Excess soluble fms-like tyrosine kinase 1 correlates with endothelial dysfunction and organ failure in critically ill COVID-19 patients

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Abstract

Excess soluble fms-like tyrosine kinase 1 (sFlt-1), a soluble inhibitor of the vascular
endothelial growth factor pathway, has been demonstrated to promote endothelial dysfunction.
Here we demonstrate that sFlt-1 plasma levels correlate with respiratory symptoms severity,
expression of endothelial dysfunction biomarker and incidence of organ failure in COVID-19 patients.

Key words: COVID-19; endothelial dysfunction; sFlt-1; critical care.

Background

Acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been responsible for a global pandemic by causing a spectrum of phenotypes varying from asymptomatic presentation to acute respiratory distress syndrome (ARDS) requiring admission in intensive care unit (ICU). The underlying mechanisms explaining 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].

The objectives of 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

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

Data collection

       Clinical data of all included patients were obtained by reviewing clinical charts and nursing records: age, sex, obesity (defined as a body mass index> 30kg/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. Biological data at admission were obtained by reviewing laboratory measurements: lymphocytes, D-Dimers, prothrombin time (PT), activated partial thromboplastin time (aPTT),

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fibrinogen, aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), creatinine, albumin and C-reactive protein (CRP). Sepsis-related Organ Failure Assessment (SOFA) scores were calculated in ICU patients at Day 0, Day 3, Day 7 and Day 14. Microsoft Excel (MS Excel 2020, v16.36) was used for data collection.

Plasma analysis

All samples were drawn in EDTA tubes, centrifuged at 1,500 x g for 15 minutes at room temperature, then frozen at -80°C within one hour of collection. Quantikine ELISA kits and quantitative controls were purchased to perform sFlt-1 and sVCAM-1 measurements (R&D systems, Minneapolis, MN, USA).

Statistical analysis

Quantitative variables are reported as the median (interquartile range) and qualitative data as number and percentage. SFlt-1 plasma levels in COVID-19 patients with mild to moderate or severe symptoms were compared using Mann Whitney test. Correlation between sFlt-1 and sVCAM-1 levels was studied using Spearman's correlation test. SFlt-1 levels in critically ill COVID-19 patients with or without organ failure were compared using the Mann Whitney test. Repeated measures correlation coefficient was calculated to assess the correlation between sFlt-1, sVCAM-1 and SOFA scores taking into account measures at day 0, 3, 7 and 14. A p value < 0.05 was considered statistically significant. All analyses were performed using XLSTAT version 2020.1.1 and R version 3.6.1

Ethics approval

All subjects provided written informed consent to participate in the study. This study was approved by ethics committee (CPP EST-III 20.04.13, April 23rd 2020) and was registered in Clinicaltrial.gov (NCT04394195).

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 to 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 COVID-19 severe patients (2120.0 vs 1355.0 ng/mL; $p<0.01$) (Figure 1A).

Next, we aimed to investigate 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; $p<0.01$) 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 association over time between sFlt-1, sVCAM-1 and SOFA scores in ICU. At ICU admission, excess sFlt-1 correlated with sVCAM-1 plasma levels (r=0.61; p<0.01) (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)

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(Figure 1D). No correlation was found between the evolution of sFlt-1 and sVCAM-1 (r= 0.22; *p*=0.17) and SOFA scores (r=0.23; *p*=0.15) over time.

Discussion

Innovative investigations are direly needed to advance our understanding of the most severe forms of COVID-19, and ultimately 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 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 (ACE2), 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 a 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

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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 correlation between sFlt-1, sVCAM-1 and SOFA score over the 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 FDA 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.

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Author contributions

VD conceptually designed the study: VD, PNG and BM supervised the study; VD, AG, MB, VC, MB, GJ and VN approached patients for inclusion; GP carried out the experiments; VD performed data acquisition; VD and LK analyzed the data; VD made the figures and drafted the manuscript; VD, PNG and BM revised the manuscript; all the authors approves the final version of the manuscript.

Conflict of interest statement

The authors declare no competing interests.

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Figure legends

Figure 1.   **SFlt-1 plasma levels correlate with endothelial dysfunction and outcomes in 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 ICU admission. C. Admission sFlt-1 plasma levels and incidence of organ failure during ICU stay. Data are presented as dots with median. 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.

Figure 1

A.

B.

Mechanical Ventilation

Vasopressor

Acute Kidney Injury

Hepatic Failure

Pulmonary Embolism

Death

C.

D.