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LETTER

Circulating Von Willebrand factor: a consistent biomarker predicting in-hospital mortality across different waves of the COVID-19 pandemic

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Dear Editor,

Circulating levels of Von Willebrand factor (VWF) have been largely described as elevated in patients with COVID-19, and its evaluation could be a valuable biomarker of disease severity [1]. VWF is produced by endothelial cells and has been proposed as one of the main actors in the microthrombotic process during severe forms of COVID-19 [2]. All along the pandemic, there were significantly fewer COVID-19-positive patients admitted to the intensive care unit (ICU), and a lower mortality rate in the Omicron wave compared to the delta and alpha waves. Moreover, a decrease in thromboembolic disease, particularly pulmonary embolism, has been observed across different waves [3]. While the pandemic appears to be winding down, and the emergency status has just been lifted by the World Health Organization, some patients continue to be burdened by COVID-19, and severe cases still require intensive care. VWF levels have primarily been studied during the initial wave of COVID-19, and to date, there are no strong data describing VWF levels across different waves of the COVID-19 pandemic. Since VWF may serve as a useful marker of disease severity and in-hospital mortality, we decided to investigate its relevance at admission during different waves of the COVID-19 pandemic.

We conducted a prospective monocentric cohort study of adult patients (≥ 18 years old) hospitalized with COVID-19 from March 8th 2020 to December 21st 2021. This study is part of the SARCODO cohort study (SARCODO: 2020-A01048-31A, NCT04624997), which aims to investigate coagulation and endothelial activity profiles in patients admitted to the European Georges Pompidou Hospital located in Paris (France) for SARS-CoV-2 infection. The institutional review board from the scientific and ethical committee of Assistance Publique—Hôpitaux de Paris (AP-HP) approved the study. Additionally, the French Ethics Committee approved the current study procedures and methods (ID RCB: 2020-A00256-33). VWF evaluation was performed at hospital admission, i.e., within the first 48 h following hospital admission for SARS-CoV-2 infection but also during the first nine days of hospitalization during waves 2–5 of pandemic. Blood samples were collected from patients using standard laboratory techniques, specifically 0.129 M trisodium citrate tubes (9NC BD Vacutainer, Plymouth, UK). Platelet-poor plasma was obtained after centrifugation twice at 2500 × g for 15 min and stored at − 80 °C until analysis. The measurement of von Willebrand factor antigen (VWF:Ag; STA Liatest, Diagnostica Stago) was performed on a STA-R® Max coagulometer (Diagnostica Stago) as previously described [1]. Data sources included office records and clinical data warehouse. Quantitative variables are accompanied by an ANOVA if they follow a normal distribution; otherwise, a Kruskal–Wallis test is used. Qualitative variables are accompanied by the Chi-square

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test. Missing data were handled by imputation using the package R mice.

The cohort comprised 572 patients with COVID-19 who were initially hospitalized in medical wards (MW, $n=304$; 53.2%) or directly hospitalized in ICU ($n=267$; 46.8%). Table 1 shows patients' demographic characteristics across different waves. The average age of patients in the total population wave was 63.5 ± 14.2 (standard deviation). The median duration of hospital stays and delay from hospital admission to in-hospital death were, respectively, 15 days (interquartile range (IQR) [8–28]), and 16 days (IQR [8–25]). In order to highlight the predictive value of VWF:Ag, we compared it to D-dimer and CRP levels at hospital admission during different waves. Using Kruskal–Wallis test, we observed that COVID-19 patients had overall significantly lower CRP and D-dimer levels compared to the patients in the first wave ($p=0.002$ and <0.001, respectively, for CRP and D-dimer). However, VWF:Ag levels at admission were not different between the waves ($p=0.36$). Next, we analyzed VWF:Ag according to the outcomes during wave 2–5 (June 16th, 2020 to December 21st, 2021). A receiver operating characteristic (ROC) curve analysis was constructed using these biomarkers for prediction of in-hospital mortality (Fig. 1A). Then, we observed that VWF:Ag had a greater but not significantly different area under the curve (AUC) of 0.68 (95% CI 0.62–0.74) compared to CRP (0.64, 95% CI 0.58–0.71) and D-dimer (0.62, 95% CI 0.55–0.69) during wave 2–5. We then compared (Table 1) positive predictive value and the negative predictive value according to different waves of COVID-19. The accuracy and clinical utility of VWF:Ag as a predictor for COVID-19 in-hospital mortality can be better understood by considering these values in the context of the specific study population. NPV has been found lower in waves 2–5 in contrast to wave 1. This result could be explained by several parameters: new standard of care (corticoids) that

Table 1 Demographic, clinical, and biological characteristics of COVID-19 patients at admission during the Five waves of pandemic

		Total from March 8th, 2020 to December 21st, 2021	Wave 1 from March 8th, 2020 to June 15th, 2020	Wave 2 from June 16th, 2020 to December 31st, 2020	Wave 3 from January 1st, 2021 to June 15th, 2021	Wave 4 from June 16th, 2021 to October 15th, 2021	Wave 5 from October 16th, 2021 to December 21st, 2021	*p* value
		$n=572$	$n=185$	$n=100$	$n=231$	$n=41$	$n=15$	
Sex—male, *n* (%)		390 (68.5)	120 (64.9)	71 (71.0)	159 (69.4)	29 (72.5)	11 (73.3)	0.74
Age—years		65 [54–74]	63 [53–73]	67 [59–76]	66 [55–73]	61.5 [48–77.25]	67 [46.5–71]	0.24
	Missing data	3	0	0	2	1	0	
Length of stay—days, median (IQR)		15 [8–28]	15 [7–27]	16 [9–37]	15 [9–27]	14 [9–24]	13 [9–30]	0.49
Admission, *n* (%)	ICU	267 (46.7)	89 (48.1)	34 (34.0)	113 (49.1)	21 (51.2)	10 (66.7)	0.042
	MW	304 (53.2)	96 (51.9)	66 (66.0)	117 (50.9)	20 (48.8)	5 (33.3)	
	Missing data	1	0	0	1	0	0	
Time to death—days, median (IQR)		16 [8–25]	14 [8–22]	23.5 [13–36.5]	15 [8.5–23.5]	13.5 [8.25–19.75]	20 [15.25–34]	0.096
VWF:Ag—%, median (IQR)		391.5 [302.75–510]	392 [283.0–506.0]	377 [288.5–488.5]	391 [312.0–513.0]	398 [337.0–523.0]	474 [353.0–521.5]	0.36
D-dimer—ng/mL, median (IQR)		1441.0 [828.0–3379.0]	2101.0 [994.3–4423.5]	1425.5 [888.8–2443.5]	1231.0 [720.0–2196.0]	1683.0 [876.0–4541.0]	1649.0 [780.5–5400.5]	<0.001
	Missing data	3	1	0	2	0	0	
CRP—mg/L, median (IQR)		86.0 [35.3–151.4]	113.3 [49.0–191.2]	86.0 [45.0–150.6]	66.8 [31.1–138.2]	65.5 [26.4–125.5]	55.2 [41–131.7]	0.002
	Missing data	106	26	8	57	11	4	
Negative predictive value (95% CI)		0.86 (0.82–0.89)	0.98 (0.95–0.98)	0.80 (0.74–0.85)				
Positive predictive value (95% CI)		0.47 (0.4–0.53)	0.52 (0.4–0.64)	0.44 (0.36–0.52)				

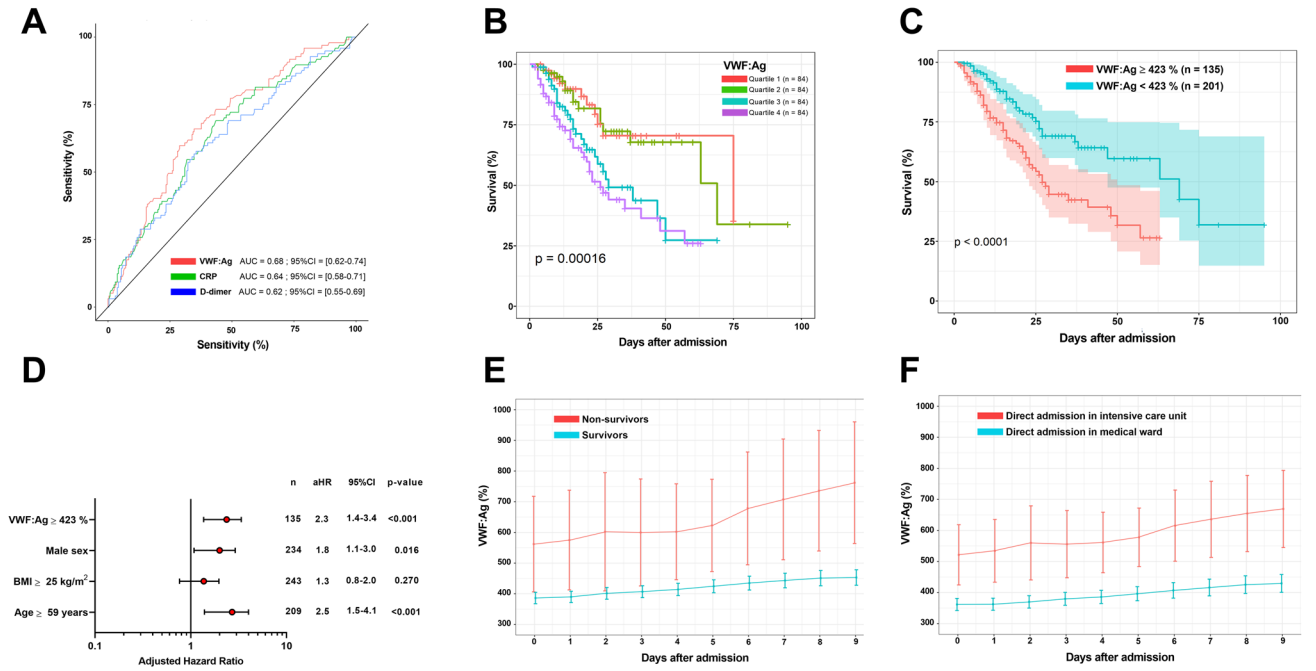

Fig. 1 VWF:Ag levels as a predictive factor for COVID-19 outcomes during wave 2–5. **A** Receiver operating curves evaluating unadjusted Von Willebrand factor antigen, CRP, and D-dimer levels' ability to predict in-hospital-mortality. The diagonal black dotted segment is the reference line. *AUC* area under the curve, *CI* confidence interval. **B** Survival curves according to Von Willebrand factor antigen using a Kaplan–Meier estimator. Data are shown for patients with quartiles of VWF: Ag. Survival curves are compared using the log-rank test. VWF:Ag = von Willebrand factor antigen. **C** Survival curves according to von Willebrand factor antigen (VWF:Ag) quartiles using a Kaplan–Meier estimator. Data are shown for patients with low VWF:Ag (< 423%) and high von Willebrand factor antigen (≥ 423%). Survival curves are compared using the log-rank test. **D** Forest plot showing the Cox proportional hazards model for Von Willebrand factor antigen adjusted for sex, body mass index, and age. Values are hazard ratios with 95% confidence intervals. *BMI* body mass index, *aHR* adjusted hazard ratio, *CI* confidence interval. **E** Temporal trends of daily VWF:Ag during the first 9 days of hospitalization according to in-hospital mortality: survivors (blue line); non-survivors (red line). **F** Temporal trends of VWF:Ag during the first 9 days of hospitalization according to initial admission at hospital: medical ward (MW; blue Line) or intensive care unit (ICU; red line)

could decrease endotheliopathy and so decrease relevance of prediction at entrance and also vaccination along periods. We then evidenced, with a log-rank test, a significant difference in the predictive capability for in-hospital mortality between the two lower quartiles and the two higher quartiles of VWF:Ag ($p = 0.00016$, Fig. 1B). Because during the first wave a VWF:Ag level over 423% at admission was significantly associated with higher in-hospital mortality in univariate but also in multivariable analysis [1], we used this cutoff to assess the relevance of VWF:Ag level in survival during wave 2–5. As shown in Fig. 1C, we demonstrated the ability of the VWF:Ag cutoff to predict in-hospital mortality ($p < 0.0001$ Fig. 1C). Finally, the ability of the VWF:Ag cutoff to predict in-hospital mortality was confirmed in a Cox proportional hazard analysis adjusted for age, sex, and body mass index (Hazard ratio, HR 2.3, 95% CI 1.4–3.4, $p < 0.001$, Fig. 1D). Last but not least, we evaluated VWF:Ag levels during the first nine days of hospitalization and they were constantly higher in non-survivors than in survivors (Fig. 1E) and also higher in patients referred to ICU than patients in MW (Fig. 1F) confirming relevance of VWF:Ag and endotheliopathy along hospitalization from June 16th, 2020 to December 21st, 2021 (waves 2–5).

This study compared the biological characteristics of COVID-19 patients with ICU referral during the first 5 waves of the COVID-19 pandemic (from March 8th, 2020, to December 21st, 2021) in a French university hospital. Despite differences in the variants and widespread vaccination efforts, we did not find a difference in VWF:Ag levels at admission in patients hospitalized for COVID-19 and found the same ability to predict severity across the different waves. This result is in line with microthrombosis being the origin of severe forms of the disease, regardless of the variants. Thus, microthrombosis could persist in severe cases, unlike macrothrombosis that decreased along the pandemic [3]. Endothelial dysfunction has been described as a consequence of inflammation rather than a direct entry of the virus into endothelial cells [4]. However, Dimmeler's group demonstrated that human coronary artery endothelial cells showed the highest viral uptake and suggested that endothelial protection may differ in patients infected with different variants [5]. Therefore, the prothrombotic potential

of different variants should be tested in further studies in endothelial cells to better understand endothelial dysfunction and coagulopathy induced by various variants. Finally, interesting study demonstrates that COVID-19 plasma enhanced VWF secretion and increased Angiopoïetin-2 expression in endothelial cells, as well as significantly enhanced in vitro endothelial cells tube formation and angiogenesis [6]. This observation indeed adds some interesting data in pathophysiology of COVID-19 endotheliopathy and angiogenic disorders observed in acute phase but also in long Covid [7].

All in all, we demonstrate here for the first time that VWF:Ag is a consistent marker of in-hospital mortality across the different waves of the COVID-19 pandemic. The reduced disease severity observed during different periods and with different variants, attributed to the emerging novel therapies and widespread vaccination efforts, does not modify the "endotheliopathy" profile of patients hospitalized with severe forms of COVID-19. Microthrombosis and endothelial dysfunction remain at the center of the pathophysiology of SARS-CoV-2 infection, and biomarkers reflecting endothelial dysfunction should be important markers to consider in the future, particularly in long COVID patients.

Author contributions DMS and JLD supervised the work. DMS wrote the paper. All others authors provided clinical or biological data and reviewed the paper.

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Declarations

Conflict of interest All authors declare that they have no conflict of interest.

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