Letter to the Editor

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Soluble fms-like tyrosine kinase-1: a potential early predictor of respiratory failure in COVID-19 patients

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To the Editor,

A main feature of the most severely affected patients with coronavirus disease 2019 (COVID-19) is hypoxemic respiratory failure from acute respiratory distress syndrome (ARDS). The presence of intussusceptive ('splitting') angiogenesis and other vascular features has been reported to distinguish the lungs of patients who died from respiratory failure due to COVID-19 from those of individuals who died from influenza [1]. Angiogenesis is chiefly regulated by vascular endothelial growth factor (VEGF) and its associated family members, whose proangiogenic activity is mediated through their engagement with VEGF receptor 1 (VEGFR1, also known as 'fms-like tyrosine kinase 1' or Flt1) and VEGFR2. Another member of the VEGF family, placental growth factor (PlGF), stimulates endothelial healing and recruitment of mononuclear bone marrow cells as well as microvascular angiogenesis through engagement with Flt1, which is expressed in many tissues including the lung [2]. Alternative splicing of Flt1 pre-mRNA creates the soluble form of this receptor, known as 'sFlt1', which binds and antagonizes VEGF and PlGF signal [3]. Excess levels of sFlt1 induce endothelial dysfunction in hypoxia and contribute to cardiovascular disease [4].

In this single-center prospective cohort study, we aimed to analyze the potential role of sFlt-1 and PlGF, together with other 'traditional' biomarkers, as predictors of respiratory failure in patients hospitalized with COVID-19.

We enrolled patients hospitalized with COVID-19 at *Hospital 12 de Octubre* (Madrid, Spain) between 03/12/2020–04/20/2020. The study was approved by the local Ethics Committee (reference#20/222 and 20/117) and adhered to the Declaration of Helsinki. Patients' main characteristics along with their progress and complications during hospitalization were extracted from electronic medical records. COVID-19 was confirmed via SARS-CoV-2 real-time reverse transcription polymerase chain reaction (nasopharyngeal swab or sputum samples). Respiratory failure was defined as the presence of an arterial oxygen partial pressure/fractional inspired oxygen ratio ≤200 mmHg or the need for mechanical ventilation (non-invasive positive pressure ventilation including high-flow nasal cannula oxygen, or invasive mechanical ventilation). Respiratory failure could occur at any time during hospital admission.

Blood samples were obtained within the first 48 h of hospitalization. Plasma sFlt1/PlG1 concentrations were

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Table 1: Baseline characteristics and main results by group.

	No respiratory failure n=59	Respiratory failure n=52	p-Value between groups
Age, years	71 [59.5, 80.5]	74 [62.0, 81.2]	0.304
Sex			
Male	31 (52.5%)	37 (71.1%)	0.053
Female	28 (47.5%)	15 (28.9%)	0.053
Signs and symptoms on admission			
Days with symptoms	7 [4, 8]	5 [4, 7]	**0.027**
Cough	48 (81.4%)	39 (75.0%)	0.491
Dyspnea	27 (45.8%)	36 (69.2%)	**0.021**
Myalgia	24 (40.7%)	15 (28.8%)	0.234
Diarrhoea	19 (32.2%)	16 (30.8%)	1.000
Expectoration	12 (20.3%)	11 (21.1%)	1.000
Vomit	9 (15.2%)	4 (7.7%)	0.251
Bronchospasm	2 (3.4%)	3 (5.8%)	0.664
Vital signs on admission			
Systolic blood pressure, mmHg	130 [121, 144]	125 [116, 138]	0.116
Heart rate, beats/min	87 [79, 98]	89 [74, 97]	0.453
Body temperature, °C	37.4 [36.9, 38.0]	37.8 [37.3, 28.4]	**0.011**
Respiratory rate, breaths/min	18 [16, 20]	21 [18, 23]	**0.001**
Arterial oxygen saturation, %	96 [93, 98]	92 [89, 94]	**<0.001**
PaO_2 (mmHg)/FiO_2 (%), mmHg	438 [345, 471]	305 [263, 433]	**0.012**
Comorbidities			
Hypertension	29 (49.2%)	34 (65.4%)	0.124
Influenza vaccination (last year)	31(52.5%)	30 (57.7%)	0.703
Dyslipidemia	19 (32.2%)	23 (44.2%)	0.240
Obesity	16 (27.1%)	18 (34.6%)	0.416
Diabetes	15 (25.4%)	14 (26.9%)	1.000
Malignancies	7 (11.9%)	9 (17.3%)	0.433
Chronic heart failure	6 (10.2%)	9 (15.2%)	0.405
Acute myocardial infarction	6 (10.2%)	6 (11.5%)	1.000
Chronic obstructive pulmonary disease	1 (1.7%)	10 (19.2%)	**0.003**
Asthma	7 (11.9%)	3 (5.8%)	0.331
Severe nephropathies	4 (6.8%)	6 (11.2%)	0.501
Liver diseases	3 (5.1%)	7 (11.9%)	0.185
Transplant	6 (10.2%)	4 (6.8%)	0.748
Treatments on admission			
Antihypertensive drugs	28 (53.8%)	32 (54.2%)	0.252
ACE inhibitors	17 (32.7%)	13 (22.0%)	0.675
ARB	10 (16.9%)	14 (23.7%)	0.250
Other	1 (1.7%)	5 (8.5%)	0.097
ASA	11 (18.6%)	10 (16.9%)	1.000
Anticoagulants	7 (11.9%)	12 (23.1%)	0.136
Steroids	5 (8.5%)	6 (10.2%)	0.753
Treatments during hospitalization			
Lopinavir/ritonavir	32 (54.2%)	37 (71.1%)	0.079
Hydroxychloroquine	55 (93.2%)	47 (90.4%)	0.737
Tocilizumab	2 (3.4%)	11 (21.1%)	**0.006**
Antibiotics	58 (98.3%)	51 (98.1%)	1.000
Steroids	16 (27.1%)	36 (69.2%)	**<0.001**
Scores			
SOFA	1.29 ± 1.82	2.28 ± 1.63	**0.005**
qSOFA	0.28 ± 0.45	0.71 ± 0.70	**<0.001**
Charlson comborbidity index	3.41 ± 2.13	4.39 ± 2.30	**0.024**
Exitus	3 (5.1%)	33 (63.5%)	**<0.001**

Table 1: (continued)

	No respiratory failure n=59	Respiratory failure n=52	p-Value between groups
eGFR (CKD-EPI, mL/min per 1.73 m^2)	20.3 [15.1, 26.1]	24.0 [18.9, 30.5]	**0.046**
Blood biochemical variables			
sFlt1, ng/L	88.2 [77.6, 98.6]	118.8 [98.3, 139.4]	**<0.001**
LDH, U/L	287 [250, 348]	404 [342, 476]	**<0.001**
CRP, mg/L	44.3 [22.0, 100.4]	105.9 [70.8, 177.9]	**0.001**
Lymphocyte count (×10^9/L)	1.1 [0.9, 1.4]	0.8 [0.6, 1.0]	**0.003**
AST/ALT	1.35 [1.03, 1.68]	1.56 [1.26, 1.86]	**0.018**
PlGF, ng/L	84.6 [58.9, 97.5]	71.4 [49.8, 87.0]	**0.022**
Albumin, g/L	37.6 [34.0, 39.8]	35.9 [32.7, 38.0]	0.066
RDW, %	13.7 [13.4, 14.5]	14.1 [13.6, 17.3]	0.143
Ferritin, µg/L	761 [336, 1,250]	1,016 [549, 2,183]	0.219
sFlt1/PlGF	4.24 [3.55, 6.00]	4.77 [3.83, 6.17]	0.219

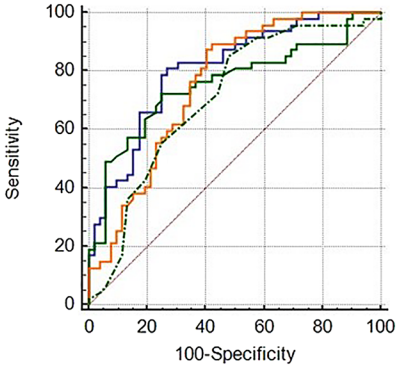
Data are mean [interquartile range] for age and vital signs and symptoms on admission, median [interquartile range] for days with symptoms, mean ± standard deviation for scores, and n (%) for the rest of variables. p-values corresponds to the comparisons between groups with the Mood´s median test or with the χ^2 or Fisher's test (for proportions). Significant p-values (<0.05) are in bold. ACE, angiotensin converting enzyme; ARB, angiotensin-II receptor blockers; ALT, alanine transaminase; ASA, acetylsalicylic acid; AST, aspartate transaminase; CKD-EPI, chronic kidney disease epidemiology collaboration equation; CRP, C-reactive protein; FiO_2, fractional inspired oxygen; eGFR, estimated glomerular filtration rate; LDH, lactate dehydrogenase; PaO_2, arterial oxygen partial pressure; PlGF, placental growth factor; RDW, red blood cell distribution width; ROC, receiver operating characteristic; sFlt1, soluble fms-like tyrosine kinase 1; SOFA, sequential organ failure assessment.

measured using automated immunoassays (Cobas e601, Roche Diagnostics, Risch-Rotkreuz, Switzerland) following manufacturer's instructions. Other major blood biomarkers associated with inflammation and/or COVID-19 were also determined (e.g., lactate dehydrogenase (LDH), C-reactive protein (CRP), lymphocyte count, albumin) as reported elsewhere [5].

To determine the clinical utility of blood biomarkers for prediction of respiratory failure (vs. not suffering this condition) in patients hospitalized with COVID-19, we used receiver operating characteristic (ROC) curve analysis. The sensitivity, specificity, positive and negative predictive values for the computed cutoff values were also calculated.

One hundred eleven hospitalized patients with confirmed COVID-19 were enrolled, of whom 52 (46.8% of total) developed respiratory failure 1–9 days following blood extraction (median, 2.5 days). Thirty-four (31% of total) of the patients died, most (n=32) having developed respiratory failure.

Significant differences were found for most blood biomarkers between patients who developed respiratory failure and those who did not (Table 1). On the other hand, four biomarkers (sFlt1, LDH, CRP and lymphocyte count) had



Biomarker		AUC (95% CI)	Significance level P	cut-off	Sensitivity	Specificity	Youden´s J statistic
sFlt1	——	0.815 (0.730-0.882)	<0.001	>95.7	82.7	72.9	0.556
LDH	——	0.776 (0.686-0.850)	0.002	>343	75.0	75.4	0.504
CRP	——	0.712 (0.661-0.830)	<0.001	>6.35	80.4	61.0	0.414
Lymphocyte	-·-·-	0.715 (0.616-0.800)	<0.001	≤0.9	72.9	56.6	0.295

Figure 1: Area under the curve (AUC), sensitivity, specificity, cut off points and Youden Index (J) values for the biomarkers with the highest AUC (>0.70) to predict occurrence of respiratory failure in patients hospitalized with COVID-19.
CRP, C-reactive protein; LDH, lactate dehydrogenase; sFlt1, soluble fms-like tyrosine kinase 1.

area under the curve values >0.7 to predict the occurrence of respiratory failure in patients hospitalized with COVID-19, with sensitivity and specificity values ranging from 75.0 to 88.2% and from 52.8 to 75.4%, respectively, and with sFlt1 showing the best predictive accuracy (Figure 1).

Endothelial disease is an essential part of the pathological response to severe COVID-19, which leads to respiratory failure, multiorgan dysfunction and thrombosis [1]. Because the vascular endothelium depends on proangiogenic factors, excess release of antiangiogenic factors (e.g., sFlt1) is a possible cause of the endothelial dysfunction observed in patients with COVID-19. Yet, sFlt1 and PlGF (an anti and proangiogenic factor, respectively) have received scant attention in the context of this disease. Giardini et al. [6] reported higher values of sFlt1 and sFlt1/PlGF ratio in patients with COVID-19–associated pneumonia than in healthy controls. Dupont et al. [7] reported high sFlt1 circulating levels in patients severely affected with COVID-19, finding a correlation between sFlt1 and an endothelial dysfunction biomarker, soluble vascular cell adhesion molecule-1. On the other hand, the finding of a potential role of angiogenic processes in patients with COVID-19 opens the door for new therapeutic approaches. Low-dose aspirin (60–100 mg/day) inhibits the expression of sFlt1 in hypoxia-induced human cytotrophoblasts [8]. It can be thus hypothesized that aspirin administration might diminish the hypoxemic respiratory failure due to COVID-19–associated ARDS in critically ill patients. In addition, sFlt1 values could guide the timing of steroid administration.

To our knowledge, this is the first study with sFlt1 as an early predictor of respiratory failure in the context of COVID-19. Our findings suggest that this biomarker might identify a subgroup of hospitalized patients with this condition who are at higher risk of developing respiratory failure, and could thus help physicians to better triage these patients.

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