





Von Willebrand factor propeptide in severe coronavirus disease 2019 (COVID-19): evidence of acute and sustained endothelial cell activation

Soracha E. Ward,^{1,*} Gerard F. Curley,^{2,*} Michelle Lavin,^{1,*}  Helen Fogarty,¹ Ellie Karampini,¹ Natalie L. McEvoy,² Jennifer Clarke,² Maria Boylan,² Razi Alalqam,² Amy P. Worrall,³  Claire Kelly,⁴ Eoghan de Barra,^{3,5} Siobhan Glavey,⁴ Cliona Ni Cheallaigh,⁶ Colm Bergin,⁶ Ignacio Martin-Loeches,^{1,6} Liam Townsend,⁶  Patrick W. Mallon,^{7,8} Jamie M. O'Sullivan,¹  and James S. O'Donnell^{1,9,10}, On behalf of the Irish COVID-19 Vasculopathy Study (ICVS) Investigators[†]

¹Irish Centre for Vascular Biology, School of Pharmacy and Biomolecular Sciences, Royal College of Surgeons in Ireland (RCSI), ²Department of Anaesthesia and Critical Care, RCSI, ³Department of Infectious Diseases, Beaumont Hospital, ⁴Department of Haematology, Beaumont Hospital, ⁵Department of Tropical Medicine and International Health, RCSI, ⁶St James's Hospital, Trinity College Dublin, ⁷Centre for Experimental Pathogen Host Research, University College Dublin, ⁸St Vincent's University Hospital, ⁹National Coagulation Centre, St James's Hospital, and ¹⁰National Children's Research Centre, Our Lady's Children's Hospital Crumlin, Dublin, Ireland

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Correspondence: Professor James O'Donnell, National Coagulation Centre, St James's Hospital, Dublin 8, Ireland.

E-mail: jamesodonnell@rcsi.ie

Dr Jamie O'Sullivan, Irish Centre for Vascular Biology, School of Pharmacy and Biomolecular Sciences, Royal College of Surgeons in Ireland, Dublin 2, Ireland.

E-mail: jamieosullivan@rcsi.ie

Summary

Endothelial cell (EC) activation plays a key role in the pathogenesis of pulmonary microvascular occlusion, which is a hallmark of severe coronavirus disease 2019 (COVID-19). Consistent with EC activation, increased plasma von Willebrand factor antigen (VWF:Ag) levels have been reported in COVID-19. Importantly however, studies in other microangiopathies have shown that plasma VWF propeptide (VWFpp) is a more sensitive and specific measure of acute EC activation. In the present study, we further investigated the nature of EC activation in severe COVID-19. Markedly increased plasma VWF:Ag [median (interquartile range, IQR) 608.8 (531–830)iu/dl] and pro-coagulant factor VIII (FVIII) levels [median (IQR) 261.9 (170–315) iu/dl] were seen in patients with severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection. Sequential testing showed that these elevated VWF–FVIII complex levels remained high for up to 3 weeks. Similarly, plasma VWFpp levels were also markedly elevated [median (IQR) 324.6 (267–524) iu/dl]. Interestingly however, the VWFpp/VWF:Ag ratio was reduced, demonstrating that decreased VWF clearance contributes to the elevated plasma VWF:Ag levels in severe COVID-19. Importantly, plasma VWFpp levels also correlated with clinical severity indices including the Sequential Organ Failure Assessment (SOFA) score, Sepsis-Induced Coagulopathy (SIC) score and the ratio of arterial oxygen partial pressure to fractional inspired oxygen (P/F ratio). Collectively, these findings support the hypothesis that sustained fulminant EC activation is occurring in severe COVID-19, and further suggest that VWFpp may have a role as a biomarker in this setting.

Keywords: coagulopathy, SARS-CoV-2, COVID-19, VWF propeptide, endothelium.

*These authors contributed equally to this study.

†See Appendix.

Introduction

Coronavirus disease 2019 (COVID-19), caused by the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2), was initially described in China in December 2019.¹ Subsequently, this virus has spread around the world, resulting in >1.1 million deaths. In some patients, SARS-CoV-2 infection leads to a severe bilateral pneumonia and significant hypoxia that is refractory to standard treatments. Coagulation activation is a hallmark of severe COVID-19.^{2,3} This coagulopathy develops at a relatively early stage. Consequently, D-dimer levels are often significantly elevated at time of initial presentation and represent an independent marker for poor clinical outcome.² Consistent with coagulation activation, high rates of deep vein thrombosis and pulmonary embolism have been associated with severe SARS-CoV-2 infection, particularly in patients requiring intensive care unit (ICU) support.⁴ In addition to these macrovascular complications, accumulating evidence suggests that microvascular occlusion within the lungs plays a critical role in COVID-19 pathogenesis. Importantly, post-mortem studies have shown widespread microthrombi throughout the pulmonary vasculature in patients with fatal COVID-19.⁵ The biological mechanisms underlying this lung-centric vasculopathy have not been clearly defined.

In normal blood vessels, the endothelial cell (EC) monolayer lining blood vessel functions to prevent pathological thrombosis. Data from autopsy studies in COVID-19 have identified marked EC apoptosis, together with loss of EC tight junction integrity in the pulmonary microvasculature.⁵ These data are interesting given that the angiotensin-converting enzyme 2 (ACE-2) receptor used by SARS-CoV-2 to gain cellular entry is expressed on EC. Moreover, electron microscopy studies have shown SARS-CoV-2 viral particles within pulmonary EC, suggesting that direct pulmonary EC infection may be important in triggering COVID-19 associated vasculopathy.⁶ Other mechanisms that may exacerbate EC damage in severe COVID-19 include pro-inflammatory cytokine generation, complement activation and severe hypoxia.

Recent studies have reported that plasma von Willebrand factor (VWF) levels are significantly increased in patients with COVID-19.⁷ *In vivo*, VWF biosynthesis is limited to EC and megakaryocytes.⁸ VWF synthesised within megakaryocytes is stored within the α -granules of their platelet progeny. Conversely, VWF synthesised in EC is either constitutively secreted into the plasma, or else stored within Weibel–Palade bodies (WPB). This stored VWF can then be secreted following EC activation, thereby facilitating tethering of platelets and leucocytes to the vessel wall. Importantly,

ultra-large VWF multimers have been shown to play a critical role in the pathogenesis underlying microvascular occlusion in several conditions including thrombotic thrombocytopenic purpura (TTP), cerebral malaria and sickle cell disease.⁹ In the present study, we sought to further investigate the nature of EC activation in patients with severe COVID-19 and in particular to characterise the biological mechanisms responsible for the markedly elevated plasma VWF levels seen in these patients.

Methods

Consecutive adult patients with COVID-19 admitted to the ICU in Beaumont Hospital were recruited between 21 March and 6 May 2020. Inclusion criteria were adults aged ≥ 18 years with a positive SARS-CoV-2 polymerase chain reaction test. The study was approved by the Beaumont Hospital Research Ethics Committee. Assent to participate in the study was obtained initially from the next of kin if patients lacked capacity and informed consent was retrospectively obtained where possible from participants once capacity was regained. Criteria for ICU admission were defined as progressive respiratory failure requiring invasive mechanical ventilation with or without the need for additional organ support including haemodialysis. All patients received low-molecular-weight heparin thromboprophylaxis as part of standard of care with dose adjustment for weight and renal function. Epidemiological, demographic, treatment and outcome data were derived from the ICU electronic patient record using a standard data collection form. Clinical severity scores including arterial oxygen partial pressure (PaO₂)/fractional inspired oxygen (FiO₂) or P/F ratio, Sequential Organ Failure Assessment (SOFA) and Sepsis-Induced Coagulopathy (SIC) scores were recorded daily. For each patient, samples were collected following admission and throughout the subsequent ICU stay. All haemostasis testing was performed in the Haemostasis Laboratory, Beaumont Hospital. Assays included prothrombin time, activated partial thromboplastin time, factor VIII activity (FVIII:C), fibrinogen and D-dimer levels. Additional coagulation parameters including plasma VWF:Ag and VWF propeptide (VWFpp) were measured using specific enzyme-linked immunosorbent assays as previously described.¹⁰ Patient subgroups, normally and non-normally distributed quantitative data were compared using the Student's *t*-test and Mann–Whitney *U*-test respectively. All data are presented as median and interquartile range (IQR). A *P* < 0.05 was considered statistically significant and data were

analysed using GraphPad Prism, version 8 (GraphPad Software Inc., La Jolla, CA, USA).

Results and discussion

A total of 28 patients (22 males and six females) were enrolled in the study, with a median (range) age of 55 (27–75) years (Table S1). In all, 20 patients (71.4%) were Caucasian, five (17.9%) were Asian, and three patients (10.7%) were of Roma ethnicity. Underlying comorbidities were identified in 23 (82.1%) of the cohort, with obesity (body mass index > 30 kg/m²; 60.7%), hypertension (50%) and chronic pulmonary disease (25%) being the most common. At time of writing, 23 patients (82%) had recovered and were discharged from hospital, with mean (range) length of ICU and hospital stay of 24 (3–56) and 39.4 (6–125) days respectively. Two patients remain in hospital and three patients have died. Despite thromboprophylaxis, six patients (21.4%) had a radiologically confirmed venous thrombotic event. In terms of clinical severity, the median (range) SOFA score was 8 (3–14) and the median (range) P/F ratio was 19 (8–39) kPa at time of ICU admission. No patient fulfilled criteria for disseminated intravascular coagulation (DIC) or SIC according to the International Society on Thrombosis and Haemostasis (ISTH) DIC/SIC criteria (Table S1).

In keeping with previous studies, plasma VWF:Ag levels were markedly increased following admission to ICU [median (IQR) 365.3 (270.8–568.2) iu/dl] in patients with severe COVID-19 requiring ICU support (Fig 1A). To further investigate the mechanism(s) underlying this observation, VWF:Ag levels were sequentially assessed in our cohort over the course of their ICU admission. We observed that plasma VWF:Ag levels increased significantly during ICU stay, peaking at a median (IQR) of 690.2 (467–848.4) iu/dl (Fig 1A). Thus, VWF levels measured during each time period were significantly higher compared to those on ICU days 1–5 ($P < 0.05$). Importantly, plasma VWF:Ag levels remained markedly elevated in all patients studied, even after 3 weeks in the ICU. Given that WPB stores within EC are limited, these novel data clearly show that severe SARS-CoV-2 infection is associated with a major sustained increased in VWF biosynthesis within EC.

In normal plasma, VWF circulates in a high affinity complex with pro-coagulant FVIII. Recent studies have shown that plasma FVIII:C is predominantly derived from hepatic EC.^{11,12} Plasma FVIII:C levels were also increased in patients with severe COVID-19 requiring ICU support (Fig 1B). Consistent with plasma VWF:Ag levels, FVIII:C levels rose significantly after 5 days in the ICU, but then remained consistently elevated for weeks (Fig 1B). Interestingly however, the absolute increase in plasma FVIII:C [median (IQR) 261.9 (170–315.7) iu/dl] was significantly lower than the rise seen in plasma VWF:Ag levels over the same time course. Consequently, the normal FVIII:C/VWF:Ag ratio of 1 was significantly reduced in patients with severe COVID-19

throughout their ICU admission (Fig 1C). Cumulatively, these data are consistent with the concept that pulmonary EC are a key source of VWF, whereas hepatic EC are more important for FVIII biosynthesis. In addition, these findings further highlight the predominantly pulmonary-centric nature of COVID-19 vasculopathy.

During post-translational modification within EC, a large 741 amino acid VWFpp is cleaved from each VWF monomer.¹³ Consequently, mature VWF and VWFpp are secreted from EC into the plasma in equimolar amounts. Moreover, VWF and VWFpp are also stored in equimolar concentrations within WPB, and are released together following EC activation. Previous studies have measured plasma VWFpp levels to gain important insights into EC activation in a number of microangiopathies (e.g. TTP and cerebral malaria).⁹ We observed that plasma VWFpp levels were markedly increased in ICU patients with severe COVID-19 (Fig 1D). Similar to plasma VWF:Ag levels, the VWFpp levels also remained consistently elevated throughout the ICU admission (Fig 1D). These novel findings support the hypothesis that SARS-CoV-2 results in a fulminant and persistent EC activation. Indeed, the absolute plasma VWF:Ag and VWFpp concentrations seen in severe COVID-19 are higher than those previously reported in patients with cerebral malaria, which is also associated with marked acute EC activation and microangiopathy.⁹

In previous studies, we and others have used the VWFpp to VWF:Ag ratio (VWFpp/VWF:Ag) to identify patients with increased or decreased VWF:Ag clearance rates.^{10,14} For example, a VWFpp/VWF:Ag ratio of >3 has been used to define patients with type 1 von Willebrand disease (VWD) due to pathological enhanced clearance (type 1C VWD). Interestingly, the VWFpp/VWF:Ag ratio was consistently reduced in the majority of patients with COVID-19 across the time course of their ICU admission, suggesting reduced VWF clearance (Fig 1E). Accordingly, estimated plasma VWF half-lives were significantly increased in all patients (Fig 1F). The biological mechanisms responsible for this attenuated VWF clearance remain unknown, but altered glycosylation of VWF derived from lung EC has previously been described in patients with pulmonary hypertension.^{15,16} Altogether, these data suggest that, in addition to the acute EC activation and enhanced VWF biosynthesis, reduced VWF clearance rates further contribute to the markedly increased plasma VWF:Ag levels seen in severe COVID-19.

Recent data suggest that elevated plasma VWF:Ag levels may be useful in identifying patients with COVID-19 who have a poor prognosis.⁷ Importantly however, previous studies have shown that plasma VWFpp levels constitute a more sensitive and specific measure of acute EC activation.^{17,18} VWFpp levels offer a number of important advantages over VWF:Ag in this context. First, VWFpp has a much shorter plasma half-life (~2 vs. 12 h).¹³ Second, unlike mature VWF, plasma VWFpp levels are not influenced by ABO blood group. Consequently, we examined whether plasma VWFpp

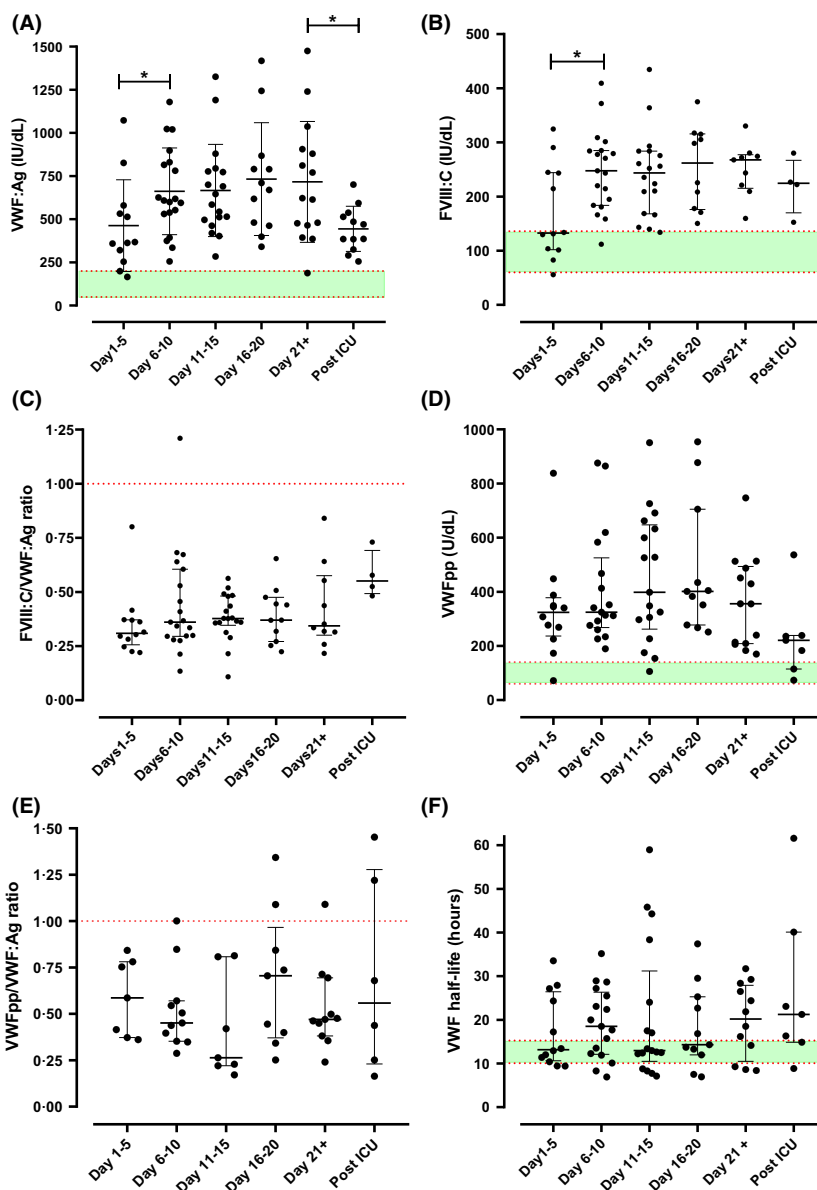


Fig 1. Plasma von Willebrand factor antigen (VWF:Ag), VWF propeptide (VWFpp) and FVIII:C levels in patients with severe coronavirus disease 2019 (COVID-19). Sequential samples were collected from patients with severe COVID-19 following their admission to the Intensive Care Unit (ICU). Data are presented as median and the interquartile range unless otherwise stated. Dotted lines represent the upper and lower limits of the local normal range (* $P < 0.05$).

levels correlated with established markers of clinical severity tools in our present cohort. In patients with COVID-19 who progressed to develop objectively confirmed venous thromboembolic complications and/or fatal outcomes, a progressive increase in plasma VWFpp but not VWF:Ag levels was observed over time (Fig 2A, B). Furthermore, significant correlations with VWFpp were also observed with the SOFA score, SIC score and the P/F ratio on admission and on follow-up across disease course (Fig 2C–E). Further studies will be required to determine whether VWFpp may have a role as a novel biomarker in severe COVID-19.

In conclusion, our present findings demonstrate that severe COVID-19 is associated with profound EC activation. Consequently, plasma VWF:Ag and VWFpp levels remain markedly elevated during the course of the disease. These data underscore the central roles played by endothelialitis and pulmonary microvascular occlusion in the pathogenesis of COVID-19. In addition, the data raise the intriguing question as to whether VWF high molecular weight multimers secreted in response to acute EC activation within the lungs may be directly involved in triggering the pulmonary microangiopathy.

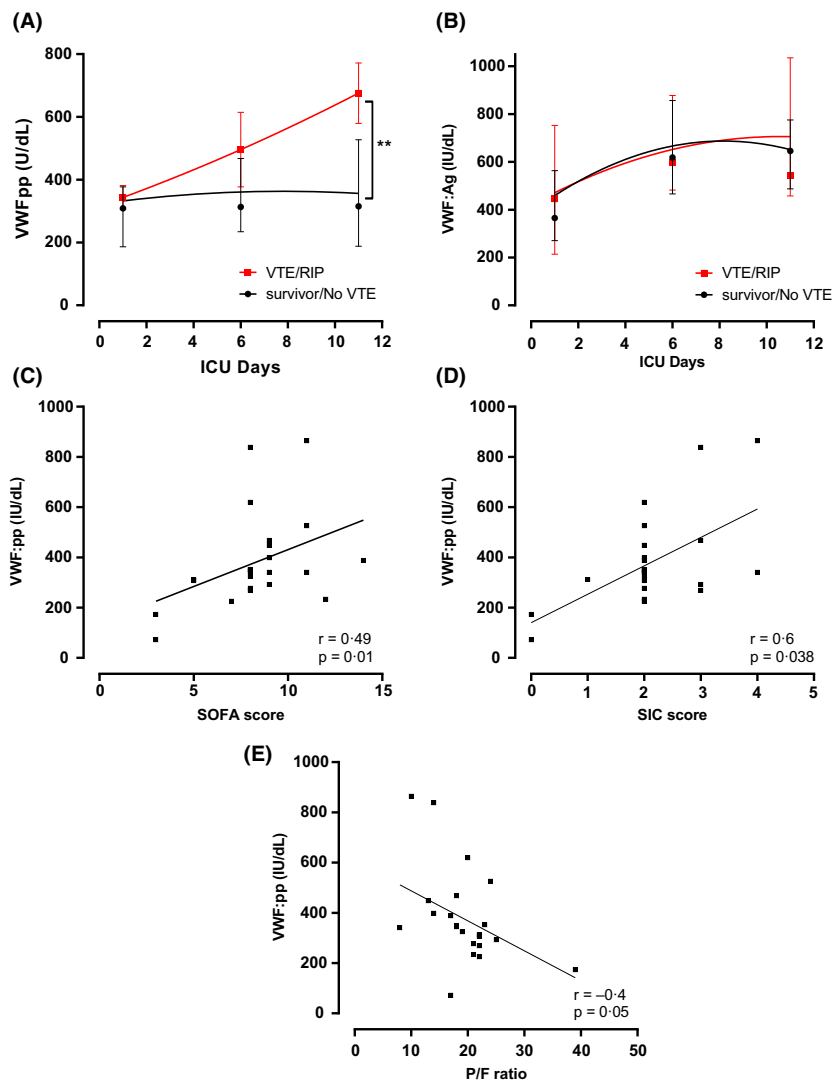


Fig 2. Plasma VWF propeptide (VWFpp) levels correlate with coronavirus disease 2019 (COVID-19) severity. (A, B) Plasma VWFpp levels were significantly higher in patients with COVID-19 who developed venous thrombosis (VTE) or those who died (RIP) compared to survivors without VTE. In contrast, plasma von Willebrand factor antigen (VWF:Ag) levels were similar. (C–E) Plasma VWFpp levels correlated with established markers of COVID-19 severity [Sequential Organ Failure Assessment (SOFA) score, Sepsis-Induced Coagulopathy (SIC) score and ratio of arterial oxygen partial pressure to fractional inspired oxygen (P/F ratio)].

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Conflict of interest

James S. O’Donnell 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. James S. O’Donnell has also received research grant

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Author contribution

Soracha E. Ward, Gerard F. Curley, Michelle Lavin, Helen Fogarty, Ellie Karampini, Natalie L. McEvoy, Jennifer Clarke, Maria Boylan, Razi Alalqam, Amy P. Worrall, Claire Kelly, Eoghan de Barra, Siobhan Glavey, Cliona Ni Cheallaigh, Colm Bergin, Ignacio Martin-Loeches, Liam Townsend, Patrick W. Mallon, Jamie M. O'Sullivan and James S. O'Donnell – 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.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Clinical and biological characteristics of patients with coronavirus disease 2019 (COVID-19) admitted to the Intensive Care Unit (ICU).

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Appendix

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

Niamh O'Connell, Kevin Ryan and Mary Byrne (National Coagulation Centre, St James's Hospital, Dublin), Roger Preston and Dermot Kenny (Irish Centre for Vascular Biology, Royal College of Surgeons in Ireland).