



Von Willebrand factor collagen-binding capacity predicts in-hospital mortality in COVID-19 patients: insight from VWF/ADAMTS13 ratio imbalance

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Abstract

Background Microthrombosis is a hallmark of COVID-19. We previously described von willebrand factor (VWF) and their high molecular weight multimers (HMWMs) as potential trigger of microthrombosis.

Objectives Investigate VWF activity with collagen-binding assay and ADAMTS13 in COVID-19.

Methods and results Our study enrolled 77 hospitalized COVID-19 patients including 37 suffering from a non-critical form and 40 with critical form. Plasma levels of VWF collagen-binding ability (VWF:CB) and ADAMTS13 activity (ADAMTS13:Act) were measured in the first 48 hours following admission. VWF:CB was increased in critical (631% IQR [460–704]) patients compared to non-critical patients (259% [235–330], $p < 0.005$). VWF:CB was significantly associated ($r = 0.564$, $p < 0.001$) with HMWMs. Moreover, median ADAMTS13:Act was lower in critical (64.8 IU/dL IQR 50.0–77.7) than non-critical patients (85.0 IU/dL IQR 75.8–94.7, $p < 0.001$), even if no patients displayed majors deficits. VWF:Ag-to-ADAMTS13:Act ratio was highly associated with VWF:CB ($r = 0.916$, $p < 0.001$). Moreover, VWF:CB level was highly predictive of COVID-19 in-hospital mortality as shown by the ROC curve analysis (AUC = 0.92, $p < 0.0001$) in which we identified a VWF:CB cut-off of 446% as providing the best predictor sensitivity–specificity balance. We confirmed this cut-off thanks to a Kaplan–Meier estimator analysis (log-rank $p < 0.001$) and a Cox-proportional Hazard model (HR = 49.1, 95% CI 1.81–1328.2, $p = 0.021$) adjusted on, BMI, C-reactive protein, and D-dimer levels.

Conclusion VWF:CB levels could summarize both VWF increased levels and hyper-reactivity subsequent to ADAMTS13 overflow and, therefore, be a valuable and easy to perform clinical biomarker of microthrombosis and COVID-19 severity.

Keywords COVID-19 · Microthrombosis · Von Willebrand factor · Collagen-binding · Multimers · ADAMTS13 · Mortality

Dear Editor,

We would like to thank Bhogal et al. [1] for their insightful response to our study demonstrated the importance of von Willebrand factor (VWF) and in particular their high

molecular weight multimers (HMWMs) [2]. They propose perspectives in COVID-19 treatment according to VWF/ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) axis involvement. VWF and ADAMTS13 form a system known to play

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a key role in microthrombosis. Indeed, VWF is paramount for both platelet adhesion to subendothelial collagen fibers and platelet aggregation under high shear [3]: VWF is released into the circulation from endothelial cells as hyperactive ultralarge VWF multimers (ULVWFMs). ADAMTS13 controls the breakdown and regulation of ULVWFMs resulting in a balanced distribution between VWF low-, intermediate-, and HMWMs [3]. Microthrombosis, in particular in lungs, is now a hallmark of COVID-19. Indeed, significant pulmonary microthrombosis could trigger major gas exchanges defects triggering acute respiratory distress syndrome [4]. As a matter of fact, we previously demonstrated that pulmonary obstruction could lead to right heart ventricle dysfunction linked to COVID-19 severity [5]. This microthrombosis is associated to endothelial dysfunction, reflected by increased levels of circulating endothelial cells, plasma angiopoietin-2, and VWF in severe COVID-19 patients [2, 5–7]. Moreover, data on ADAMTS13 activity (ADAMTS13:Act) in severe COVID-19 patients are conflicting, some authors reporting strictly normal [8], slightly decreased [9], or even greatly diminished values [10].

In the present study, we intend to highlight VWF hyperactivity through a rapid and easy-to-perform VWF collagen-binding (VWF:CB) assay. We further explored the cause of the abovementioned VWF hyperactivity in COVID-19, which still remains elusive, through ADAMTS13:Act assessment. In this monocentric cross-sectional study, we explored 77 COVID-19 patients hospitalized including 37 suffering from a non-critical form and 40 with critical form. Patients were all included and COVID-19 severity stratified as previously described [2]. All laboratory assays were performed on samples collected less than 48 h following patients' hospitalization. Determination of D-dimer and C-reactive protein (CRP) levels, platelet count, VWF:antigen (VWF:Ag), and VWF multimeric profile were achieved as previously described [2]. The VWF:CB was determined using the Asserachrom VWF:CB ELISA kit (Stago, Asnières-sur-Seine, France). ADAMTS13:Act was assessed using the Technozyme ADAMTS13 activity ELISA (Technoclone, Vienna, Austria). Both ELISA kits were used according to the manufacturer's instructions.

Among the 77 COVID-19 patients hospitalized and studied here, 33 (83%) critical patients and 25 (68%) non-critical patients were male. Critical patients were not significantly older than non-critical patients (critical: median age of 62 years, interquartile range [IQR] 53–72; non-critical: 63 years IQR 52–72, $p=0.85$). Conversely, critical patients had significantly higher median body mass index (BMI) than non-critical patients (critical: 28.9 kg/m², IQR 26.9–34.9; non-critical: 25.1 kg/m² IQR 23.6–29.5, $p=0.005$). There was no significant difference in median platelet count between critical (307 G/L, IQR 199–375)

and non-critical patients (275 G/L IQR 199–375). It is to be noted that we previously described that critical patients displayed higher levels of plasma VWF:Ag associated with a higher proportion of HMWMs compared with non-critical COVID-19 patients [2].

VWF:CB levels on admission were significantly higher in critical (median 631% IQR [460–704]) than in non-critical (259% [235–330], $p<0.005$) COVID-19 patients (Fig. 1a). Moreover, the VWF:CB-to-VWF:Ag ratio, an indirect and quantitative way to measure VWF multimers size, was still significantly higher in critical (median 1.16 IQR 0.96–1.28) than in non-critical (0.91, IQR 0.85–1.07, $p=0.0001$) COVID-19 patients, therefore, in favor of VWF hyperactivity in critical patients (Fig. 1b). Consistently, both VWF:CB and the VWF:CB-to-VWF:Ag ratio were strongly positively associated with HMWMs of VWF in our cohort (Spearman correlation coefficient $r=0.564$ and $r=0.731$, respectively, $p<0.0001$ for both) (Fig. 1c and d). Therefore, the VWF:CB assay appears to be an adequate surrogate method to appreciate VWF multimers levels.

Plasma ADAMTS13:Act levels at admission were significantly lower in critical (median 64.8 IU/dL IQR 50.0–77.7) than in non-critical COVID-19 patients (85.0 IU/dL IQR 75.8–94.7, $p<0.001$) (Fig. 1e). Nevertheless, only 3 (7.5%) critical patients displayed ADAMTS13:Act levels under normal values (*i.e.*, 40–130 UI/dL), while all non-critical patients showed ADAMTS13:Act levels in the normal range. Moreover, VWF:Ag-to-ADAMTS13:Act ratio is higher in critical (median 8.27 IQR 6.20–11.1) than non-critical patients, (3.46 IQR 2.61–4.30, $p<0.001$) supporting a potential saturation of ADAMTS13 in critical COVID-19 patients (Fig. 1f). This result means that for a single unit of plasma ADAMTS13, there was approximately three times more and eight times more circulating VWF:Ag, in non-critical and critical COVID-19 patients, respectively. Notably, the VWF:Ag-to-ADAMTS13:Act ratio was strongly associated with VWF:CB (Spearman correlation coefficient $r=0.916$, $p<0.001$), suggesting that VWF:CB summarize both quantitative (*i.e.*, plasma VWF:Ag increase) and qualitative VWF abnormalities (VWF excess of HMWMs consequential to ADAMTS13 overflow, Fig. 1g).

Finally, we evaluated the discriminatory ability between survivors and non-survivors of VWF:CB using a ROC curve (AUC = 0.92, $p<0.0001$, Fig. 2a). Therefore, we estimated a cut-off value to predict in-hospital mortality. Upon admission, a VWF:CB level of 446% provided an optimal sensitivity–specificity balance, with an excellent sensitivity of 95.2% (95% confidence interval [CI] 73.3–99.8%) associated with an acceptable specificity of 76.8% (95% CI 64.2–85.9%) regarding COVID-19-related mortality. The ability of this VWF:CB cut-off to predict in-hospital mortality was further validated in a Kaplan–Meier

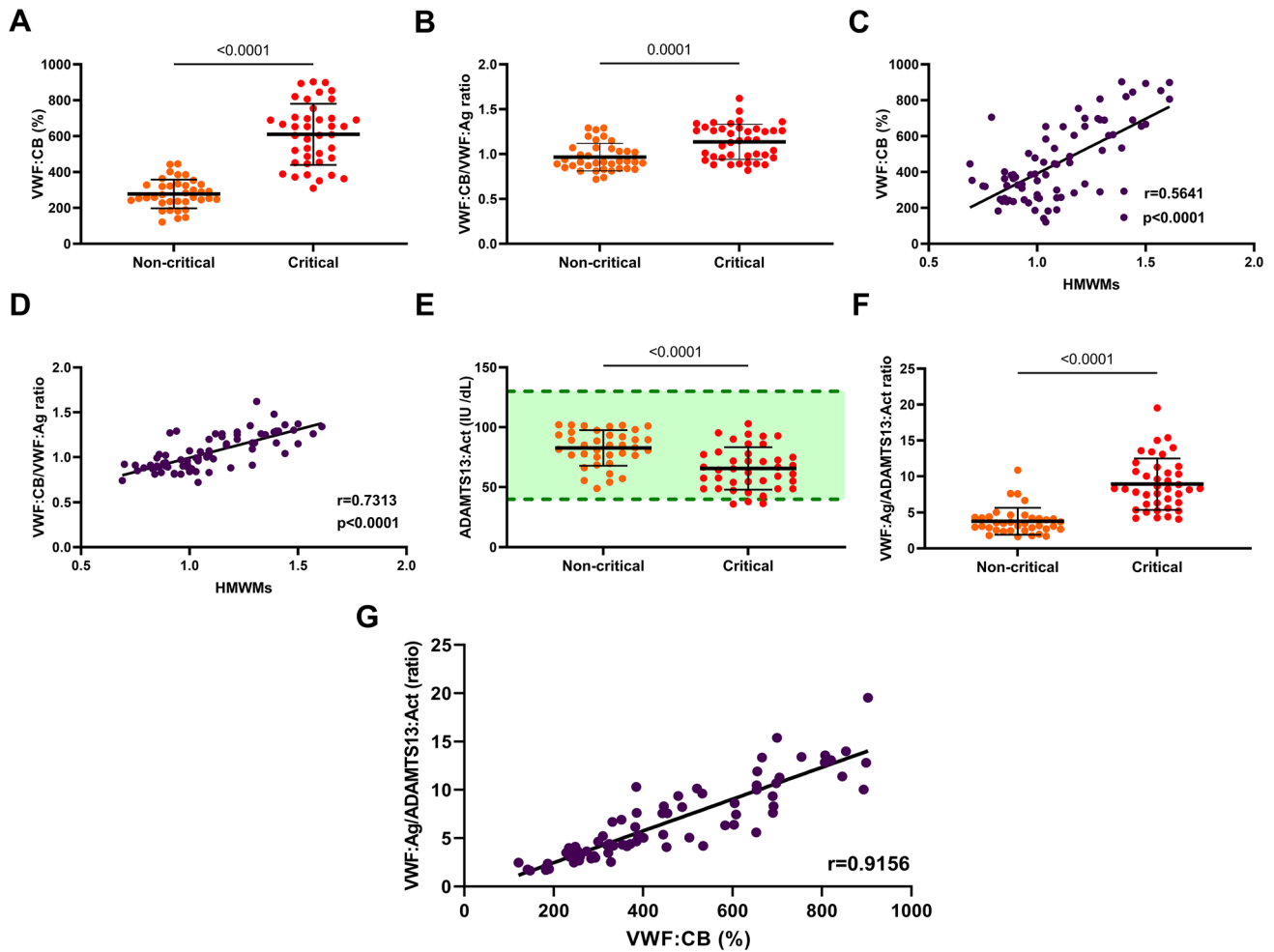


Fig. 1 Levels and association of von Willebrand factor—ADAMTS13 system-related biomarkers according to critical and non-critical COVID-19 patients. Data points indicate individual measurements, whereas horizontal bars represent the means with standard deviations. Green-shaded area indicate the normal ranges of values. For comparison between severity groups, p value comes from the Mann–Whitney test. For association between two variables in the whole cohort, p value and correlation coefficient (r) comes from the Spearman correlation and the solid black line was

obtained through simple linear regression. Plasma levels of von Willebrand factor:collagen binding (VWF:CB) (a) and VWF:CB-to-VWF:antigen (VWF:Ag) ratio (b). Association between VWF high molecular weight multimers (HMWMs) proportion and VWF:CB (c) or VWF HMWMs and VWF:CB-to-VWF:Ag ratio (d). Plasma levels of ADAMTS13 activity (ADAMTS13:Act) (e) and VWF:Ag-to-ADAMTS13 ratio (f). Association between VWF:Ag-to-ADAMTS13 ratio and VWF:CB (g)

estimator ($p < 0.001$, Fig. 2b) and a Cox-proportional hazard analysis adjusted for BMI, D-dimer, and CRP (Hazard ratio [HR] 49.1, 95% CI 1.81–1328.2, $p = 0.021$, Fig. 2c).

Our study highlights increased circulating hyperactive VWF and ADAMTS13 overflow in COVID-19 severity. This imbalance could be at the origin of microthrombosis. Moreover, VWF:CB appears to sum up VWF global thrombogenic impact and is a powerful predictor of COVID-19-related mortality which reinforces this hypothesis.

VWF:CB assay is initially a diagnostic test that quantifies the binding capacity of VWF-A1 and -A3 domain to collagen binding, the main subendothelial matrix component in

order to improve the diagnosis and differentiation of qualitative variant of von Willebrand disease, the most common inherited bleeding disorder [3]. VWF activity is commonly assessed using VWF ristocetin cofactor assays, which evaluate the binding between the VWF-A1 domains to platelet membrane receptor GPIb. VWF:CB activity is a more sensitive test exploring HMWMs of VWF abnormalities [11]. Consistently, we observed a marked increase of VWF:CB in critical COVID-19 patients, even after normalization to VWF:Ag levels, confirming an intrinsic VWF hyperactivity, previously described using HMWMs evaluation [2]. VWF multimers quantification is a complex and time-consuming

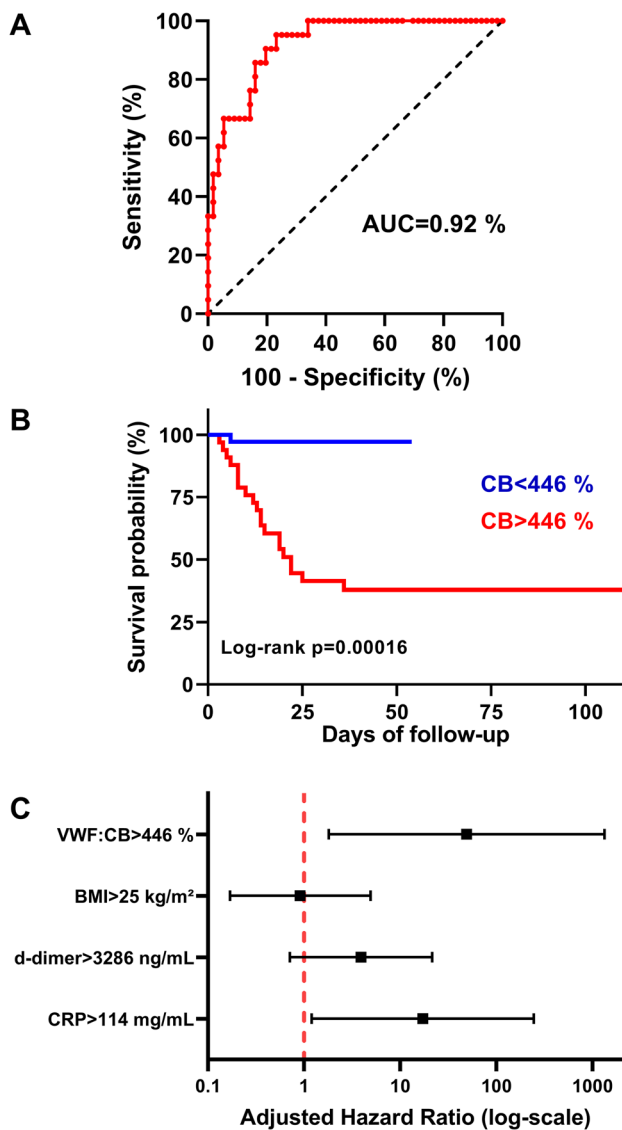


Fig. 2 Association between von Willebrand factor collagen-binding admission level and in-hospital mortality in COVID-19 patients. **a** Receiver operating curves evaluating von Willebrand factor collagen-binding (VWF: CB) ability to predict in-hospital-mortality estimated by the area under the curve (AUC) value. The diagonal black-dotted segment is the reference line. **b** Survival curves according to VWF:CB level using a Kaplan–Meier estimator. Data are shown for patients with low VWF:CB (<446%) and high von VWF:CB (>446%). Survival curves are compared using the log-rank test. **c** Forest plot showing the Cox-proportional hazards model for VWF:CB adjusted for age, body mass index (BMI), D-dimer, and C-reactive protein (CRP). For each variable, black squares represent hazard ratios (HR) and solid black lines represent HR 95% confidence intervals

method. Thus, VWF:CB could represent a valuable, easy-to-perform assay to highlight VWF hyperactivity in COVID-19.

In addition, we found lower ADAMTS13:Act in critical COVID-19 patients than in non-critical ones even if only few patients present values above normal, a finding

consistent with some previous reports [9]. Severe deficiency in ADAMTS13 (activity < 10 IU/dL) is a signature for thrombotic thrombocytopenic purpura (TTP) diagnosis, a life-threatening thrombotic condition caused by the subsequent accumulation of hyperactive HMWMs causing disseminated thrombi formation occluding arterioles and capillaries [12]. Reports of small platelets-rich thrombi within the microvasculature of SARS-CoV-2-infected patients [13] makes it tempting to draw a parallel between TTP and COVID-19 associated coagulopathy. However, the normal platelet count in nearly all COVID-19 patients in our cohort associated with the scarcity of ADAMTS13:Act true deficits make this hypothesis difficult to accept. Thus, more than a decrease in ADAMTS13:Act levels, we observed a major discrepancy between ADAMTS13:Act and VWF:Ag levels in critical patients, suggesting an overflow of ADAMTS13 capacity. Although TTP arises from severe decreased in ADAMTS13:Act, thrombotic events have been associated to slight decrease if associated with a major increase of circulating VWF level, in particular in myocardial infarction, ischemic stroke, and cancer-associated venous thrombosis [14]. Moreover, Warlo et al. demonstrated a correlation between increased VWF:Ag-to-ADAMTS13:Act ratio and high residual platelet reactivity despite aspirin treatment, suggesting that imbalance in VWF-ADAMTS13 system could also promote arise of hyper-reactive platelets [14]. The mechanisms underlying altered VWF:Ag-to-ADAMTS13 ratio in COVID-19 is still unknown. ADAMTS13 could be consumed at a higher rate due to increased VWF processing, and/or inflammatory cytokines could suppress ADAMTS13 biosynthesis in the liver [15].

All in all, VWF:CB is significantly associated to VWF:Ag-to-ADAMTS13 ratio and HMWMs of VWF. VWF:CB could summarize both VWF qualitative and quantitative anomalies in COVID-19 and therefore be a valuable, easy-to-perform, mirror of VWF global thrombotic involvement in microthrombosis formation. Our results not only highlight the potential for plasma VWF:CB to discriminate COVID-19 patient's severity and/or in-hospital mortality but also may help targeting patients for new therapeutic approaches such as (i) recombinant ADAMTS13, (ii) caplacizumab (an anti-von Willebrand factor humanized single-variable-domain immunoglobulin targeting the A1 domain of von Willebrand factor), (iii) N-acetyl cysteine known to reduce the size of VWF multimers.

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during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest All author declares that they have no conflict of interest.

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