Clinical Infectious Diseases

BRIEF REPORT

Excess Soluble fms-like Tyrosine Kinase 1 Correlates With Endothelial Dysfunction and Organ Failure in Critically Ill Coronavirus Disease 2019 Patients

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Excess soluble fms-like tyrosine kinase 1 (sFlt-1), a soluble inhibitor of vascular endothelial growth factor pathway, has been demonstrated to promote endothelial dysfunction. Here, we demonstrate that sFlt-1 plasma levels correlate with respiratory symptom severity, expression of endothelial dysfunction biomarker, and incidence of organ failure in coronavirus disease 2019 patients.

Clinical Trials Registration. NCT04394195.

Keywords. COVID-19; endothelial dysfunction; sFlt-1; critical care.

Acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for a global pandemic by causing a spectrum of phenotypes that vary from asymptomatic presentation to acute respiratory distress syndrome that requires admission to an intensive care unit (ICU). The underlying mechanisms to explain why some coronavirus disease 2019 (COVID-19) patients develop life-threatening symptoms while others do not remain incompletely elucidated. Several arguments indicate that the most severe forms of COVID-19 may be related to an endothelial injury [1, 2].

The soluble fms-like tyrosine kinase 1 (sFlt-1) is a splice variant of the receptor 1 for vascular endothelial growth factor A (VEGF-A) that lacks the cytoplasmic and transmembrane domains. By binding to its circulating ligand with high affinity, sFlt-1 inhibits the VEGF-A pathway and impairs endothelial cell homeostasis [3]. Overexpression of sFlt-1 has been well demonstrated to promote endothelial dysfunction, notably during preeclampsia [4].

Our objectives in this study were to compare admission sFlt-1 levels in COVID-19 patients with mild to moderate and severe symptoms and to analyze sFlt-1 levels in critically ill COVID-19 patients with or without organ failure.

METHODS

Study Design and Participants

Patient samples were collected from our local COVID-19 biobank, which is a single-center prospective cohort of adult patients hospitalized at the University Hospital of Reims (northeastern France) between 25 March 2020 and 25 April 2020. Our cohort includes patients with COVID-19 confirmed by reverse-transcription polymerase chain reaction assay and without bacterial coinfection. All included patients had to meet 1 of the following criteria suggestive of lower respiratory tract infection: radiographic infiltrates by imaging study, peripheral oxygen saturation ≤94% on room air, or the need for supplemental oxygen. The severe COVID-19 group included patients hospitalized in an ICU, requiring either high-flow oxygen therapy or mechanical ventilation. The mild to moderate COVID-19 group included patients hospitalized in the department of infectious disease, requiring standard oxygen therapy. Patient samples were obtained at the following time points: admission (day 0), day 3, day 7, and day 14.

Data Collection

Clinical data for all included patients were obtained by reviewing clinical charts and nursing records. Data were collected for the following: age, sex, obesity (defined as a body mass index >30 kg/m^2), hypertension, diabetes, use of mechanical ventilation, use of vasopressor (defined as norepinephrine >0.1 µg/kg/min), incidence of acute kidney injury (AKI; defined as stage 3 based on the Kidney Disease Improving Global Outcomes Classification), incidence of hepatic failure (defined as serum bilirubin level >20 µmol/L), incidence of pulmonary embolism (diagnosed by computed tomography pulmonary angiography), and ICU mortality. The following biological data at admission were obtained by reviewing laboratory measurements: lymphocytes, D-dimers, prothrombin time, activated partial thromboplastin time, fibrinogen, aspartate aminotransferase, alanine aminotransferase, creatinine, albumin, and C-reactive protein. Sepsis-related Organ Failure Assessment (SOFA) scores were calculated in ICU patients at day 0, day 3, day 7, and day 14. Microsoft Excel (2020, v16.36) was used for data collection.

Received 15 June 2020; editorial decision 9 July 2020; accepted 13 July 2020; published online July 16, 2020.

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Clinical Infectious Diseases® 2021;72(10):1834–7

DOI: 10.1093/cid/ciaa1007

Plasma Analysis

All samples were drawn in EDTA tubes, centrifuged at 1500 × g for 15 minutes at room temperature, and then frozen at −80°C within 1 hour of collection. Quantikine enzyme-linked immunosorbent assay kits and quantitative controls were purchased to perform sFlt-1 and soluble vascular adhesion molecule 1 (sVCAM-1) measurements (R&D systems, Minneapolis, MN).

Statistical Analyses

Quantitative variables are reported as the median (interquartile range), and qualitative data are reported as number and percentage. SFlt-1 plasma levels in COVID-19 patients with mild to moderate or severe symptoms were compared using the Mann-Whitney test. Correlation between sFlt-1 and sVCAM-1 levels was studied using the Spearman correlation test. SFlt-1 levels in critically ill COVID-19 patients with or without organ failure were compared using the Mann-Whitney test. The repeated measures correlation coefficient was calculated to assess the correlations between sFlt-1, sVCAM-1, and SOFA scores taking into account measures at days 0, 3, 7, and 14. A P value < .05 was considered statistically significant. All analyses were performed using XLSTAT version 2020.1.1 and R version 3.6.1.

Ethics Approval

All patients provided written informed consent to participate in the study. This study was approved by ethics committee (CPP EST-III).

RESULTS

We included 46 severe and 10 mild to moderate COVID-19 patients. Among severe patients, 22 had blood samples drawn at day 0, 19 at day 3, 23 at day 7, and 21 at day 14 (see Supplementary Table 1). At admission, severe patients showed higher plasma levels of sFlt-1 compared with patients with mild to moderate respiratory symptoms (616.0 vs 442.0 pg/mL). Similarly, plasma levels of endothelial dysfunction biomarker sVCAM-1 were found to be higher in severe COVID-19 patients (2120.0 vs 1355.0 ng/mL; P < .01; Figure 1A).

Next, we aimed to determine if plasma levels of sFlt-1 at admission were associated with outcomes throughout ICU stay in severe COVID-19 patients. SFlt-1 plasma levels at day 0 were associated with the need for mechanical ventilation (877.0 vs 485.5 pg/mL), the need for vasopressor support (1810.0 vs 590.0 pg/mL), stage 3 AKI incidence (2900.0 vs 581.0 pg/mL; P < .01), and death (1810.0 vs 487.0 pg/mL) during ICU stay (Figure 1C). No association was found between admission sFlt-1 plasma levels and the incidence of hepatic failure or pulmonary embolism.

Finally, we aimed to assess the evolution and the potential associations over time between sFlt-1, sVCAM-1, and SOFA scores in the ICU. At ICU admission, excess sFlt-1 correlated with sVCAM-1 plasma levels (r = 0.61; P < .01; Figure 1B). Evolution of both sFlt-1 and sVCAM-1 showed maximal circulating levels at day 3 among critically ill patients (905.0 pg/mL and 2330.0 ng/mL, respectively; Figure 1D). No correlation was found between the evolution of sFlt-1 and sVCAM-1 (r = 0.22; P = .17) and SOFA scores (r = 0.23; P = .15) over time.

DISCUSSION

Innovative investigations are direly needed to advance our understanding of the most severe forms of COVID-19 and, ultimately, to develop new therapeutic paradigms that offer safe and effective alternatives to ICU care. We focused specifically on sFlt-1 and report that high sFlt-1 circulating levels are associated with a severe COVID-19 phenotype. Moreover, we found a correlation between sFlt-1 and the endothelial dysfunction biomarker sVCAM-1 in critically ill COVID-19 patients at ICU admission.

SARS-CoV-2 binds to and downregulates angiotensin-converting enzyme 2, which leads to an increase in angiotensin II bioavailability [5]. The interaction between angiotensin II and its receptor AT1 has been found to promote sFlt-1 upregulation during hypoxia [6]. Moreover, animal models have previously demonstrated that excess sFlt-1 reduces the phosphorylation of endothelial nitric oxide (NO) synthase, which leads to decreased NO formation and an increase in oxidative stress and angiotensin sensitivity [7]. Interestingly, patients at higher risk of developing severe phenotypes of COVID-19 (with hypertension, obesity, and diabetes) are well known to exhibit chronically lower NO bioavailability [8, 9].

Our results suggest an association between sFlt-1 upregulation and organ failure occurrence in critically ill COVID-19 patients. High sFlt-1 levels have been previously reported in patients with bacterial sepsis. Similarly, biological markers of sepsis-associated endothelial dysfunction and sepsis-induced immunosuppression have also been noticed in COVID-19 patients [10]. Taken together, these data suggest that the disease mechanism of COVID-19 involves common pathogenic processes with bacterial sepsis and support the hypothesis of a viral sepsis [11]. However, compared with previous reports of bacterial sepsis, we noticed drastically higher sFlt-1 levels in COVID-19 patients despite the absence of bacterial coinfection [12]. While admission sFlt-1 levels were associated with severe respiratory symptoms and both sVCAM-1 levels and the incidence of organ failure during ICU stay, our results did not find any correlations between sFlt-1, sVCAM-1, and SOFA score over time in critically ill patients. The fact that no further correlation was found over time may suggest that, if available, a potential therapeutic intervention should be considered in the early stages of the natural disease evolution.

While our results need to be replicated in larger cohorts, this study may be clinically relevant because excess sFlt-1–associated endothelial dysfunction is potentially reversible using a phosphodiesterase-5 inhibitor. The ability of the US Food

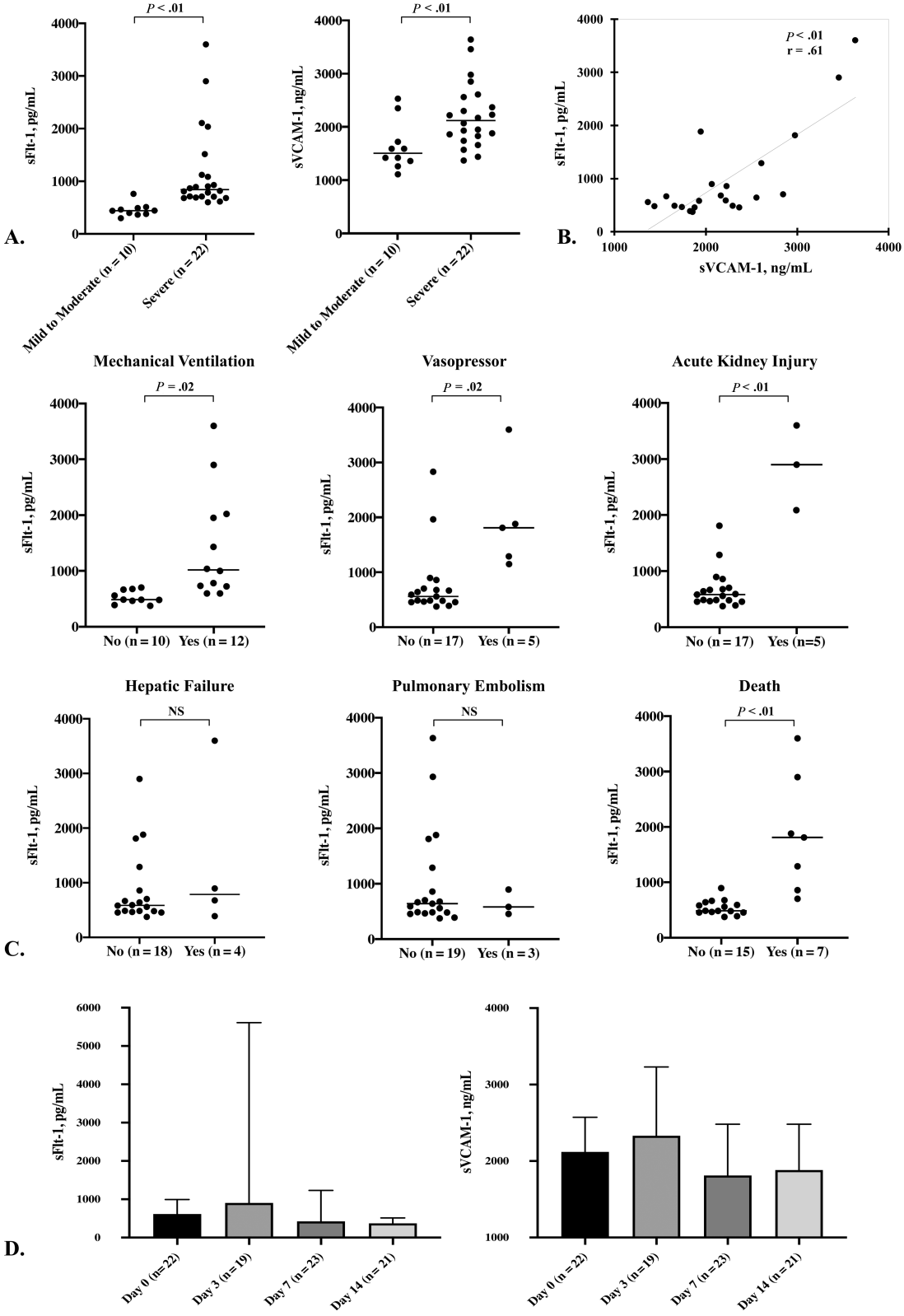

Figure 1. sFlt-1 plasma levels correlate with endothelial dysfunction and outcomes in coronavirus disease 2019 (COVID-19) patients. *A*, Admission sFlt-1 and sVCAM-1 plasma levels in COVID-19 patients in relation to the severity of respiratory symptoms. *B*, Correlation between sFlt-1 and sVCAM-1 plasma levels at intensive care unit (ICU) admission. *C*, Admission sFlt-1 plasma levels and incidence of organ failure during ICU stay. Data are presented as dots with median. The Mann-Whitney test was used for comparison. *D*, Evolution of sFlt-1 and sVCAM-1 throughout ICU stay. Data are presented as median and interquartile range. Abbreviations: NS, not significant; sFlt-1, soluble fms-like tyrosine kinase 1; sVCAM, soluble vascular adhesion molecule 1.

and Drug Administration–approved sildenafil to increase cGMP levels and enhance NO signaling makes it of particular interest for patients at high risk of developing severe COVID-19 [7]. Both its safety and efficacy remain to be established in COVID-19.

In conclusion, our findings strongly suggest that excess sFlt-1 could be an important determinant of COVID-19–associated endothelial and organ dysfunction.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. V. D. conceptually designed the study. V. D., P. N., and B. M. supervised the study. V. D., A. G., M. B., V. C., M. B., G. J., and V. N. approached patients for inclusion. G. P. carried out the experiments. V. D. performed data acquisition. V. D. and L. K. analyzed the data. V. D. created the figures and drafted the manuscript. V. D., P. N. G., and B. M. revised the manuscript. All the authors approved the final version of the manuscript.

Acknowledgments. The authors thank the patients and their families who made this study possible; Maryse Strady and Clotilde Fauvet for sample management; and Grace Stockton for English-language proofreading.

Potential conflicts of interest. The authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

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