

Circulating markers of angiogenesis and endotheliopathy in COVID-19

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Abstract

Increase in thrombotic and microvascular complications is emerging to be a key feature of patients with critical illness associated with COVID-19 infection. While endotheliopathy is thought to be a key factor of COVID-19-associated coagulopathy, markers indicative of this process that are prognostic of disease severity have not been well-established in this patient population. Using plasma profiling of patients with COVID-19, we identified circulating markers that segregated with disease severity: markers of angiogenesis (VEGF-A, PDGF-AA and PDGF-AB/BB) were elevated in hospitalized patients with non-critical COVID-19 infection, while markers of endothelial injury (angiopoietin-2, FLT-3L, PAI-1) were elevated in patients with critical COVID-19 infection. In survival analysis, elevated markers of endothelial injury (angiopoietin-2, follistatin, PAI-1) were strongly predictive of in-hospital mortality. Our findings demonstrate that non-critical and critical phases of COVID-19 disease may be driven by distinct mechanisms involving key aspects of endothelial cell function, and identify drivers of COVID-19 pathogenesis and potential targets for future therapies.

Keywords

COVID-19, endotheliopathy, angiogenesis

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Despite over 40 million infected and over 1 million deaths globally, the pathophysiological factors that determine the wide spectrum of clinical outcomes in COVID-19 remain inadequately defined. Importantly, patients with underlying cardiovascular disease have been found to have worse clinical outcomes,¹ and autopsy findings of endotheliopathy in COVID-19 have accumulated.² Nonetheless, circulating vascular markers associated with disease severity and mortality have not been reliably established. To address this limitation and better understand COVID-19 pathogenesis, we report plasma profiling of factors related to the vascular system from patients admitted to our health system with confirmed COVID-19 diagnoses, which revealed significant increases in

markers of angiogenesis and endotheliopathy in hospitalized patients.

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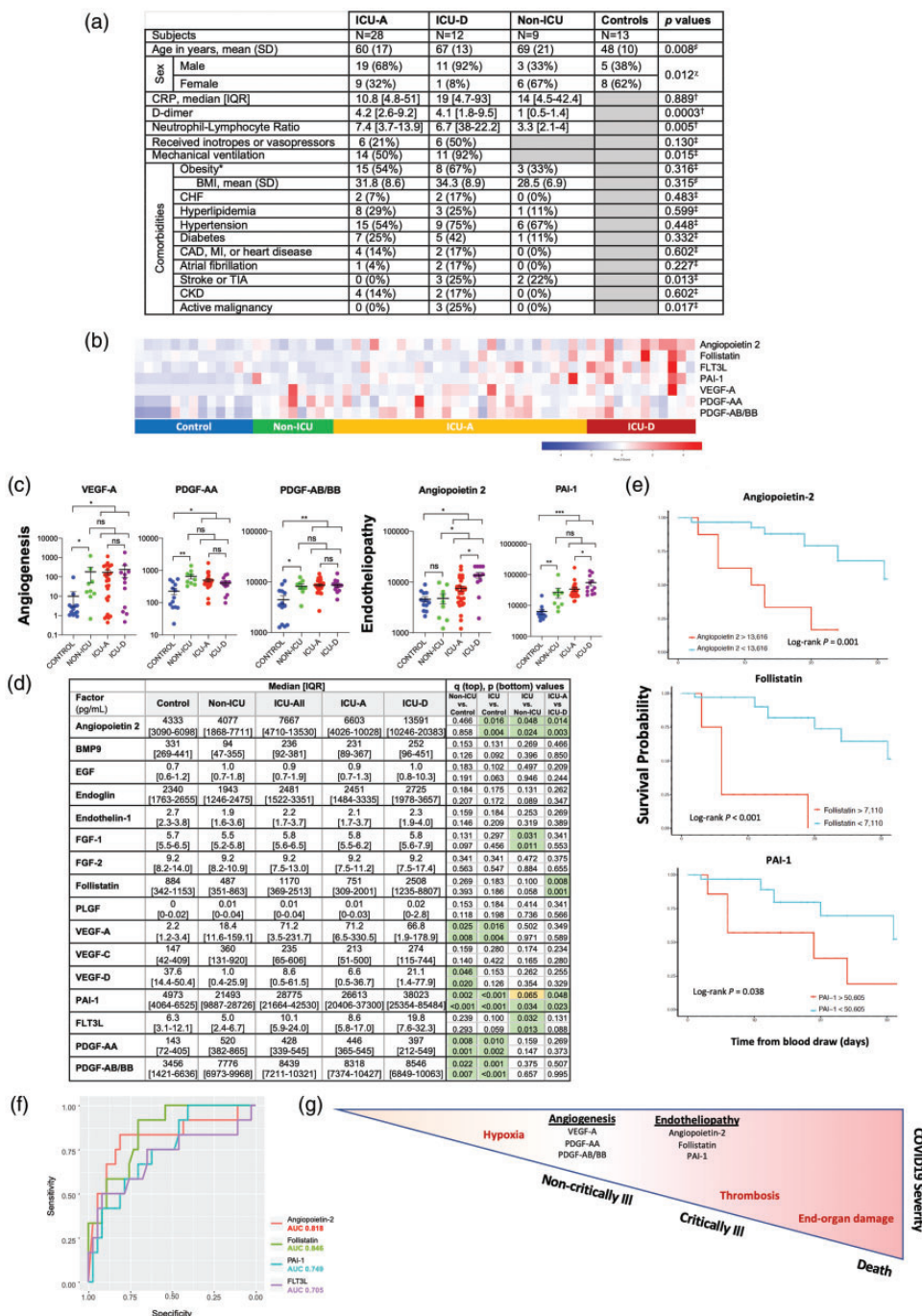


Figure 1. Analyses of vascular biomarkers across the spectrum of COVID-19 disease severity. (a) Subject demographics for ICU patients with COVID-19 (classified by alive (A) and died (D)), non-ICU patients with COVID-19, and controls. *Obesity is defined as BMI > 30. ^yOne-way ANOVA. ^zGroup-wise Pearson's Chi² test; ^fKruskal-Wallis test. [†]Fisher's exact test. SD, standard deviation; BMI, body-mass index; CHF, congestive heart failure; CAD, coronary artery disease; MI, myocardial infarction; TIA, transient ischemic attack; CKD, chronic kidney disease. (b) Heatmap indicating relative protein levels detected in each subject (columns) for proteins (rows) that had statistically significant differences between groups. (c) Comparisons of absolute plasma protein levels (pg/mL) for select markers between control, non-ICU, and ICU groups, as well as ICU survivors vs. non-survivors; differences were tested in two-sided t-tests (with Welch correction for unequal variances, where applicable) and two-tailed Mann-Whitney U-tests for samples that did and did not conform to the normal distribution, respectively. Significance levels are expressed as q-values ('adjusted p-values'; *q < 0.05, **q < 0.01, ***q < 0.0001). (d) Raw values of vascular markers, p and q values for tests evaluating differences between subject cohorts; original tests with p < 0.05 were accepted as significant if corresponding q was less than the false discovery rate of 0.05 (highlighted green). (e) Kaplan-Meier curves for angiopoietin-2, follistatin, and PAI-1, indicating that patients with elevated circulating levels of these proteins had a significantly higher likelihood of in-hospital mortality. (f) Receiver operating characteristic (ROC) curves for angiopoietin-2, follistatin, PAI-1, and FLT-3L. (g) Schematic model of vascular processes in the progression of COVID-19 pathogenesis.

The Institutional Review Board approved the study and waived the need for consent. Blood collected between April 13 and April 24, 2020 from critically ill patients (defined by admission to intensive care unit at blood draw, designated 'ICU', n=40) and non-critically ill patients ('non-ICU', n=9) with COVID-19 was used for analyses (Fig. 1a). To ensure a maximum separation of illness severity between the cohorts and to reduce bias where possible, we preferentially included ICU patients who were intubated and non-ICU patients with minimal oxygen requirement. Based on our treatment algorithm at the time of enrollment, the ICU cohort was much more likely to receive tocilizumab than the non-ICU cohort (38/40 vs. 2/9 patients). Blood from 13 asymptomatic, non-hospitalized, presumed SARS-CoV-2-negative individuals comprised mostly of healthcare workers were collected and served as controls. Blood was collected in sodium citrate tubes, and plasma supernatant was used for analysis. The biomarker profiling was conducted at Eve Technologies (Calgary, Canada) utilizing laser bead-based multiplexed assays to detect multiple proteins simultaneously. Of the critically ill patients, 12 had died by the time of last follow-up on 23 May 2020, while 25 were discharged and 3 remained hospitalized. All of the non-ICU patients with COVID-19 were discharged home, and none progressed to critical illness.

We assessed 16 circulating markers related to vascular function. A heatmap was generated using Heatmapper (heatmapper.ca) to represent the concentrations of those biomarkers that were differentially regulated (Fig. 1b). We compared protein levels in (1) non-ICU vs. controls, (2) ICU vs. controls, (3) ICU vs. non-ICU, and (4) ICU-Alive vs. ICU-Died (Fig. 1c). We made three key observations relating vascular biomarkers with the severity of disease. First, multiple pro-angiogenic factors, including VEGF-A, PDGF-AA, and PDGF-AB/BB, were significantly elevated in non-ICU patients with COVID-19 compared to controls, which may be contributing to the described vascular remodeling in COVID-19 (Fig. 1c).² Second, in ICU patients with COVID-19 compared to controls, we found significantly increased levels of angiotensin-2 and PAI-1, which when present at high levels in circulation likely reflect endotheliopathy supporting data implicating endothelial involvement in critical illness (Fig. 1c).^{2,3} Other markers of angiogenesis and endothelial dysfunction also differed between the groups (Fig. 1d). Third, markers of endotheliopathy—angiotensin-2, follistatin, and PAI-1—were elevated in ICU patients who died compared to those who survived (Fig. 1d). Kaplan-Meier survival analyses using unbiased cutpoints determined by maximally selected rank statistics⁴ revealed that these markers of endotheliopathy segregated significantly with in-hospital mortality (Fig. 1e). Moreover, we generated receiver operating characteristic curves for each marker's ability to predict mortality, and found that only four factors (angiotensin-2, follistatin, PAI-1, and FLT-3L), all of which can be considered as markers of endotheliopathy,

had areas under the curves greater than 0.7, suggesting that they may be important predictors of death in patients with COVID-19 (Fig. 1f).

Overall, we have identified circulating vascular markers that track with disease severity. Markers of angiogenesis increased in all hospitalized patients with COVID-19, which may reflect the presence of hypoxemia and inflammation. Markers of endotheliopathy rose particularly in critically ill patients and those who ultimately died from the illness. Endothelial dysfunction and injury may drive key features of critical illness, including thrombosis and multi-organ failure (Fig. 1g). Moreover, recent findings of pulmonary angiopathy in patients with severe COVID-19 highlight the likely involvement of endotheliopathy contributing to the pulmonary perfusion defects, coagulopathy, and thrombosis.⁵

Limitations of our study include the fact that: (1) controls, who were mostly healthcare workers, were on average younger and without comorbidities, and (2) some of the changes in biomarkers may be due to non-vascular causes, such as metabolic disease. Future comparisons of these and other biomarkers, including with non-COVID-19 hospitalized cohorts, would advance our findings.

While other biomarkers, including D-dimer, troponin, and B-type natriuretic peptide, have been associated with mortality in COVID-19,⁶ there is currently a paucity of vascular-specific biomarkers that can help identify vascular pathology and provide prognostic value. Given the emerging evidence for endothelial and vascular involvement in COVID-19, development of tests to detect vascular injury may be critical to guide the use of novel therapeutic strategies, including those that protect the endothelium. Further mechanistic studies to understand the causes of endothelial injury, vascular dysfunction, and thrombosis promise to provide pivotal insights into COVID-19 pathogenesis and guide clinical management.

Author contributions

ABP, MLM, GG, AIL, and HJC designed the studies, data analyses, and wrote the manuscript. CC, HZ, PB, AP, RG, and JMK collected and processed the samples. JB and DvD carried out the computational analyses. CHW, CP, CSD, SH, and JH contributed to data analysis and data interpretation.

Conflict of interest

The author(s) declare no conflicts of interest.

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Ethical approval

This work is approved by Yale University IRB #s (IRB#2000027792 and IRB#1401013259).

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