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BRIEF REPORT

ADAMTS13 regulation of VWF multimer distribution in severe COVID-19

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Abstract

Background: Consistent with fulminant endothelial cell activation, elevated plasma von Willebrand factor (VWF) antigen levels have been reported in patients with COVID-19. The multimeric size and function of VWF are normally regulated through A Disintegrin And Metalloprotease with ThrombSpondin Motif type 1 motif, member 13 (ADAMTS-13)--mediated proteolysis.

Objectives: This study investigated the hypothesis that ADAMTS-13 regulation of VWF multimer distribution may be impaired in severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) infection contributing to the observed microvascular thrombosis.

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Irish COVID-19 Vasculopathy Study (iCVS) investigators listed in the Appendix.

Patients and Methods: Patients with COVID-19 (*n* = 23) were recruited from the Beaumont Hospital Intensive Care Unit (ICU) in Dublin. Plasma VWF antigen, multimer distribution, ADAMTS-13 activity, and known inhibitors thereof were assessed.
Results: We observed markedly increased VWF collagen-binding activity in patients with severe COVID-19 compared to controls (median 509.1 versus 94.3 IU/dl). Conversely, plasma ADAMTS-13 activity was significantly reduced (median 68.2 IU/dl). In keeping with an increase in VWF:ADAMTS-13 ratio, abnormalities in VWF multimer distribution were common in patients with COVID-19, with reductions in high molecular weight VWF multimers. Terminal sialylation regulates VWF susceptibility to proteolysis by ADAMTS-13 and other proteases. We observed that both N- and O-linked sialylation were altered in severe COVID-19. Furthermore, plasma levels of the ADAMTS-13 inhibitors interleukin-6, thrombospondin-1, and platelet factor 4 were significantly elevated.
Conclusions: These findings support the hypothesis that SARS-CoV-2 is associated with profound quantitative and qualitative increases in plasma VWF levels, and a multifactorial down-regulation in ADAMTS-13 function. Further studies will be required to determine whether therapeutic interventions to correct ADAMTS-13-VWF multimer dysfunction may be useful in COVID-microvascular thrombosis and angiopathy.

KEYWORDS
ADAMTS-13, COVID-19, multimers, SARS-CoV-2, von Willebrand factor

1 | INTRODUCTION

The severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) pandemic has already been responsible for more than 2 million deaths caused by severe bilateral pneumonia, venous thromboembolism, and microvascular thrombosis that results in significant hypoxia. Although the pathogenic mechanisms contributing to this pneumonia remain poorly understood, autopsy studies in COVID-19 patients have reported disseminated thrombosis, microangiopathy, and angiogenesis within the pulmonary microvasculature.[1,2] These post-mortem studies also highlighted significant endothelial cell (EC) damage within the lungs, including loss of EC tight junctions, separation of EC from basement membranes, and EC apoptosis. Interestingly, EC expresses the angiotensin-converting enzyme 2 receptor and electron microscopy analysis has demonstrated the presence of SARS-CoV-2 within EC.[3] A number of other pathways have also been implicated in EC injury in patients with severe COVID-19 (e.g., hypoxia, complement pathway activation, and pro-inflammatory cytokine production).[4]

Clinical studies have demonstrated that COVID-19 is associated with a characteristic coagulopathy. Given the normal role of ECs in regulating thrombin generation, von Willebrand factor (VWF) release and platelet adhesion and activation, it seems likely that EC damage is critical in triggering this coagulopathy and propensity for thrombosis. Consistent with that concept, we and others have observed markedly elevated plasma VWF antigen (VWF:Ag) and VWF propeptide (VWF:pp) levels in patients with severe COVID-19.[5,6]

Essentials

- COVID-19 is associated with a characteristic coagulopathy and acute endothelial cell activation.
- Patients with severe COVID-19 present with elevated plasma von Willebrand factor (VWF) levels and changes in multimer distribution.
- COVID-19 results in a modest reduction in A Disintegrin And Metalloprotease with ThrombSpondin Motif type 1 motif, member 13 (ADAMTS-13) activity, accompanied by increased levels of ADAMTS-13 inhibitors.
- Therapeutic interventions targeting ADAMTS-13-VWF multimer dysfunction may be beneficial in COVID--microvascular thrombosis.

Indeed, patients with severe SARS-CoV-2 had VWF:pp levels higher than those seen in other fulminant vascular disorders such as thrombotic thrombocytopenic purpura (TTP). The striking increases in plasma VWF levels are sustained over weeks and recent studies have reported that higher plasma VWF:Ag and VWF:pp levels correlate with worse clinical outcomes in patients with severe COVID-19.[5] Critically, however, it remains unclear whether the increased VWF levels play any direct role in modulating microvascular occlusion within the lungs, or whether they merely represent a biomarker of acute EC activation and damage.

VWF multimer distribution is a key determinant of its functional activity. High molecular weight multimers (HMWM) bind both collagen and platelet glycoprotein Ib alpha (GPIbα) with significantly higher affinity compared to low molecular weight multimers (LMWM). In normal plasma, VWF multimer distribution is regulated by the metalloprotease ADAMTS-13 (A Disintegrin And Metalloprotease with ThrombSpondin Motif type 1 motif, member 13). Dysregulation of VWF proteolysis by ADAMTS-13 is important in the etiology of a number of microangiopathies that share similar features to severe COVID-19 (notably TTP and cerebral malaria).[7] Although several studies have reported elevated plasma VWF levels in severe COVID-19,[6,8–12] the data on VWF multimer patterns is less clear. To gain further insights into the potential dysregulation of the VWF--ADAMTS-13 axis in COVID-19, we investigated VWF multimer distribution, as well as a series of factors shown to influence the susceptibility of VWF to ADAMTS-13 proteolysis.

2 | METHODS

Following study approval by the Hospital Research Ethics Committee, adult patients with COVID-19 were recruited from the Beaumont Hospital Intensive Care Unit (ICU) in Dublin (demographic and clinical details can be found in Table S1 in supporting information). A local control group of 10 healthy individuals (median age of 46 years, 72% male) was utilized for comparison in this study. All participants had a positive SARS-CoV-2 polymerase chain reaction result and all received low molecular weight heparin thromboprophylaxis. Blood samples were collected into 3.2% sodium citrate on admission to ICU. Plasma VWF:Ag and VWF collagen binding activity (VWF:CB) were determined by ELISA and VWF sialylation assessed using modified lectin-binding arrays as before.[13] VWF multimer analysis was performed as previously described.[7] ADAMTS-13 activity was quantified using a commercial FRETS-VWF73 assay (Peptides International, Inc.). Plasma interleukin (IL-) 6, thrombospondin (TSP-) 1, and platelet factor 4 (PF4) levels were measured using commercial ELISA kits (R&D Systems). Normally and non-normally distributed quantitative data were compared using the Student's t-test and Mann-Whitney U test, respectively. Data were analyzed using GraphPad Prism 8 and a *P*-value of <.05 was considered statistically significant.

3 | RESULTS AND DISCUSSION

Twenty-three patients (19 males and 5 females) were included in the study with a median age of 55 (range 37–72) years. Underlying comorbidities were evident in 22 patients (96%) with a median comorbidity count of 3 (range 0–4). Four patients developed radiologically confirmed venous thrombosis during their inpatient stay and two patients developed pulmonary embolism following discharge. At time of writing 19 patients (82.6%) have been discharged from hospital with median length of ICU and hospital stay of 15.5 (range 2–79) and 21 (range 6–125) days, respectively. Three patients died during the study.

In keeping with previous reports, plasma VWF:Ag levels were markedly elevated in patients with severe COVID-19 compared to healthy controls (median 580 IU/dl [interquartile range (IQR) 360–798; $P < .0001$]; Figure 1A). The VWF:CB assay is a functional qualitative assay sensitive to alterations in HMWM. We observed that VWF:CB was also significantly increased in COVID-19 patients compared to controls (median 509 IU/dL [IQR 385–879; $P < .0001$]; Figure 1B). However, significant inter-patient variability was seen in the VWF:CB/VWF:Ag ratio (Figure 1C) and despite the marked increase in VWF:Ag levels, the VWF:CB/VWF:Ag ratio was not significantly increased compared to healthy controls. Interestingly, previous studies have reported that increases in qualitative VWF ristocetin cofactor activity (VWF:RCo) in COVID-19 patients are consistently lower compared to increases in quantitative plasma VWF:Ag levels in the same patients.[6,8,11,12,14,15] Together, these data suggest that COVID-19 infection is not only associated with a marked quantitative increase in plasma VWF levels, but also impacts VWF functional activity.

To further investigate the mechanism(s) underlying this observation, VWF multimer distribution was analyzed in patients with severe COVID-19. A number of important differences were observed in patients compared to normal control plasma. In particular, a relative decrease in HMWM was a common feature seen in 18/23 (78%) of patients studied (Figure 1D,E). Recent studies have reported differences in HMW multimers in patients with severe COVID-19. In keeping with our findings, Doevelaar et al. reported loss of the largest VWF multimers in 75% of patients with severe COVID-19.[16] Similarly, Mancini et al. found that HMWM decreased progressively with increasing intensity of supportive care required.[8] In contrast, however, Philippe et al. reported significantly higher VWF HMWM in critical COVID-19 patients compared to non-critical controls.[9] Further studies will be necessary to elucidate the explanations underlying these findings. However, it seems likely that they may relate in part to differences in the patient cohorts studied, the timing of sample collection, and the methods utilized for VWF multimer analysis. A similar loss of HMWM has been described in patients with acute TTP where it has been attributed to consumption of hyperactive HMWM multimers binding to platelet GPIbα causing platelet adhesion and subsequent aggregation leading to microthrombi and blood vessel occlusion.[17] It seems possible that similar consumption of HMWM VWF may be occurring in COVID coagulopathy. Importantly, ultra-large (UL-) VWF multimers were not observed in any of our patients with severe COVID-19, which is consistent with previous studies.[8,16] However, an increase in LMWM VWF and a smearing of the normal VWF triplet pattern were observed on multimer analysis for all COVID-19 patients (Figure 1D). Taken together, these data suggest that severe COVID-19 is associated with consumption of HMWM, but also that it impacts in other ways upon normal VWF proteolysis *in vivo*.

Following secretion from EC, ultra-large VWF multimers normally undergo partial proteolysis by ADAMTS-13. Consequently, we

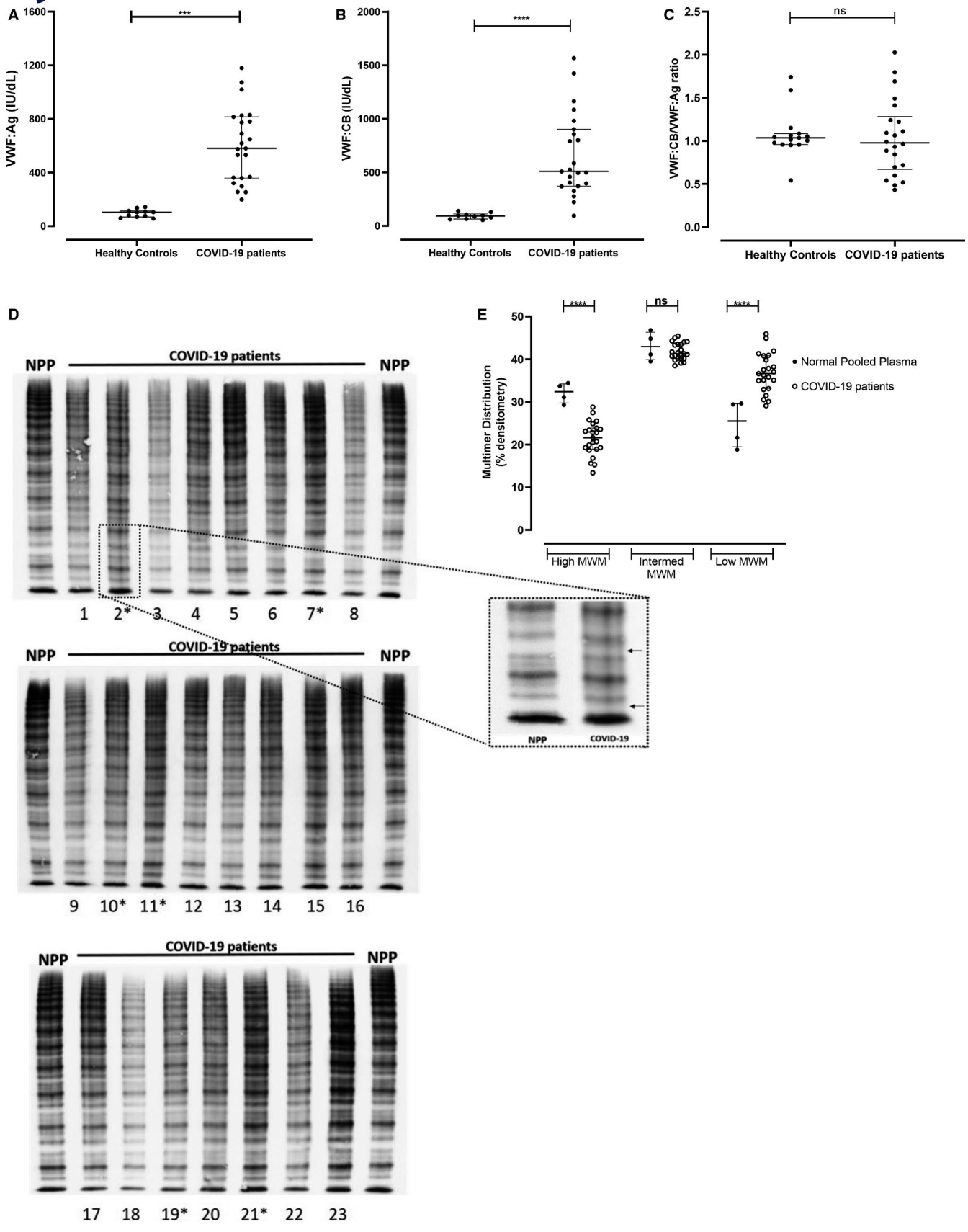

FIGURE 1 A, Plasma von Willebrand factor antigen (VWF:Ag), B, collagen binding activity, and D,E, multimer distribution in patients with severe COVID-19. Plasma samples were collected from patients with severe COVID-19 following admission to the intensive care unit and compared to healthy controls. Data are presented as median and the interquartile range unless otherwise stated (***$P < .001$). Asterisk symbols on multimer lanes indicate pronounced abnormal smearing of triplet structure bands, also shown in inset image. MWM, molecular weight multimers; NPP, normal pooled plasma; VWF:CB, VWF collagen binding activity

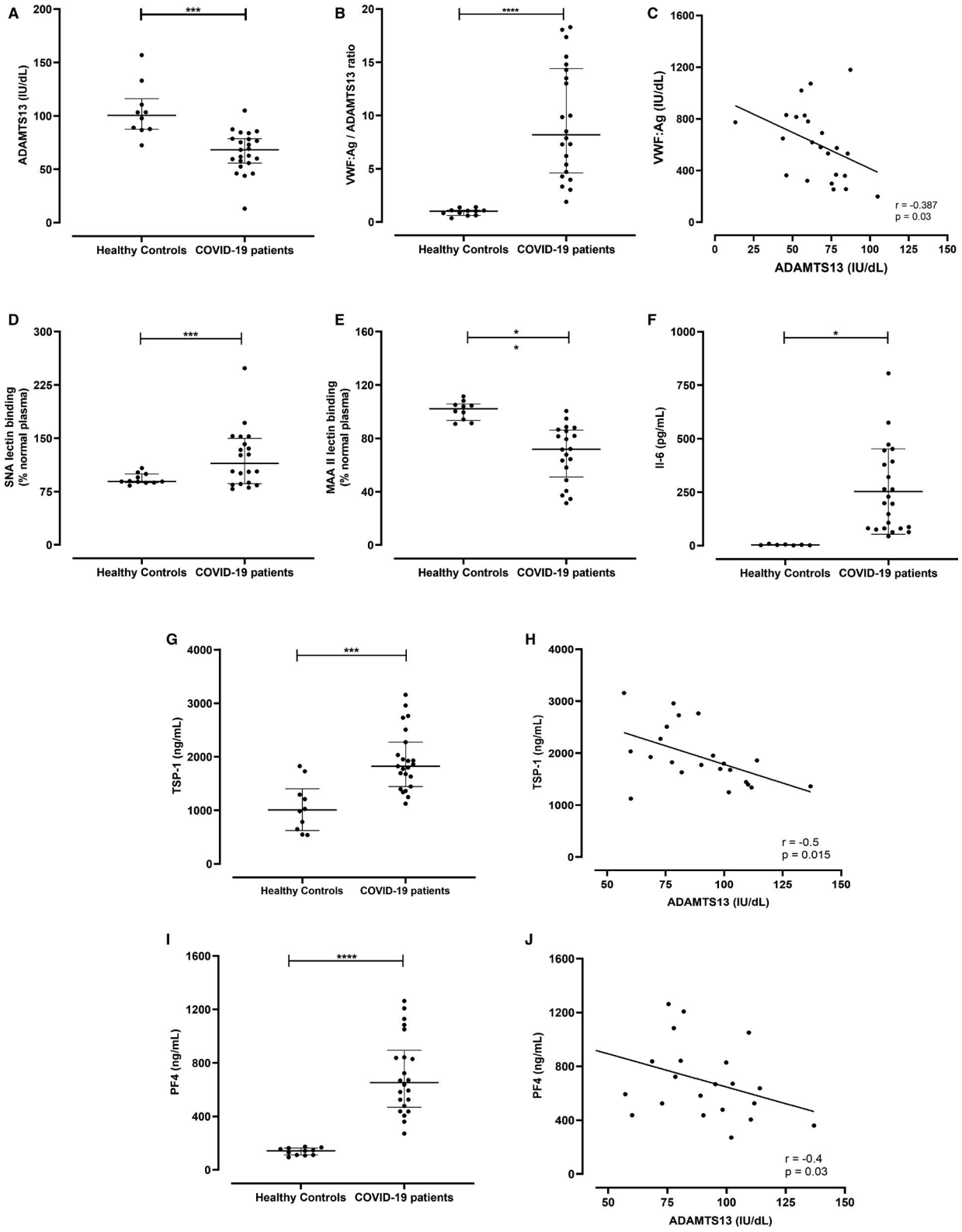

FIGURE 2 A Disintegrin And Metalloprotease with ThrombSpondin Motif type 1 motif, member 13 (ADAMTS-13) regulation of von Willebrand factor (VWF) multimers in patients with severe COVID-19. A–C, ADAMTS-13 activity was reduced in patients with severe COVID-19 resulting in an increased VWF/ADAMTS-13 ratio with a direct inverse correlation. D & E, Altered N- and O-linked VWF sialylation was observed in COVID-19 patients. F–J, Elevated levels of ADAMTS-13 inhibitors, IL-6, TSP-1, and PF4 were observed and TSP-1 and PF4 levels correlated significantly with ADAMTS-13 activity. IL-6, interleukin-6; MAA, *Maackia amurensis*; PF4, platelet factor 4; SNA, *Sambucus nigra* agglutinin; TSP-1, thrombospondin-1. **P* < .05, ***P* < .01, ****P* < .001, *****P* < .0001

next investigated ADAMTS-13 activity in patients with SARS-CoV-2 infection. In keeping with previous studies, significantly reduced ADAMTS-13 activity was seen in patients with severe COVID-19 compared to controls (median 68.2% [IQR 56–78; $P < .001$]; Figure 2A). Reduced plasma ADAMTS-13 levels have previously been reported in association with other types of sepsis including *Plasmodium falciparum* malaria and Dengue virus. Lower ADAMTS-13 activity in severe COVID-19 is likely to be attributable in part to reduced hepatic ADAMTS-13 synthesis. However, increased ADAMTS-13 consumption or clearance leading to deficiency has also been previously associated with markedly elevated plasma VWF levels, which is a hallmark of severe COVID-19. Moderately reduced ADAMTS-13 levels were a consistent finding in severe COVID-19 infection in which all our patients had residual ADAMTS-13 activity levels above 50 IU/dl compared to TTP patients, whose levels are reduced to less than 10 IU/dl. However, under high shear induced blood flow conditions, much higher ADAMTS-13 levels are required to effectively cleave ultra large VWF multimers released from EC that could potentially mediate COVID-19 thrombosis.[18]

This mechanism may be important when considered in the context of the massively increased plasma VWF levels. In contrast to other types of sepsis, severe COVID-19 thus results in a major increase in the VWF/ADAMTS-13 ratio (Figure 2B) and a significant inverse correlation was observed between VWF:Ag levels and ADAMTS-13 activity (Figure 2C). Moreover, this imbalance between substrate and enzyme under high shear stress is likely to be even more pronounced in the lung microvasculature where EC damage is most evident.

ADAMTS-13 regulates multimer distribution by specifically cleaving VWF at the Tyr1605-Met1606 bond within the A2 domain. This cleavage site is flanked by two N-linked glycan sites (N1515 and N1574 in A2). Terminal sialic acid residues on these N-glycan chains have been shown to regulate the susceptibility of VWF to ADAMTS-13 proteolysis.[19] The *Sambucus nigra* agglutinin (SNA) lectin has specific affinity for terminal α2-6 linked sialic acid. This type of sialic acid accounts for approximately 80% of the total sialylation on human VWF and is predominantly expressed on N-glycans.[20] We observed significantly enhanced SNA binding to VWF in patients with severe COVID-19 compared to controls (median 114.7% [IQR 87–144; $P < .05$]; Figure 2D). In contrast, *Maackia amurensis* lectin *II* (MAA-II; affinity for terminal α2-3 linked sialic acid on VWF O-glycans) binding to VWF was significantly reduced in COVID-19 patients (median 71.8% [IQR 55–85; $P < .05$]; Figure 2E). Collectively, these data suggest that the post-translational processing of VWF within EC is altered in severe COVID-19, which is perhaps unsurprising given the marked increase in VWF biosynthesis and EC damage that are features of the condition. Altered VWF sialylation in severe COVID-19 has important biological relevance. First, α2-6 sialylation expression on VWF has been shown to influence susceptibility to proteolysis by ADAMTS-13 and other proteases.[19] In particular, sialyation has been shown to protect VWF from proteolysis by plasmin[21] and recent studies have reported markedly elevated plasmin generation in patients with severe COVID-19.[22] Second, hypersialylation also results in an extended VWF half-life, which has recently been reported in patients with severe COVID-19.[5] Finally, platelet-derived VWF has significantly reduced α2-6 sialic acid expression compared to EC-derived VWF.[23] Consequently, our data suggest that the markedly elevated plasma VWF levels observed in SAR-CoV-2 patients predominantly result from EC rather than platelet activation.

In addition to VWF glycosylation, a number of other putative regulators of ADAMTS-13 activity have been reported. For example, high levels of IL-6, TSP-1, and PF4 have been shown to inhibit ADAMTS-13.[24-26] We observed that plasma levels of IL-6 were significantly elevated in patients with severe COVID-19 compared to controls (median 198.1 pg/ml [IQR 80–385; $P < .05$]; Figure 2F). Although plasma IL-6 levels were increased, absolute concentrations did not reach those necessary to inhibit ADAMTS-13 activity (>50 ng/ml).[24] Similarly, plasma TSP-1 levels were also elevated in COVID-19 patients (median 1824.9 ng/ml [IQR 1537–2153; $P < .001$]) and TSP-1 levels inversely correlated with ADAMTS-13 activity[25] (Figure 2H). Finally, plasma PF4 levels were significantly elevated in patients with severe COVID-19 compared to healthy controls (median 652.6 ng/ml, [IQR 270.6–1263]; $P < .0001$; Figure 2I). Moreover, PF4 levels also were inversely correlated with ADAMTS-13 activity ($P = .03$; Figure 2J). These data suggest that high concentrations TSP-1 released from perturbed vascular EC or elevated PF4 levels secreted from activated platelets may inhibit *in vivo* ADAMTS-13 activity in patients with severe COVID-19. Furthermore, coagulation and fibrinolytic cascade activation are prominent features of COVID-19 coagulopathy. This is relevant as previous studies have demonstrated that ADAMT-13 is susceptible to proteolytic inactivation by both thrombin and plasmin.[21,27] Consequently, our data support the hypothesis that ADAMTS-13 regulation of VWF multimer distribution is impacted through multiple mechanisms in patients with severe COVID-19.

In conclusion, our findings demonstrate that SARS-CoV-2 is associated with profound quantitative and qualitative increases in plasma VWF levels. In addition, severe COVID-19 also downregulates ADAMTS-13 function through a variety of different mechanisms. Importantly, consumption of HMWM--VWF is a common feature in patients with severe COVID-19 infection requiring ICU support. Thus, plasma VWF multimer distribution in these patients is similar to that typically associated with acute TTP. Further studies will be required to determine whether therapeutic interventions targeted at reversing ADAMTS-13 dysfunction may constitute a useful adjunctive measure in the management of COVID-induced microangiopathy.

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CONFLICTS OF INTEREST

J.S.O'D has served on the speaker's bureau for Baxter, Bayer, Novo Nordisk, Sobi, Boehringer Ingelheim, Leo Pharma, Takeda, and Octapharma. He has also served on the advisory boards of Baxter, Sobi, Bayer, Octapharma, CSL Behring, Daiichi Sankyo, Boehringer Ingelheim, Takeda, and Pfizer. J.S.O'D has also received research grant funding awards from 3M, Baxter, Bayer, Pfizer, Shire, Takeda, and Novo Nordisk. PWH has received honoraria and/or travel grants from Gilead Sciences, ViiV Healthcare, Bristol Myers Squibb, and MSD. RIB has served on the speaker's bureau for Bayer and also served on the scientific advisory boards of Roche and Janssen-Cileg. RIB's institution has received research grant funding from Bayer, Takeda, Pfizer, Daiichi Sankyo, CSL Behring, Roche, Amgen, Celgene, Rigel Pharmaceuticals, Abbvie, Sanofi, MorphoSys AG, Acerta Pharma, Jansen-Cileg, Bristol-Myers Squibb, Boehringer Ingelheim, Portola, Technoclone, and Alexion. SEW, HF, EK, ML, SS, RD, HM, SG, CNC, CB, IML, GFC, UB, and JMO'S, have nothing to disclose.

AUTHOR CONTRIBUTIONS

Contribution: SEW, HF, EK, ML, SS, RD, HM, SG, CNC, CB, IML, PWM, GFC, RIB, UB, JMO'S, JSO'D: conception, patient enrollment, data collection, and interpretation. All authors contributed to literature review, final draft writing, and critical revision. All the authors have participated sufficiently in this work, take public responsibility for the content, and have made substantial contributions to this research.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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APPENDIX 1

Additional investigators of the Irish COVID-19 Vasculopathy Study (ICVS).

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