombocytopenia is a relatively common complication of preeclampsia and is believed to result from increased platelet activation and consumption[54]. No relationship was observed between VWF:Ag or VWF:Ag/ADAMTS13 ratio and thrombocytopenia. However, VWF activity correlated positively with % fall in platelets, as did VWF:Ac/VWF:Ag ratio. It would appear that while overall preeclampsia is associated with increased VWF levels with reduced VWF:Ac/VWF:Ag ratios, patients with preserved VWF:Ac/VWF:Ag ratios are more likely to develop thrombocytopenia. Hulstein et al[35] demonstrated increased amounts of what they termed ‘active’ VWF, with greater GPIb binding capacity, in HELLP compared to

preeclampsia without HELLP. Based on positive correlation between propeptide/mature VWF ratio and levels of 'active' VWF, they postulated that acute, as opposed to chronic, endothelial activation is the source of the 'active' VWF. Extending this idea to our findings, it may be the patients with greater fall in platelet count had more acutely activated endothelium which produced more 'active' VWF, explaining the higher VWF:Ac/VWF:Ag ratios. The positive correlation between sFlt-1/PlGF ratio and the % fall in platelets also supports the concept that thrombocytopenia is related to endothelial activation.

The main limitation of this study is the sample size. Although the changes in the VWF/ADAMTS13 axis in preeclampsia were significant enough to be clearly demonstrated in the small sample, it may be that the observed lack of association with clinical features, or angiogenic imbalance, is merely due to sample size. The small size of the history of preeclampsia group and earlier GA at bloods limit the validity of the observed normal ratio in that group, and further investigation of the ratio in women who develop preeclampsia, before and after the event, is warranted.

In summary, this study builds on existing evidence for a role of the VWF/ADAMTS13 axis in the pathophysiology of preeclampsia, and specifically demonstrates marked increases in both VWF:Ag and VWF:Ag/ADAMTS13 ratio, with a reduction in VWF activity:antigen ratio, and a mild reduction in ADAMTS13 activity. In addition, associations between thrombocytopenia, increased VWF activity, and increased sFlt-1/PlGF ratio were demonstrated. Preeclampsia remains a leading cause of maternal/fetal morbidity/mortality, and improved risk tools, therapeutic and preventative strategies are needed[55]. Larger prospective studies with repeated blood sampling would help determine whether alterations in VWF/ADAMTS13 axis might precede onset of the clinical syndrome and have utility in risk prediction models; as well clarifying whether levels at diagnosis might have prognostic value, and whether alterations in the axis may persist and contribute to long term cardiovascular/thrombotic risk.

The main reason to measure ADAMTS13 in preeclampsia remains the exclusion of TTP in cases with diagnostic uncertainty. However demonstration of a VWF/ADAMTS13 imbalance in preeclampsia, even in the absence of severe ADAMTS13 deficiency, is also of value as it

raises the question of whether administration of recombinant ADAMTS13 might restore VWF/ADAMTS13 homeostasis (for example as demonstrated *ex vivo* in the setting of severe COVID-19 infection[56]), and ameliorate the clinical picture, raising the possibility of a potential therapeutic avenue to be explored in preeclampsia.

Authorship details:

LN designed the study, recruited participants, collected samples/data, performed laboratory tests and data analysis, and wrote the manuscript. MT, RdG and ALD provided intellectual input. ALD, AJD and KM assisted with participant recruitment. DS assisted with laboratory tests. GA provided statistical advice. As senior author, MS designed the study, interpreted the data, and wrote the manuscript. All authors reviewed the manuscript and approved the final version of the paper.

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Competing interests:

LN has served on an advisory board for Alexion; MT has served on advisory boards for Ablynx, Sanofi, Bayer, has received speaker fees for Bayer, Sanofi and Anthos and received consultancy fees from Bayer. MS has received speaker fees and has served on advisory boards for Alexion, Novartis, Takeda, Sanofi, and Octapharma and has received research grants from Shire and Alexion. The remaining authors have no conflicts of interest to declare.

References

1 Abalos E, Cuesta C, Grosso AL, Chou D, Say L. Global and regional estimates of preeclampsia and eclampsia: a systematic review. *Eur J Obstet Gynecol Reprod Biol*. 2013; **170**: 1-7. 10.1016/j.ejogrb.2013.05.005.

2 Jim B, Karumanchi SA. Preeclampsia: Pathogenesis, Prevention, and Long-Term Complications. *Seminars in nephrology*. 2017; **37**: 386-97. 10.1016/j.semnephrol.2017.05.011.

3 Chappell LC, Cluver CA, Kingdom J, Tong S. Pre-eclampsia. *Lancet (London, England)*. 2021; **398**: 341-54. 10.1016/S0140-6736(20)32335-7.

4 Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, Hall DR, Warren CE, Adoyi G, Ishaku S, International Society for the Study of Hypertension in P. The hypertensive disorders of pregnancy: ISSHP classification, diagnosis & management recommendations for international practice. *Pregnancy hypertension*. 2018; **13**: 291-310. 10.1016/j.preghy.2018.05.004.

5 Scheres LJJ, Lijfering WM, Groenewegen NFM, Koole S, de Groot CJM, Middeldorp S, Cannegieter SC. Hypertensive Complications of Pregnancy and Risk of Venous Thromboembolism. *Hypertension (Dallas, Tex : 1979)*. 2020; **75**: 781-7. 10.1161/HYPERTENSIONAHA.119.14280.

6 Bellamy L, Casas JP, Hingorani AD, Williams DJ. Pre-eclampsia and risk of cardiovascular disease and cancer in later life: systematic review and meta-analysis. *BMJ (Clinical research ed)*. 2007; **335**: 974. 10.1136/bmj.39335.385301.BE.

7 Kelliher S, Maguire PB, Szklanna PB, Weiss L, Ewins K, O'Doherty R, Angelov D, Ni Ainle F, Kevane B. Pathophysiology of the Venous Thromboembolism Risk in Preeclampsia. *Hamostaseologie*. 2020; **40**: 594-604. 10.1055/a-1162-3905.

8 Gestational Hypertension and Preeclampsia: ACOG Practice Bulletin, Number 222. *Obstetrics and gynecology*. 2020; **135**: e237-e60. 10.1097/AOG.0000000000003891.

9 Fakhouri F, Scully M, Provot F, Blasco M, Coppo P, Noris M, Paizis K, Kavanagh D, Pene F, Quezada S, Hertig A, Kissling S, O'Brien P, Delmas Y, Alberio L, Winer N, Veyradier A, Cataland S, Fremeaux-Bacchi V, Loirat C, Remuzzi G, Tsatsaris V. Management of thrombotic microangiopathy in pregnancy and postpartum: report from an international working group. *Blood*. 2020; **136**: 2103-17. 10.1182/blood.2020005221.

10 Maynard SE, Min J-Y, Merchan J, Lim K-H, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *Journal of Clinical Investigation*. 2003; **111**: 649-58. 10.1172/JCI200317189.

11 Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA. Circulating angiogenic factors and the risk of preeclampsia. *The New England journal of medicine*. 2004; **350**: 672-83. 10.1056/NEJMoa031884.

12 Duhig KE, Myers J, Seed PT, Sparkes J, Lowe J, Hunter RM, Shennan AH, Chappell LC, group Pt. Placental growth factor testing to assess women with suspected pre-eclampsia: a multicentre, pragmatic, stepped-wedge cluster-randomised controlled trial. *Lancet (London, England)*. 2019; **393**: 1807-18. 10.1016/S0140-6736(18)33212-4.

13 Zeisler H, Llurba E, Chantraine F, Vatish M, Staff AC, Sennstrom M, Olovsson M, Brennecke SP, Stepan H, Allegranza D, Dilba P, Schoedl M, Hund M, Verlohren S. Predictive Value of the sFlt-1:PlGF Ratio in Women with Suspected Preeclampsia. *The New England journal of medicine*. 2016; **374**: 13-22. 10.1056/NEJMoa1414838.
14 Newell LF, Holtan SG. Placental growth factor: What hematologists need to know. *Blood reviews*. 2017; **31**: 57-62. 10.1016/j.blre.2016.08.004.
15 Gill JC, Endres-Brooks J, Bauer PJ, Marks WJ, Jr., Montgomery RR. The effect of ABO blood group on the diagnosis of von Willebrand disease. *Blood*. 1987; **69**: 1691-5.
16 Zheng XL. Structure-function and regulation of ADAMTS-13 protease. *Journal of thrombosis and haemostasis : JTH*. 2013; **11 Suppl 1**: 11-23. 10.1111/jth.12221.
17 Furlan M, Robles R, Solenthaler M, Wassmer M, Sandoz P, Lammle B. Deficient activity of von Willebrand factor-cleaving protease in chronic relapsing thrombotic thrombocytopenic purpura. *Blood*. 1997; **89**: 3097-103.
18 Scully M, Cataland S, Coppo P, de la Rubia J, Friedman KD, Kremer Hovinga J, Lammle B, Matsumoto M, Pavenski K, Sadler E, Sarode R, Wu H. Consensus on the standardization of terminology in thrombotic thrombocytopenic purpura and related thrombotic microangiopathies. *Journal of thrombosis and haemostasis : JTH*. 2017; **15**: 312-22. 10.1111/jth.13571.
19 Sanchez-Luceros A, Meschengieser SS, Marchese C, Votta R, Casais P, Woods AI, Nadal MV, Salviu MJ, Lazzari MA. Factor VIII and von Willebrand factor changes during normal pregnancy and puerperium. *Blood coagulation & fibrinolysis : an international journal in haemostasis and thrombosis*. 2003; **14**: 647-51. 10.1097/00001721-200310000-00005.
20 Sanchez-Luceros A, Farias CE, Amaral MM, Kempfer AC, Votta R, Marchese C, Salviu MJ, Woods AI, Meschengieser SS, Lazzari MA. von Willebrand factor-cleaving protease (ADAMTS13) activity in normal non-pregnant women, pregnant and post-delivery women. *Thrombosis and haemostasis*. 2004; **92**: 1320-6. 10.1160/th03-11-0683.
21 Mannucci PM, Canciani MT, Forza I, Lussana F, Lattuada A, Rossi E. Changes in health and disease of the metalloprotease that cleaves von Willebrand factor. *Blood*. 2001; **98**: 2730-5.
22 Scully M, Thomas M, Underwood M, Watson H, Langley K, Camilleri RS, Clark A, Creagh D, Rayment R, McDonald V, Roy A, Evans G, McGuckin S, Ni Ainle F, Maclean R, Lester W, Nash M, Scott R, P OB. Thrombotic thrombocytopenic purpura and pregnancy: presentation, management, and subsequent pregnancy outcomes. *Blood*. 2014; **124**: 211-9. 10.1182/blood-2014-02-553131.
23 Joly BS, Darmon M, Dekimpe C, Dupont T, Dumas G, Yvin E, Beranger N, Vanhoorelbeke K, Azoulay E, Veyradier A. Imbalance of von Willebrand factor and ADAMTS13 axis is rather a biomarker of strong inflammation and endothelial damage than a cause of thrombotic process in critically ill COVID-19 patients. *Journal of thrombosis and haemostasis : JTH*. 2021; **19**: 2193-8. 10.1111/jth.15445.
24 Mancini I, Baronciani L, Artoni A, Colpani P, Biganzoli M, Cozzi G, Novembrino C, Boscolo Anzoletti M, De Zan V, Pagliari MT, Gualtierotti R, Aliberti S, Panigada M, Grasselli G, Blasi F, Peyvandi F. The ADAMTS13-von Willebrand factor axis in COVID-19 patients. *Journal of thrombosis and haemostasis : JTH*. 2021; **19**: 513-21. 10.1111/jth.15191.
25 Prasannan N, Heightman M, Hillman T, Wall E, Bell R, Kessler A, Neave L, Doyle A, Devaraj A, Singh D, Dehbi HM, Scully M. Impaired exercise capacity in post-COVID-19

syndrome: the role of VWF-ADAMTS13 axis. *Blood advances*. 2022; **6**: 4041-8. 10.1182/bloodadvances.2021006944.
26 Taylor A, Vendramin C, Singh D, Brown MM, Scully M. von Willebrand factor/ADAMTS13 ratio at presentation of acute ischemic brain injury is predictive of outcome. *Blood advances*. 2020; **4**: 398-407. 10.1182/bloodadvances.2019000979.
27 Singh K, Kwong AC, Madarati H, Kunasekaran S, Sparring T, Fox-Robichaud AE, Liaw PC, Kretz CA. Characterization of ADAMTS13 and von Willebrand factor levels in septic and non-septic ICU patients. *PloS one*. 2021; **16**: e0247017. 10.1371/journal.pone.0247017.
28 Sonneveld MA, de Maat MP, Portegies ML, Kavousi M, Hofman A, Turecek PL, Rottensteiner H, Scheiflinger F, Koudstaal PJ, Ikram MA, Leebeek FW. Low ADAMTS13 activity is associated with an increased risk of ischemic stroke. *Blood*. 2015; **126**: 2739-46. 10.1182/blood-2015-05-643338.
29 Sonneveld MA, Kavousi M, Ikram MA, Hofman A, Rueda Ochoa OL, Turecek PL, Franco OH, Leebeek FW, de Maat MP. Low ADAMTS-13 activity and the risk of coronary heart disease - a prospective cohort study: the Rotterdam Study. *Journal of thrombosis and haemostasis : JTH*. 2016; **14**: 2114-20. 10.1111/jth.13479.
30 Deng L, Bremme K, Hansson LO, Blomback M. Plasma levels of von Willebrand factor and fibronectin as markers of persisting endothelial damage in preeclampsia. *Obstetrics and gynecology*. 1994; **84**: 941-5.
31 Molvarec A, Rigo J, Jr., Boze T, Derzsy Z, Cervenak L, Mako V, Gombos T, Udvardy ML, Harsfalvi J, Prohaszka Z. Increased plasma von Willebrand factor antigen levels but normal von Willebrand factor cleaving protease (ADAMTS13) activity in preeclampsia. *Thrombosis and haemostasis*. 2009; **101**: 305-11.
32 Zhang D, Xiao J, Huang H, Chen J, Liu T, Yin Z, Gao D, Liu Q, Ai J, Chen S. Von Willebrand factor antigen and ADAMTS13 activity assay in pregnant women and severe preeclamptic patients. *J Huazhong Univ Sci Technolog Med Sci*. 2010; **30**: 777-80. 10.1007/s11596-010-0657-4.
33 Stepanian A, Cohen-Moatti M, Sanglier T, Legendre P, Ameziane N, Tsatsaris V, Mandelbrot L, de Prost D, Veyradier A. Von Willebrand factor and ADAMTS13: a candidate couple for preeclampsia pathophysiology. *Arteriosclerosis, thrombosis, and vascular biology*. 2011; **31**: 1703-9. 10.1161/atvbaha.111.223610.
34 Alpoim PN, Gomes KB, Godoi LC, Rios DR, Carvalho MG, Fernandes AP, Dusse LM. ADAMTS13, FVIII, von Willebrand factor, ABO blood group assessment in preeclampsia. *Clin Chim Acta*. 2011; **412**: 2162-6. 10.1016/j.cca.2011.07.030.
35 Hulstein JJ, van Runnard Heimel PJ, Franx A, Lenting PJ, Bruinse HW, Silence K, de Groot PG, Fijnheer R. Acute activation of the endothelium results in increased levels of active von Willebrand factor in hemolysis, elevated liver enzymes and low platelets (HELLP) syndrome. *Journal of thrombosis and haemostasis : JTH*. 2006; **4**: 2569-75. 10.1111/j.1538-7836.2006.02205.x.
36 Aref S, Goda H. Increased VWF antigen levels and decreased ADAMTS13 activity in preeclampsia. *Hematology (Amsterdam, Netherlands)*. 2013; **18**: 237-41. 10.1179/1607845412y.0000000070.
37 Lattuada A, Rossi E, Calzarossa C, Candolfi R, Mannucci PM. Mild to moderate reduction of a von Willebrand factor cleaving protease (ADAMTS-13) in pregnant women with HELLP microangiopathic syndrome. *Haematologica*. 2003; **88**: 1029-34.
38 Yoshida Y, Matsumoto M, Yagi H, Isonishi A, Sakai K, Hayakawa M, Hori Y, Sado T, Kobayashi H, Fujimura Y. Severe reduction of free-form ADAMTS13, unbound to von

Willebrand factor, in plasma of patients with HELLP syndrome. *Blood advances*. 2017; **1**: 1628-31. 10.1182/bloodadvances.2017006767.
39 Kokame K, Nobe Y, Kokubo Y, Okayama A, Miyata T. FRETS-VWF73, a first fluorogenic substrate for ADAMTS13 assay. *British journal of haematology*. 2005; **129**: 93-100. 10.1111/j.1365-2141.2005.05420.x.
40 Nadar SK, Al Yemeni E, Blann AD, Lip GY. Thrombomodulin, von Willebrand factor and E-selectin as plasma markers of endothelial damage/dysfunction and activation in pregnancy induced hypertension. *Thrombosis research*. 2004; **113**: 123-8. 10.1016/j.thromres.2004.02.010.
41 Pourrat O, Coudroy R, Pierre F. ADAMTS13 deficiency in severe postpartum HELLP syndrome. *British journal of haematology*. 2013; **163**: 409-10. 10.1111/bjh.12494.
42 Mannucci PM, Capoferri C, Canciani MT. Plasma levels of von Willebrand factor regulate ADAMTS-13, its major cleaving protease. *British journal of haematology*. 2004; **126**: 213-8. 10.1111/j.1365-2141.2004.05009.x.
43 Reiter RA, Varadi K, Turecek PL, Jilma B, Knobl P. Changes in ADAMTS13 (von-Willebrand-factor-cleaving protease) activity after induced release of von Willebrand factor during acute systemic inflammation. *Thrombosis and haemostasis*. 2005; **93**: 554-8. 10.1160/TH04-08-0467.
44 Crawley JT, Lam JK, Rance JB, Mollica LR, O'Donnell JS, Lane DA. Proteolytic inactivation of ADAMTS13 by thrombin and plasmin. *Blood*. 2005; **105**: 1085-93. 10.1182/blood-2004-03-1101.
45 Weiner CP. Preeclampsia-eclampsia syndrome and coagulation. *Clin Perinatol*. 1991; **18**: 713-26.
46 Cao WJ, Niiya M, Zheng XW, Shang DZ, Zheng XL. Inflammatory cytokines inhibit ADAMTS13 synthesis in hepatic stellate cells and endothelial cells. *Journal of thrombosis and haemostasis : JTH*. 2008; **6**: 1233-5. 10.1111/j.1538-7836.2008.02989.x.
47 Bernardo A, Ball C, Nolasco L, Moake JF, Dong JF. Effects of inflammatory cytokines on the release and cleavage of the endothelial cell-derived ultralarge von Willebrand factor multimers under flow. *Blood*. 2004; **104**: 100-6. 10.1182/blood-2004-01-0107.
48 Sharma A, Satyam A, Sharma JB. Leptin, IL-10 and inflammatory markers (TNF-alpha, IL-6 and IL-8) in pre-eclamptic, normotensive pregnant and healthy non-pregnant women. *American journal of reproductive immunology (New York, NY : 1989)*. 2007; **58**: 21-30. 10.1111/j.1600-0897.2007.00486.x.
49 Xiao J, Feng Y, Li X, Li W, Fan L, Liu J, Zeng X, Chen K, Chen X, Zhou X, Zheng XL, Chen S. Expression of ADAMTS13 in Normal and Abnormal Placentae and Its Potential Role in Angiogenesis and Placenta Development. *Arteriosclerosis, thrombosis, and vascular biology*. 2017; **37**: 1748-56. 10.1161/atvbaha.117.309735.
50 Thorp JM, Jr., White GC, 2nd, Moake JL, Bowes WA, Jr. von Willebrand factor multimeric levels and patterns in patients with severe preeclampsia. *Obstetrics and gynecology*. 1990; **75**: 163-7.
51 von Krogh AS, Kremer Hovinga JA, Romundstad PR, Roten LT, Lammle B, Waage A, Quist-Paulsen P. ADAMTS13 gene variants and function in women with preeclampsia: a population- based nested case- control study from the HUNT Study. *Thrombosis research*. 2015; **136**: 282-8. 10.1016/j.thromres.2015.06.022.
52 Blackshear JL, Wysokinska EM, Safford RE, Thomas CS, Stark ME, Shapiro BP, Ung S, Johns GS, Chen D. Indexes of von Willebrand factor as biomarkers of aortic stenosis severity

(from the Biomarkers of Aortic Stenosis Severity [BASS] study). *Am J Cardiol*. 2013; **111**: 374-81. 10.1016/j.amjcard.2012.10.015.
53 Ward SE, Fogarty H, Karampini E, Lavin M, Schneppenheim S, Dittmer R, Morrin H, Glavey S, Ni Cheallaigh C, Bergin C, Martin-Loeches I, Mallon PW, Curley GF, Baker RI, Budde U, O'Sullivan JM, O'Donnell JS, Irish C-VSi. ADAMTS13 regulation of VWF multimer distribution in severe COVID-19. *Journal of thrombosis and haemostasis : JTH*. 2021; **19**: 1914-21. 10.1111/jth.15409.
54 Pishko AM, Levine LD, Cines DB. Thrombocytopenia in pregnancy: Diagnosis and approach to management. *Blood reviews*. 2020; **40**: 100638. 10.1016/j.blre.2019.100638.
55 Myatt L. The prediction of preeclampsia: the way forward. *American journal of obstetrics and gynecology*. 2022; **226**: S1102-S7 e8. 10.1016/j.ajog.2020.10.047.
56 Turecek PL, Peck RC, Rangarajan S, Reilly-Stitt C, Laffan MA, Kazmi R, James I, Dushianthan A, Schrenk G, Gritsch H, Ewenstein BM, Mellgard B, Erdlenbruch W, Jain N, Binder NB, Mumford AD. Recombinant ADAMTS13 reduces abnormally up-regulated von Willebrand factor in plasma from patients with severe COVID-19. *Thrombosis research*. 2021; **201**: 100-12. 10.1016/j.thromres.2021.02.012.