Moreover, our assay is not able to identify non vital endothelial cells that already underwent apoptosis after viral injury and, therefore, our analysis might not fully depict the entirety of endothelial damage.

It would also be of interest to extend our evaluation to the circulating endothelial progenitor cells (CEPs) compartment in order to identify possible correlation between CEC and CEP counts and to investigate angiogenic activity.

Finally, from a therapeutic perspective, it will be useful to assess the value of CEC monitoring during treatment with low molecular weight heparin and other drugs under investigation for COVID-19. Complement inhibitors such as eculizumab and IFX-1, and the endothelial protecting drug defibrotide, could limit the extension of endothelial damage and the progression of respiratory failure in COVID-19.

The results of clinical trials (NCT04335201; NCT04288713, NCT04333420) investigating these agents are eagerly awaited.

CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

M.E.N. and G.M. contributed equally as first authors, and L.A., R.B. and M.B. contributed equally as last authors. L.A., G.A.I., R.B. and M.B. are designed the study: M.E.N., G.M., L.P., M.S. and A.D.S. collected data: A.T., C.P., and E.C. performed flow cytometry, V.V.F. performed statistical analysis, M.E.N., G.M., and L.A. wrote paper: all authors approved the final version.

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SUPPORTING INFORMATION

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Increased sFLT-1/PlGF ratio in COVID-19: A novel link to angiotensin II-mediated endothelial dysfunction

To the Editor:

COVID-19 represents a worldwide emergency. A growing body of evidence indicates the presence of a severe form of coagulopathy,[1,2] which accounts for part of the excess of mortality of the syndrome. The pathogenesis of this coagulopathy is incompletely understood at present. Simply, SARS-CoV-2 enters lung cells using angiotensin-converting-enzyme 2 (ACE2),[3] which is expressed on epithelial cells in the

lungs, in the small bowel, and on endothelial cells of virtually every organ. So, ACE2 is a key regulator of the renin-angiotensin-system (RAS) which converts angiotensin-II (AngII) into angiotensin 1-7 (Ang1-7). So, AngII binds its type 1 and 2 receptors (AT1 and AT2) and exerts pro-inflammatory, oxidative, vasoconstrictive and pro-fibrotic effects. Ang1-7, by binding the Mas receptor (MasR), mediates vasodilatory, anti-inflammatory and anti-oxidant effects.[4]

Elevated values of vascular growth factors (VEGFs) and AngII, due to SARS-CoV-1 binding to ACE2 and to its downregulation, increase vascular permeability and inflammation and drive acute lung injury (ALI).[5] Thus, AngII also directly mediates endothelial cells activation, perturbation and apoptosis.[6]

There is a well-known model of AngII-mediated endothelial dysfunction (ED): the preeclampsia (PE) syndrome. In a seminal publication by Gant et al.,[7] pregnancy was determined to be a state of relative insensitivity to AngII, a physiological adaptation that contributes to low systemic vascular resistance. Conversely, women who later develop preeclampsia remained sensitive to AngII, and exhibit an imbalanced proportion of anti-angiogenic and pro-angiogenic soluble plasmatic factors. The most promising markers are PlGF (placental growth factor) and its decoy receptor sFlt-1(soluble fms-like tyrosine kinase 1). The PE-affected women display a high sFlt-1/PlGF ratio that is associated with adverse outcome. We speculated that also in COVID-19 patients AngII could mediate an abnormal secretion of sFlt-1 and an ensuing high sFlt-1/PlGf ratio, causing a pathological imbalance between angiogenic and anti-angiogenic factors and subsequent ED. In this report the values of sFlt-1 and the related PlGF were measured in COVID-19 patients and in two control groups.

The sFlt-1/PlGF values were determined in a cohort of consecutive patients admitted to San Gerardo Hospital for pneumonia, either COVID-19 positive (19 patients) or negative (12 patients). All patients signed an informed consent for collection of biological materials. Six patients in the COVID-19+ group were intubated and nine were in continuous positive airway pressure (CPAP) therapy. Four patients in the COVID-19 negative group were receiving CPAP treatment. All patients were tested post admission (day two to 17), and all but one (in the COVID-19 negative group) were receiving prophylactic treatment with enoxaparin.

Leftover serum and plasma specimens (BD Vacutainer STT II Advance tube REF 366881 BD Plymouth UK) were obtained by centrifugation and immediately stored at −20°C until analysis.

Both sFLt-1 and PlGF were measured on the Cobas e601 platform (Roche Diagnostics) using the electrochemiluminescence immunoassay principle. No reference ranges for sFlt1 (pg/mL) and PlGF (pg/mL) exist outside pregnancy. The C-reactive protein (CRP) was measured on the Cobas c702 platform (Roche Diagnostics) using an immunoturbidimetric method. Normal values are < 5 mg/L. The D-dimer values were measured on an ACL TOP 500 platform (Instrumentation Laboratory) using a chromogenic method. Normal values are < 250 ng/mL.

All values are expressed as mean plus or minus (+/−) Standard Error (SE). Statistical analysis and graphs were analyzed using GraphPad Prism6 (GraphPad Software, Inc.). The Mann-Whitney non-parametric statistical test was utilized to compare different groups, and *P* values < .05 were considered significant.

We obtained sFlt-1 values in 19 consecutive COVID-19 infected patients (all hospitalized with pneumonia and elevated D-dimers), in 12 age and sex matched COVID-19 negative patients hospitalized with pneumonia, and in a group of 18 healthy blood donors. Blood samples were obtained in the COVID-19 positive group after 7.8 (+/− 1.5) days from admission and in the COVID-19 negative group after 9.6 (+/− 1.4) days (*P* = .2).

The D-dimer values ranged between 1029 and 37 826 ng/mL (mean +/− SE: 7558 +/− 2806) in the COVID-19 positive patients, and were within the normal range in the other group. As shown in Table 1, values of sFlt-1 were significantly higher in COVID-19 positive patients as compared to patients with COVID-19 negative pneumonia, and healthy donors. The PLGF values did not change significantly in the two groups but the sFlt1/PLGF ratio increases from 5.0 to 14.1 (*P* = .001). In Figure S1 sFlt-1, and PLGF values as well as sFlt1/PLGF ratio results are presented for two COVID-19 positive patients that were followed over a period of time of 8 days

TABLE 1 sFlt-1, PlGF, sFlt-1/PlGF ratio, CPR and Vitamin D values in patients affected by COVID-19 positive or negative pneumonia and in healthy donors (HD)

Variable (mean +/− SE)	Covid-19 positive pneumonia	Covid-19 negative pneumonia	Healthy donors (HD)	*P*value COVID-19pos vs COVID-19 neg	*P* value COVID-19pos vs HD
Age (years)	63.7 +/−3.6	68.5+/−1.2	49.5+/−1.8	NS	<.001
M/F	14/19	6/12	11/18	NS	NS
CRP (mg/L)	109+/−31	58+/−28	3.0+/−1.0	NS	<.001
sFlt1 (pg/mL)	320.7+/−45	117.2+/−7.5	90.5+/−2.1	.001	<.001
PlGF (pg/mL)	25.7+/−2.6	26.4+/−0.5	12.8+/−0.3	NS	<.001
sFlt1/PlGF ratio	14.1+/−1.4	5.0+/−0.4	7.3+/−0.3	<.001	.001
Vitamin D (ng/mL)	11.8+/−1.4	15.9+/−3.8	16.0+/−0.9	NS	.004

NS, not significant.

following hospital admission. They show a strong and early increase in blood values of sFlt-1 with a parallel increase in the sFlt-1/PlGF ratio.

SARS-CoV-2 binds to and down-regulates ACE2, resulting in an increase in AngII, which acts through its receptors (AT1 and AT2) and directly causes ED.[8] Preeclampsia represents a model of AngII mediated ED. Trophoblasts are resistant to AngII in normal pregnancy, while they remain sensitive in women who later develop preeclampsia. Preeclampsia develops in two stages: an earlier placental dysfunction (including deficient placentation) and a later maternal syndrome (systemic vascular inflammation). Several studies have demonstrated an imbalance between angiogenic factors (ie, PlGF) and antiangiogenic factors (ie, s-Flt1).[9] Thus sFlt1, a soluble inhibitor of vascular endothelial growth factors (VEGFs), is induced upon Ang II Type 1 receptor (AT1) activation by AngII in response to hypoxia.[10] sFlt1 is an endothelial decoy receptor that acts as a trap for VEGFs, like placental growth factor (PlGF). Also, sFlt-1 mediates endothelial damage by impairing nitric oxide (NO) production and, more importantly, it sensitizes endothelial cells to AngII,[11] thus starting a positive loop.

We speculate that in response to ACE2 depletion and the consequent imbalance of AngII/Ang1-7 a hypoxia driven secretion of sFlt-1 occurs, leading to global endothelial damage. There are previously described high values of sFlt-1 in septic patients, with VEGFs values rapidly increasing in the first 48 hours from the onset of fever.[12] These authors did not measure PlGF, and the ratio between sFlt1 and VEGFs did not change over time. In COVID-19, the endothelial damage possibly due to direct infection through ACE2, results in increased sFlt-1/PlGF ratio, likely due to a pathological imbalance between AngII and Ang1-7.

Our data offer a link between ACE2 downregulation and an AngII/sFlt-1 mediated ED, a model that strictly resembles preeclampsia. These results could also offer an explanation to the pathogenesis of acute global vascular damage because ACE2 is throughout the vascular system expressed by endothelial cells.

These data also provide a rationale for AngII-targeted therapy, and/or the use of aspirin[13] to decrease sFlt-1 production and to counteract the COVID-19-related coagulopathy. The sFlt-1 and PlGF tests are already performed in many hospitals for risk stratification among women presenting for hypertensive disorders in pregnancy, and reference ranges based on gestational age are readily available. The use of the sFlt-1/PlGF ratio in COVID-19 could therefore provide a simple clinical tool to stratify the intensity of ED.

Since sFlt-1 is produced only by endothelial cells or monocytes, it remains to be demonstrated if these cells are directly infected by COVID-19, or whether their excessive sFlt-1 production represents a response to increased levels of AngII produced elsewhere, or to inflammatory mediators. A recent publication documented the presence of viral particles inside endothelial cells obtained from advanced cases of COVID-19 infection.[14]

Prospective serial collection of samples from patients during COVID-19 infection are currently under way and will be needed to confirm these preliminary observations, in addition to the direct study of endothelial cells.

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CONFLICT OF INTEREST

The authors declare no potential conflict of interests.

AUTHOR CONTRIBUTIONS

Dr V.G. wrote the first draft of the paper. Dr A.C. conceived the project and collected samples. Dr E.C. supplied clinical material. Dr M.C. conceived the project and performed lab tests. Dr P.V. supervised results. Prof C.G.-P. finalized the manuscript and supervised results. All authors read the manuscript and approved it.

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Immune thrombocytopenia due to COVID-19 during pregnancy

To the Editor:

In April 2020, during the 2019 novel coronavirus disease (COVID-19) pandemic caused by the virus SARS-CoV-2, a pregnant patient was diagnosed with immune thrombocytopenia (ITP) triggered by COVID-19.

The 41-weeks-pregnant woman, with no significant past medical history, presented to the obstetric physician due to contractions. She had a sore throat but no other flu-like symptoms. She had no signs of easy bruising or bleeding. Her vitals at presentation were a temperature of 36·4°C, respiration rate of 16/min, peripheral oxygen saturation (SpO_2) of 98%, blood pressure of 115/80 mmHg, and pulse of 93/min. General laboratory examinations were performed, which showed a platelet count of 16 × 10E09/L. Two weeks earlier, the platelet counts were 98 × 10E09/L. The patient was suspected to have immune thrombocytopenia (ITP). Additional test with direct monoclonal antibody immobilization of platelet antigens (MAIPA) showed platelet auto-antibodies against glycoprotein V. Throat and nose swabs were positive for SARS-CoV-2.

The patient was diagnosed with a first presentation of ITP, most likely triggered by COVID-19. Treatment with intravenous immunoglobulin (IVIG) for 2 days was initiated. In order to be able to safely perform epidural anesthesia for the labor, 2 units of donor thrombocytes were administered. Her platelet counts increased to 80 × 10E09/L. Epidural anesthesia was complicated by hypotension with a suboptimal cardiotocography. Therefore, an urgent caesarian section was performed and a healthy daughter was born. Few hours later, she became hypoxic with a peripheral oxygen saturation of 91% without dyspnea. A chest CT showed infiltrates in the left lower lobe with ground-glass opacities, typical of COVID-19 (Figure S1). Within 24 hours, the peripheral oxygen saturation increased to 100% while breathing room air. Four days later, she was discharged without flu-like symptoms and with stable platelet counts of 82 × 10E09/L that normalized 3 weeks later (315 × 10E09/L). Her newborn daughter did not develop any symptoms of COVID-19. The newborn's platelets were 158 × 10E9/L at birth, but decreased to 41 × 10E09/L 5 days after birth. However, her platelets increased spontaneously thereafter, reaching 198 × 10E09/L at 3 weeks.

About 80% of patients infected with SARS-CoV-2 are asymptomatic or have mild flu-like symptoms.[1] While mainly a respiratory disease, COVID-19 can trigger widespread systemic pathology, ranging from thrombo-embolism, cardiovascular injury, hyper-inflammatory syndrome, immune-mediated pathology, and multi-organ failure.[2,3] Interestingly, COVID-19 has some unique aspects interfering with the immune system which are rarely observed in other respiratory viral infections.[4] Lymphopenia and, at the same time, a cytokine storm, which is reflected by elevated levels of acute phase reactants, show an affected innate and adaptive immune system and are thought to predict disease severity. Similar to other viral infections,[5] SARS-CoV-2 can also trigger ITP and probably autoimmune hemolytic anemia.[6] Our patient developed a COVID-19 induced ITP that was confirmed by a positive MAIPA. This case-report shows that COVID-19 can induce ITP even in patients with mild symptoms. Recently, Zulfiqar et al. reported a case of suspected ITP in a patient admitted due to COVID-19.[7] The patient had normal platelet counts at admission, but dropped gradually to 1 × 10E09/L in 8 days. However, no auto-antibodies against glycoproteins were found and no response to IVIG was observed in that patient.

As SARS-CoV-2 is now very widespread, we suggest testing for SARS-CoV-2 in patients suspected of a (relapsed) ITP, even in the absence of respiratory symptoms.