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High levels of von Willebrand factor with reduced specific activities in hospitalized patients with or without COVID-19

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Abstract

The COVID-19 pandemic is often accompanied by severe respiratory illness and thrombotic complications. Von Willebrand Factor (VWF) levels are highly elevated in this condition. However, limited data are available on the qualitative activity of VWF in COVID-19. We measured plasma VWF levels quantitatively (VWF antigen) and qualitatively (ristocetin-induced platelet agglutination, glycoprotein IbM (GPIbM) binding, and collagen binding). Consistent with prior reports, VWF antigen levels were significantly elevated in hospitalized patients with or without COVID-19. The GPIbM and collagen binding activity-to-antigen ratios were significantly reduced, consistent with qualitative changes in VWF in COVID-19. Of note, critically ill hospitalized patients without COVID-19 had similar reductions in VWF activity-to-antigen ratios as patients with COVID-19. Our data suggest that qualitative changes in VWF in COVID-19 may not be specific to COVID-19. Future studies are warranted to determine the mechanisms responsible for qualitative changes in VWF in COVID-19 and other critical illnesses.

- VWF levels were increased in COVID-19 compared to healthy controls.
- VWF activity-to-antigen ratios were decreased in COVID-19 compared to healthy controls.
- There were no differences in VWF activity-to-antigen ratios between hospitalized patients with or without COVID-19.
- These findings are consistent with qualitative changes in VWF in systemic inflammation which are not specific to COVID-19.
- Future studies are needed to define possible roles of changes in conformation or multimer length in the qualitative changes in VWF in systemic inflammation.

Keywords von Willebrand factor · COVID-19 · Ristocetin · Glycoprotein IbM · Collagen binding

Introduction

COVID-19 is associated with an increased risk of thrombosis at all levels of the vascular tree.[1] Patients with COVID-19 have an increased incidence of macrovascular thrombi such as deep venous thrombosis, pulmonary embolism, and stroke.[2] It has also been suggested that the severe pulmonary disease in some patients may be related to microvascular thrombi.[3, 4] The clear association of increased thrombotic risk after an inflammatory stimulus in this disease highlights the interconnectedness between thrombosis and inflammation, or thromboinflammation.[5] The thrombotic mechanisms in COVID-19 remain to be fully clarified; one of the mediators suggested to participate is von Willebrand factor (VWF). [6, 7] VWF bridges platelets to the subendothelial extracellular matrix at sites of vascular

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injury in hemostasis and is a mediator of thromboinflammatory responses.[8] High levels of VWF have been associated with an increased risk for thrombosis.[9] We hypothesized that plasma VWF would be more active in patients with COVID-19 compared to healthy controls and examined VWF antigen and activity levels.

Materials and methods

Sample collection: Blood was collected from patients diagnosed with COVID-19 and admitted to the acute medical ward or intensive care unit, patients in the intensive care unit without COVID-19, and from healthy donors. All procedures were approved by the Institutional Review Board (IRB) of Baylor College of Medicine. Platelet poor plasma was isolated, aliquoted, and stored at -80 °C. Samples were rapidly thawed at 37°C prior to assays.

Measures of VWF antigen: VWF antigen levels were measured by ELISA as previously described [10] with the following modifications. The capture and detection anti-VWF antibodies were used at a concentration of 4.1 µg/mL and a dilution of 1:15,000, respectively. ELISA standards were generated with normal pooled plasma (George King Biomedical, 0010).

Measures of VWF activity: VWF activities were measured using three methods. The first method utilized light transmission agglutination of fixed platelets suspended in plasma in the presence of ristocetin, with outcomes of primary slope (rate of agglutination) and maximum agglutination (size of clot). Fixed platelets (Bio/Data Corporation) were resuspended at a final concentration of $200 \times 10^3/\mu L$, with 30 µL of plasma in a final volume of 250 µL. Intermediate concentrations of ristocetin between 0.6–0.7 mg/mL were used, lower than the clinically available assay which uses a concentration of 1 mg/mL. The lower concentration was chosen to determine whether VWF from COVID-19 patients was more active.[11] Second, VWF platelet-binding activity was additionally measured with a recombinant variant of the platelet VWF receptor, glycoprotein Ibα (GPIbM), in an ELISA format similar to Flood et al.[12] Briefly, amino acid substitutions, D251Y and M255V, were introduced by site-directed mutagenesis (Quik-change XL, Agilent) into a mammalian expression vector for glycocalicin, the ectodomain of glycoprotein Ibα (M1-V305), that is C-terminally fused to tandem tags (AviTag, V5, and 6xHis). A mammalian expression vector for biotin ligase was constructed by subcloning a PCR amplicon of *birA* from XL1-Blue MRF' cells (Agilent) into pcDNA3.1 (ThermoFisher). Human embryonic kidney cells 293T (HEK293T) cells were transiently co-transfected with the expression vectors for GPIbM and biotin ligase using polyethylenimine as previously described [10] and cultured in the presence of 50µM biotin. Biotinylated GPIbM was partially purified from conditioned media by immobilized metal affinity chromatography (Ni-NTA agarose beads, Genesee Scientific), concentrated on centrifugal filters (10 kDa Amicon Ultra-15, Millipore), and stored as aliquots at -80 °C. Biotinylated GPIbM was immobilized onto Maxisorp plates (ThermoFisher) coated with streptavidin (ThermoFisher) at 1 µg/mL in bicarbonate/carbonate coating buffer (71mM $NaHCO_3$ + 29mM Na_2CO_3, pH = 9.2) to enable an ELISA-based assessment of VWF platelet-binding activity. Plasma VWF bound to immobilized, biotinylated GPIbM was detected as described for VWF antigen measurements. Third, VWF collagen-binding activity was measured with an ELISA-based method using human type III collagen as previously described.[13] The VWF GPIbM- and collagen-binding activity assays used normal pooled plasma for ELISA standards as above.

Data analysis: Comparison of means between two groups was performed with an unpaired Student's t-test. A p value ≤ 0.05 was considered statistically significant. Means and standard deviations (SD) are reported.

Results

COVID-19 patients: Multiple measures of VWF activity were significantly increased compared to healthy controls (Online Resource 1). VWF activity measured by ristocetin-induced agglutination was significantly elevated in COVID-19 when compared to healthy controls, using both primary slope (156% +/- 36% vs. 104% +/- 17%, Online Resource 1a) and max agglutination (121% +/- 18% vs. 102% +/- 9%, Online Resource 1b). The GPIbM-binding activity was also elevated in COVID-19 when compared to healthy controls (476% +/- 277% vs. 106% +/- 37%, Online Resource 1c). The collagen binding assay revealed that hospitalized COVID-19 patients had increased collagen binding compared to healthy controls (516% +/- 202% vs. 116% +/- 37%, Online Resource 1d). VWF antigen levels from hospitalized patients with COVID-19 were also significantly elevated compared to controls (477% +/- 172% vs. 95% +/- 31%). However, the VWF specific activities were significantly reduced in patients with COVID-19 relative to healthy controls (Fig. 1). Both the primary slope (0.36 +/- 0.11 vs. 1.18 +/- 0.40, Fig. 1a) and max agglutination (0.28 +/- 0.08 vs. 1.17 +/- 0.38, Fig. 1b) to antigen ratios demonstrated a lower activity per unit of VWF antigen using ristocetin-induced agglutination as a marker of activity. Similarly, the VWF GPIbM activity-to-antigen ratio (GPIbM:Ag) was significantly reduced in patients with COVID-19 (0.88 +/- 0.27 vs. 1.20 +/- 0.20, Fig. 1c). The

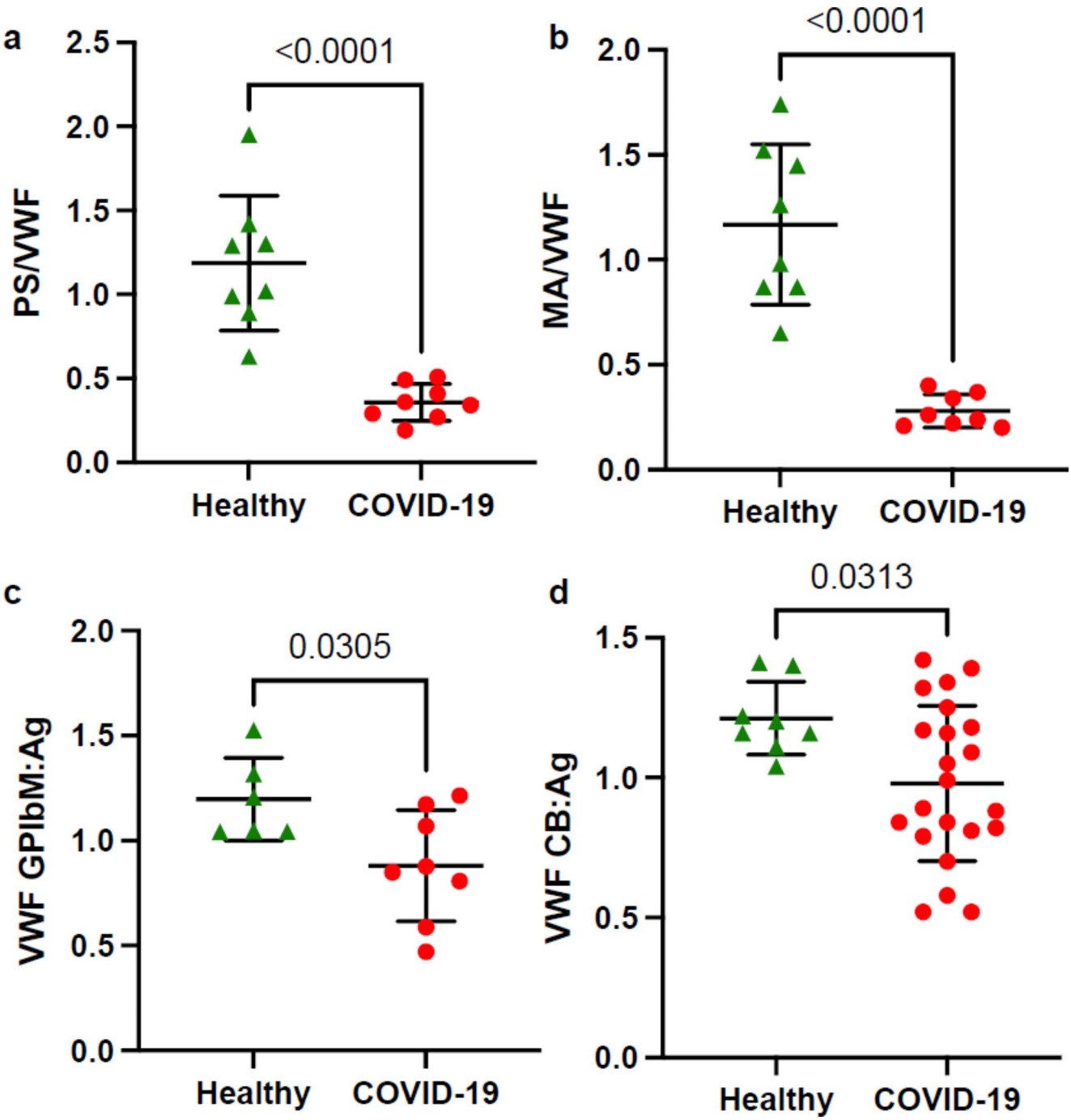

Fig. 1 Activity to Antigen Ratios (a) Primary slope over antigen ratio (PS/VWF) compared between healthy controls and hospitalized patients with COVID-19 (b) Max agglutination over antigen ratio (MA/VWF) compared between healthy controls and hospitalized patients with COVID-19 (c) GPIbM activity over antigen ratio compared between healthy controls and hospitalized patients with COVID-19 (d) Type III collagen binding normalized to VWF antigen ratios compared between healthy controls and hospitalized patients with COVID-19. Bars show mean with SD.

VWF collagen binding activity-to-antigen ratio (CB:Ag) was also significantly lower in hospitalized patients with COVID-19 compared to healthy controls. (0.98 +/- 0.28 vs. 1.21 +/- 0.13, Fig. 1d).

Critically Ill Controls: To determine whether the reduction in VWF specific activities were specific to COVID-19, we measured the VWF GPIbM and collagen binding activities of plasma from critically ill patients hospitalized without a diagnosis of COVID-19 (Fig. 2). Similar to patients diagnosed with COVID-19, critically ill patients without COVID-19 had markedly elevated VWF antigen levels (503% +/- 191% vs. 95% +/- 31%), GPIbM activity levels

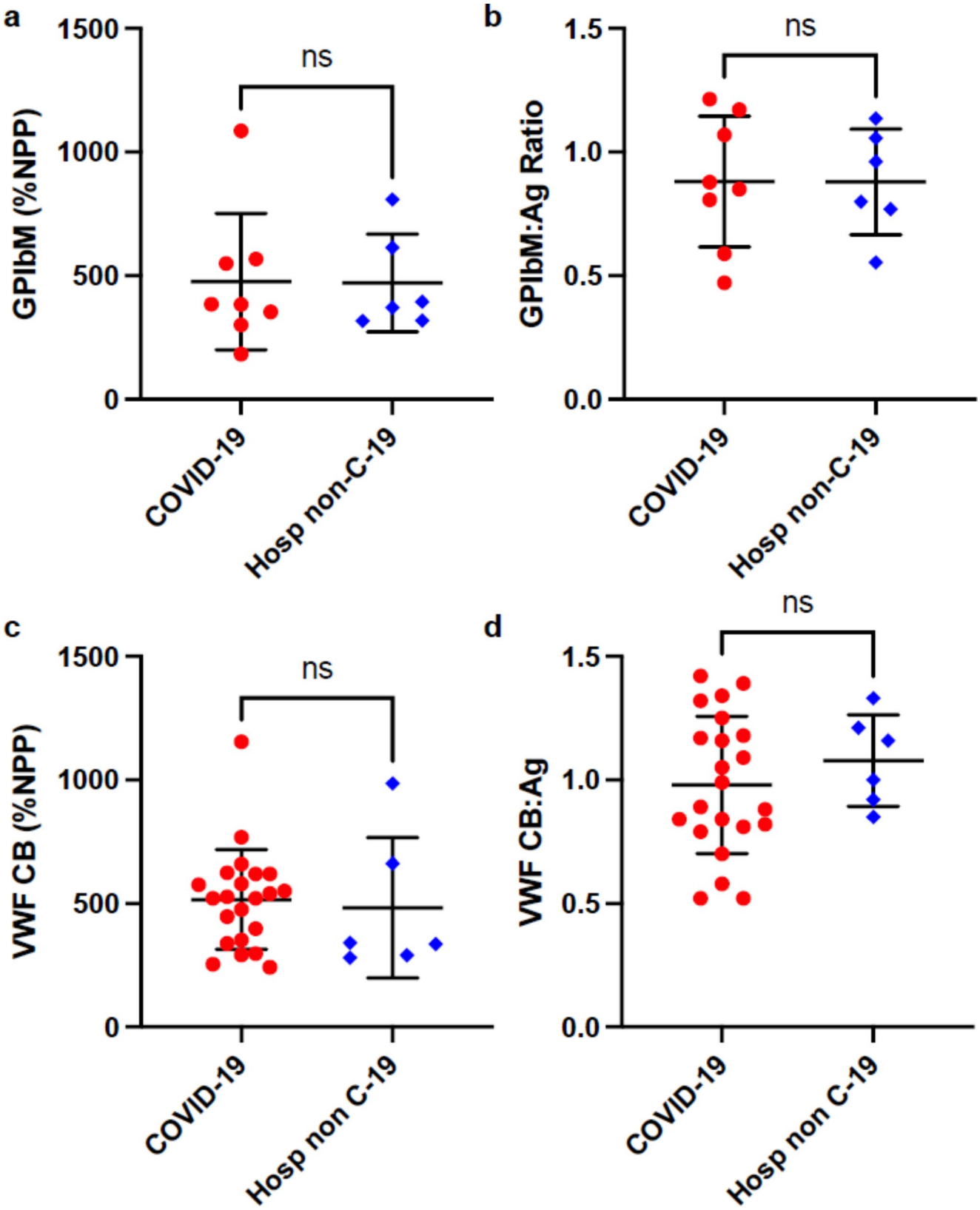

Fig. 2 Measures of VWF parameters in hospitalized patients with COVID-19 or without COVID-19 (Hosp non C-19). (a) VWF GPIbM-binding activity (b) VWF GPIbM-binding activity normalized to VWF antigen level (c) VWF collagen-binding activity (d) VWF collagen-binding activity normalized to VWF antigen levels. Bars show mean with SD

Table 1 Articles discussing VWF activity-to antigen-ratios

Report	Groups Compared	Total subjects	Measure of Activity	Results
de Cristofaro [24]	COVID pneumonia (CP) v. Viral pneumonia (VP)	20	VWF:RCo/VWF:Ag	CP 1.01 [0.95–1.02] v. VP 0.90 [0.85–0.91] **P = 0.0001**
Mancini [7]	COVID patients: high flow nasal cannula (low) v. positive pressure (intermediate) v. intubation (high)	50	VWF:RCo/VWF:Ag	Low 0.88 (0.76–0.93) v. intermediate 0.87 (0.79–0.93) v. high 0.81 (0.79–0.85) **p = 0.118**
Pascreau [25]	Controls v. COVID (home, non-ICU, ICU)	91	Not specified	Control 0.92 (0.84–1.0) v. Home 0.75 (0.73–0.99) v. non-ICU 0.79 (0.73–0.85) v. ICU 0.78 (0.72–0.88) **P < 0.001 for control v. non-ICU p < 0.01 for control v. ICU**
Philippe [23]	Non-COVID-19 v. COVID (Outpatient, Non-critical, critical)	237	VWF:Rco/VWF:Ag	Non-C19 0.83 (0.80–0.86) v. outpatient 0.80 (0.71–0.84) v. non-ICU 0.81 (0.72–0.90) v. ICU 0.77 (0.66–0.91) **P = 0.16**, unclear which groups being compared

(470% +/- 198% vs. 106% +/- 37%, Fig. 2a), and collagen binding activity (482% +/- 284% vs. 116% +/- 37%, Fig. 2c) compared to healthy controls. The VWF levels (503% +/- 191% vs. 477% +/- 172%), VWF GPIbM:Ag (0.88 +/- 0.21 vs. 0.88 +/- 0.27, Fig. 2b), and VWF CB:Ag (1.08 +/- 0.18 vs. 0.98 +/- 0.28, Fig. 2d) for these critically ill patients were no different than hospitalized patients with COVID-19.

Discussion

VWF is acutely secreted into the circulation as multimers upon an inflammatory stimulation. Circulating VWF levels remain elevated in chronic inflammation [9]. Our findings demonstrate that patients with COVID-19 have increased VWF antigen levels as well as increased VWF activities as demonstrated by ristocetin cofactor activity, GPIbM binding, and collagen binding (Fig. 1) which is consistent with findings reported by other authors.[14–17] Of interest, these patients had reduced VWF activity-to-antigen ratio. There are limited studies regarding activity-to-antigen ratios, two of the four which reported significant differences (Table 1). De Cristofaro et al. found that the VWF ristocetin cofactor activity-to-antigen ratios (VWF RCo:Ag) were lower in COVID pneumonia compared to viral pneumonia while Pascreau et al. found that VWF RCo:Ag were lower in hospitalized COVID compared to healthy controls.[18, 19] The reduced GPIbM:Ag and CB:Ag ratios in this report may reflect a shift in VWF multimer size as both the GPIbM-binding and collagen-binding assays have a greater avidity for high molecular weight VWF multimers [12, 20]. Consistent with this view, Mancini et al. and Ward et al. reported a decrease in high molecular weight multimers [7, 21]. The possibility of other conformational changes in VWF that may lead to diminished activity-to-antigen ratios cannot be excluded.

The reduction in VWF specific activities we show in this manuscript do not appear to be specific for COVID-19. Our data show that COVID-19 is associated with reduced VWF activity-to antigen ratios, and the VWF parameters were comparable between critically ill patients without COVID-19 and hospitalized patients with COVID-19 (Fig. 2). Comparable reductions in VWF activity-to-antigen ratio have been reported in other inflammatory conditions [22, 23]. These findings support the notion that systemic inflammation per se may account for the qualitative changes in VWF function.

Limitations of our study include relatively small sample numbers in some groups and limited clinical correlates of disease. We provide an analysis of VWF activities in COVID-19 utilizing a variety of approaches: ristocetin cofactor activity, GPIbM binding, and collagen binding, and we express these as a function of VWF antigen. We show evidence of a reduction in VWF activity relative to VWF antigen, not only in COVID-19, but also in critical illness unrelated to COVID-19. Further investigation is warranted to delineate the precise mechanisms responsible for the qualitative changes in VWF evident in patients with acute inflammation, including COVID-19 and critically ill hospitalized patients.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11239-022-02679-5.

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