Circulating Levels of Tissue Plasminogen Activator and Plasminogen Activator Inhibitor-1 Are Independent Predictors of Coronavirus Disease 2019 Severity: A Prospective, Observational Study

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A substantial pool of evidence suggests that the pathophysiology of the severe form of coronavirus disease 2019 (COVID-19) is mediated, in part, by a hypercoagulable state termed COVID-19-associated coagulopathy (CAC),[1–3] and characterized by micro- and macrovascular thrombosis.[4,5] Approximately 27% of critically ill intensive care unit (ICU)-admitted COVID-19 patients develop venous thromboembolism during admission,[6] while 4.4% experience arterial thrombotic complications.[7] Autopsy studies on COVID-19 patients have demonstrated extensive presence of microthrombi within pulmonary capillaries, associated with diffuse alveolar damage and neoangiogenesis.[8,9] Other organs may also be affected by this form of thrombotic microangiopathy, resulting in multiple organ dysfunction syndrome and eventually death.[10] Laboratory profiles seen in patients with severe COVID-19 (elevated D-dimers and fibrin degradation products [FDPs], and prolonged prothrombin time) are also consistent with a hypercoagulable state.

The role of the fibrinolytic system in CAC is not yet well established. It has been hypothesized that patients with severe COVID-19 have elevated levels of antifibrinolytic proteins, which create a hypofibrinolytic state, with subsequent failure of clot lysis. This hypothesis is supported by a recent thromboelastographic study by Wright and colleagues where a complete lack of lysis of clot at 30 minutes was reported in approximately 57% of ICU-admitted COVID-19 patients.[11] Henry and colleagues recently demonstrated that ICU-admitted COVID-19 patients had lower levels of circulating plasminogen than non-ICU patients, suggesting an exhaustion of fibrinolysis.[12] Recent small clinical studies have also demonstrated potential therapeutic effects of supplementing the fibrinolytic system, where exogenous administration of tissue plasminogen activator (tPA) resulted in improvement of respiratory function and reduced mortality in critically ill COVID-19 patients with acute respiratory distress syndrome (ARDS).[13,14]

Herein, we performed a prospective observational study to determine whether circulating plasma levels of plasminogen activator inhibitor-1 (PAI-1) and tPA may be significantly

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elevated in patients with severe COVID-19. We additionally determined the diagnostic performance of baseline PAI-1 and tPA for predicting progression to severe disease.

Adults (>18 years old) presenting to the University of Cincinnati Medical Center Emergency Department (ED) with symptoms of COVID-19 with a positive reverse transcription-polymerase chain reaction test for COVID-19 on nasopharyngeal swab were included. This study was approved by the Institutional Review Board of the University of Cincinnati. Blood samples were collected via routine draws for clinical indications in the ED. Circulating levels of tPA and PAI-1 were determined using enzyme-linked immunosorbent assay (Technozym, Diapharma, West Chester, OH). The clinical course of these patients was then monitored for 30 days. The severity of COVID-19 at the time of presentation to the ED was the primary outcome of this study, while peak severity of COVID-19 within 30 days of index visit to the ED or hospital discharge was the secondary outcome. Severe disease was defined as a composite of (1) partial pressure of oxygen in arterial blood/fraction of inspired oxygen ≤ 300 mm Hg or (2) patients requiring noninvasive ventilation using high flow nasal devices/mechanical ventilation/vital life support/ICU admission, or admission to the ICU.

Analysis of the data was performed using Prism 7 (Graph-Pad Software, San Diego, CA) and SPSS (IBM Statistics Software Version 25, Armonk, NY). Categorical data were reported as frequencies (%) while continuous data were reported as median and interquartile range (IQR). Comparison of baseline tPA and PAI-1 levels between patients with and without severe COVID-19 at the time of index ED visit was performed using the Mann–Whitney U-test. The diagnostic performance of baseline tPA and PAI-1 for predicting peak 30-day severity of COVID-19 was assessed using receiver operating characteristic (ROC) curve, with calculation of the area under the curve (AUC) and its 95% confidence interval (95% CI). Logistic regression was performed to estimate the effect of elevated tPA and PAI-1 (≥ 4 and >52.3 ng/mL respectively, based on ROC curve analysis) on the secondary outcome of peak 30-day severity of COVID-19, adjusting for age, sex, and comorbidities, and to calculate adjusted odds ratios (ORs) with the corresponding 95% Wald CI. Additionally, correlation between circulating levels of tPA/PAI-1 and inflammatory biomarkers (interleukin [IL]-6, IL-10, tumor necrosis factor α [TNFα], ferritin and C-reactive protein [CRP]) as well as platelet counts was performed using Spearman's correlation.

A total of 52 patients were included in this study. Of these, 46 had nonsevere disease at the time of index ED presentation, while six had severe disease. The median age in the severe group was 57 (IQR: 47–67) versus 50.5 (IQR: 37.8–66) years in the nonsevere group ($p = 0.445$). Males made up 83% of the severe group and 54.3% of the nonsevere group ($p = 0.181$). The comorbidities of these patients are presented in ►**Fig. 1A**. Patients with severe disease had significantly higher levels of PAI-1 than those with the nonsevere disease (115.6 [IQR: 49.5–357] vs. 47.6 [IQR: 27.9–88.3] ng/mL; $p = 0.045$) (►**Fig. 1B**). Circulating tPA levels were also elevated in patients with severe disease compared with those with nonsevere disease, although this did not achieve statistical significance
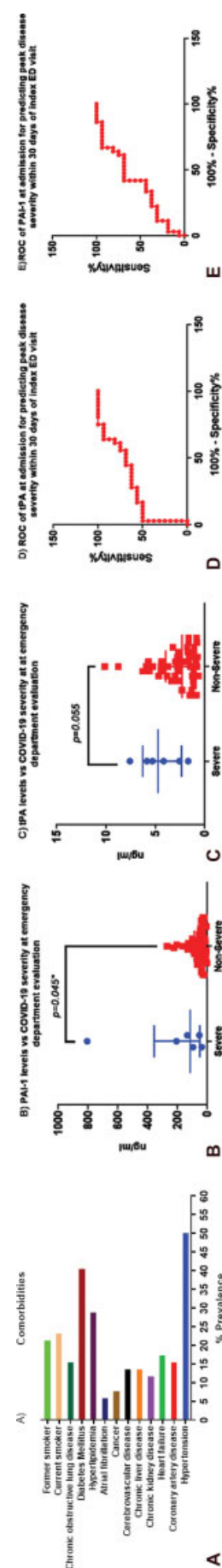
Fig. 1 Comorbidities in the included COVID-19 patients (A). Plasminogen activator inhibitor 1 (PAI-1) plasma levels were significantly higher in severe than in the nonsevere group (B). No significant differences were noted in the levels of plasma tissue plasminogen activator (tPA) between severe and nonsevere patients (C). Receiver operating characteristic (ROC) curves of tPA and PAI-1 at emergency department evaluation for predicting peak disease severity within 30 days of index visit to the emergency department (D, E).

Table 1 Age-, sex-, and comorbidity-adjusted multivariable logistic regression for tPA and PAI-1 as independent predictors of severe COVID-19 within 30 days

Variable	Estimate	Standard error	Odds ratio (95% CI)	p-Value
Elevated tPA	1.897	0.788	6.67 (1.42–31.21)	0.016[a]
Elevated PAI-1	2.053	0.897	7.79 (1.34–45.21)	0.022[a]

Abbreviations: CI, confidence interval; PAI-1, plasminogen activator inhibitor 1; tPA, tissue plasminogen.
[a]Significant at the value of 0.05.

(4.7 [IQR: 2.4–6.3] vs. 2.3 [IQR: 1.2–4.0] ng/mL; $p = 0.055$) (►**Fig. 1C**). A total of 16 patients developed severe disease within 30 days of index ED presentation. ROC curves were generated for tPA and PAI-1 at the time of initial ED evaluation for predicting peak disease severity within 30 days of index ED visit. The AUC of tPA was 0.73 (95% CI: 0.58–0.89; $p = 0.008$). Analysis of ROC data determined that a tPA cut-off of ≥ 4 ng/mL was associated with 0.56 sensitivity and 0.83 specificity, respectively (►**Fig. 1D**). The AUC for PAI-1 was 0.63 (95% CI: 0.47–0.79; $p = 0.142$), with ≥ 52.3 ng/mL cut-off displaying 0.69 sensitivity and 0.58 specificity (►**Fig. 1E**). Multivariable logistic regression (adjusted for age, sex, and comorbidities) for tPA and PAI-1 levels above the ROC cut-offs (i.e., ≥ 4 and ≥ 52.3 ng/mL, respectively) as independent predictors of peak 30-day severity revealed that elevated tPA and PAI-1 levels were associated with increased odds of developing severe disease (OR = 6.67, 95% CI: 1.42–31.21, $p = 0.016$ and OR = 7.79, 95% CI: 1.34–45.21, $p = 0.022$, respectively) (►**Table 1**). Circulating tPA positively correlated with IL-10 ($r = 0.431$, 95% CI: 0.168–0.637; $p = 0.002$), TNFα ($r = 0.424$, 95% CI: 0.159–0.631; $p = 0.002$), serum ferritin ($r = 0.471$, 95% CI: 0.219–0.664; $p < 0.001$), and lactate dehydrogenase ($r = 0.443$, 95% CI: 0.183–0.641; $p < 0.001$), while PAI-1 positively correlated with IL-10 ($r = 0.318$, 95% CI: 0.038–0.551; $p = 0.023$), TNFα ($r = 0.310$, 95% CI: 0.029–0.546; $p = 0.027$), and platelet counts ($r = 0.347$, 95% CI: 0.061–0.058; $p = 0.016$) (►**Table 2**).

Taken together, these findings suggest that the fibrinolytic system plays a significant role in the pathophysiology of COVID-19. In normal lung physiology, the pulmonary alveolar space (PAS) is thought to have a profibrinolytic milieu.[15] Previous studies demonstrated that suppression of the fibrinolytic system is a key feature of non-COVID-19 ARDS.[16,17] PAI-1 may be produced by alveolar macrophages, with elevated levels during lung injury and inflammation.[18] Moreover, the observed positive correlation between PAI-1 levels and platelet counts suggests that platelets are a substantial source of PAI-1 in these patients. Finally, genetic[19] and metabolic factors (increased abdominal adipose tissue)[20] may also influence increased production of PAI-1.

The findings of elevated PAI-1 levels in patients with severe COVID-19 as seen in the current and one other study,[21] combined with recent thromboelastographic investigations,

Table 2 Correlation between tPA/PAI-1 and various inflammatory markers and platelet counts

	IL-6	IL-10	TNFα	Ferritin	LDH	CRP	Platelet count
tPA	r = 0.277 (95% CI: − 0.01–0.522); p = 0.051	r = 0.431 (95% CI: 0.168–0.637); p = 0.002[a]	r = 0.424 (95% CI: 0.159–0.631); p = 0.002[a]	r = 0.471 (95% CI: 0.219–0.664); p < 0.001[a]	r = 0.443 (95% CI: 0.183–0.641); p < 0.001[a]	r = 0.105 (95% CI: −0.185–0.376); p = 0.464	r = −0.151 (95% CI: −0.424–0.148); p = 0.307
PAI-1	r = 0.271 (95% CI − 0.165–0.517); p = 0.057	r = 0.318 (95% CI: 0.038–0.551); p = 0.023[a]	r = 0.310 (95% CI: 0.029–0.546); p = 0.027[a]	r = 0.164 (95% CI: − 0.123–0.425); p = 0.246	r = 0.124 (95% CI: −0.162–0.391); p = 0.381	r = 0.0127 (95% CI: −0.162–0.396); p = 0.375	r = 0.347 (95% CI: 0.061–0.058); p = 0.016[a]

Abbreviations: CRP, C-reactive protein; IL-10, interleukin 10; IL-6, interleukin 6; LDH, lactate dehydrogenase; r, Spearman's rho; TNFα, tumor necrosis factor α.
[a]Significant at significance level of 0.05.

suggest dysregulation of the fibrinolytic system and a phenomenon of "fibrinolytic shutdown" or "temporary exhaustion" in COVID-19 patients.[11,21] These phenomena would result in reduced clot lysis and persistence of thrombi within the capillary network. The presence of "fibrinolytic shutdown" seems to be inconsistent with the observations of elevated D-dimer levels in patients with severe COVID-19,[22] since formation of D-dimers and FDP generation would need an intact and efficient fibrinolytic system. This discrepancy can, however, be attributed to temporal changes in levels of tPA and PAI-1, as well as to insufficient hyperfibrinolysis characterized by systemic suppression and local (i.e., within the pulmonary tissue) hyperactive fibrinolysis derangement, as recently pinpointed by Ji et al.[23] This is in concordance with recent observations by Henry et al where COVID-19 patients progressing to severe disease had lower levels of plasminogen compared with discharged patients.[12] This suggests that the high D-dimer levels observed in patients with severe COVID-19 may come from an initial rise in tPA/plasmin. This hypothesis is further supported by observations by Wright and colleagues where patients who met the criteria of hypofibrinolysis based on thromboelastographic findings had a marked decline in D-dimer levels after an initial rise.[11] Our findings also suggest that elevated tPA and PAI-1 are associated with poor outcomes among COVID-19 patients, consistent with previous reports.

In their preclinical study on nonhuman primates, Biemond and colleagues[24] demonstrated that a bolus injection of bacterial endotoxin resulted in rapid increase in tPA and plasmin levels within the first 2 hours. PAI-1 levels remained constant during this period, after which a rapid increase was observed with simultaneous decrease in tPA and plasmin activities. The authors noted that the fibrinolytic system was suppressed for the remainder of the study due to high PAI-1 levels.[24] Thus, we suspect in COVID-19 that the initial rise in tPA is driven by inflammation, after which remnant tPA is complexed to PAI-1 and rendered inactive, while any subsequent tPA released is insufficient to overcome the increase in circulating PAI-1.

Since upregulation of PAI-1 levels is a common feature of thrombotic microangiopathies (TMAs) such as primary and secondary thrombotic thrombocytopenic purpura and hemolytic uremic syndrome,[25–27] the observations of the current study support the presence of a TMA phenomenon in COVID-19. This is further supported by the positive correlation observed between PAI-1 and proinflammatory cytokines such as TNFα, which are important mediators of endothelial damage, a hallmark of TMA. When combined with previous observations of reduced ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) in COVID-19, a picture strongly suggestive of TMA-like phenomenon emerges.[28]

Our study was limited by the small sample size. In the analysis of PAI-1, we noted an outlier among the six severe cases that could have biased the statistical finding. This was however delimited by ROC and regression analysis for 30-day peak COVID-19 severity in which there were more patients in the severe group (16 patients) with no obvious outliers. We measured circulating plasma tPA and PAI-1, which may not

be reflective of the actual levels within the PAS. Further, we did not perform serial measurements of PAI-1/tPA. Future studies should focus not only on circulating but also on local (pulmonary) levels of tPA/PAI-1, as well as their temporal changes along the clinical course of COVID-19 patients to provide further clarification of changes in the fibrinolytic system in COVID-19 patients.

In conclusion, patients with severe COVID-19 have significantly elevated plasma levels of PAI-1. Elevated levels of tPA and PAI-1 are independent predictors of progression to severe COVID-19. The evidence provided suggests that fibrinolytics should continue to be investigated as therapeutic avenue in patients with COVID-19.

Conflict of Interest
None declared.

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