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Suppression of Fibrinolysis and Hypercoagulability, Severity of Hypoxemia, and Mortality in COVID-19 Patients: A Retrospective Cohort Study

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EDITOR'S PERSPECTIVE

What We Already Know about This Topic

- The host defense response to COVID-19 is thromboinflammation, a pathophysiologic response that produces hypercoagulability to minimize acute infectious injury
- However, in patients without previous immunity and/or vaccination, thrombosis, acute lung injury, and multiorgan injury can progress, necessitating intensive care unit–level care and potentially the need for extracorporeal membrane oxygenation

What This Article Tells Us That Is New

- Based on measured biomarkers including increased plasminogen activator inhibitor 1 levels and viscoelastic testing, fibrinolytic suppression was associated with severe hypoxemia, worsening respiratory failure, and thrombotic events
- These findings further support the role of fibrinolytic shutdown as a major pathophysiologic cause of microcirculatory thrombosis and worse outcomes in severe COVID-19

ABSTRACT

Background: COVID-19 causes hypercoagulability, but the association between coagulopathy and hypoxemia in critically ill patients has not been thoroughly explored. This study hypothesized that severity of coagulopathy would be associated with acute respiratory distress syndrome severity, major thrombotic events, and mortality in patients requiring intensive care unit–level care.

Methods: Viscoelastic testing by rotational thromboelastometry and coagulation factor biomarker analyses were performed in this prospective observational cohort study of critically ill COVID-19 patients from April 2020 to October 2020. Statistical analyses were performed to identify significant coagulopathic biomarkers such as fibrinolysis-inhibiting plasminogen activator inhibitor 1 and their associations with clinical outcomes such as mortality, extracorporeal membrane oxygenation requirement, occurrence of major thrombotic events, and severity of hypoxemia (arterial partial pressure of oxygen/fraction of inspired oxygen categorized into mild, moderate, and severe per the Berlin criteria).

Results: In total, 53 of 55 (96%) of the cohort required mechanical ventilation and 9 of 55 (16%) required extracorporeal membrane oxygenation. Extracorporeal membrane oxygenation–naïve patients demonstrated lysis indices at 30 min indicative of fibrinolytic suppression on rotational thromboelastometry. Survivors demonstrated fewer procoagulate acute phase reactants, such as microparticle-bound tissue factor levels (odds ratio, 0.14 [0.02, 0.99]; $P = 0.049$). Those who did not experience significant bleeding events had smaller changes in ADAMTS13 levels compared to those who did (odds ratio, 0.05 [0, 0.7]; $P = 0.026$). Elevations in plasminogen activator inhibitor 1 (odds ratio, 1.95 [1.21, 3.14]; $P = 0.006$), D-dimer (odds ratio, 3.52 [0.99, 12.48]; $P = 0.05$), and factor VIII (no clot, 1.15 ± 0.28 *vs.* clot, 1.42 ± 0.31; $P = 0.003$) were also demonstrated in extracorporeal membrane oxygenation–naïve patients who experienced major thrombotic events. Plasminogen activator inhibitor 1 levels were significantly elevated during periods of severe compared to mild and moderate acute respiratory distress syndrome (severe, 44.2 ± 14.9 ng/ml *vs.* mild, 31.8 ± 14.7 ng/ml and moderate, 33.1 ± 15.9 ng/ml; $P = 0.029$ and 0.039, respectively).

Conclusions: Increased inflammatory and procoagulant markers such as plasminogen activator inhibitor 1, microparticle-bound tissue factor, and von Willebrand factor levels are associated with severe hypoxemia and major thrombotic events, implicating fibrinolytic suppression in the microcirculatory system and subsequent micro- and macrovascular thrombosis in severe COVID-19.

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The coagulopathy engendered by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is marked by thrombotic events, most notably in critically ill patients requiring life support measures in the intensive care unit (ICU).[1,2] In patients requiring veno-venous extracorporeal membrane oxygenation (ECMO) support for respiratory distress, coronavirus disease 2019 (COVID-19)–associated

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coagulopathy has the potential to further increase the elevated risk of hemostatic complications associated with ECMO.[3,4] Beyond the macrothrombotic events like stroke (cerebrovascular accident), pulmonary embolism, and deep vein thrombosis, postmortem studies also report the presence of microthrombi in the pulmonary vasculature of COVID-19 patients.[5–8] Endothelial cell damage through direct viral infection and local inflammatory response at the alveolar–capillary interface is theorized to contribute to this pathology.[9,10] Inflamed and injured endothelium releases procoagulant factors, such as von Willebrand factor (vWF), and antifibrinolytic factors, such as plasminogen activator inhibitor 1, which may contribute locally and systemically to COVID-19–associated coagulopathy.

Coagulation biomarkers, including elevated vWF, fibrinogen, and D-dimer, were shown early in the pandemic to predict worse outcomes and could distinguish disease severity and inform anticoagulation intensity.[11–13] More recently, plasminogen activator inhibitor 1 elevation was reported in patients requiring supplemental oxygen compared to those on room air, as well as in patients who died compared to those who survived.[14,15] Despite these reports, the association between coagulopathy and respiratory distress, the key driver of mortality in critically ill patients with COVID-19, remains to be determined. To further characterize the association between coagulopathy and hypoxemia in SARS-CoV-2 infection, we performed a prospective observational cohort study of 55 patients admitted to a quaternary care hospital's ICU for COVID-19 management. We hypothesized that the severity of coagulopathy would be associated with worse clinical outcomes including acute respiratory distress syndrome (ARDS) severity, major thrombotic events, and mortality in patients requiring ICU-level care.

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Materials and Methods

Patient Population

From April 2020 to October 2020, 55 SARS-CoV-2 positive patients requiring ICU-level care were screened and enrolled in the study. Eligible patients were men and women ages 18 yr old and above who were admitted to an adult ICU at a quaternary hospital center with SARS-CoV-2 infection confirmed by polymerase chain reaction testing. Eighty-nine percent of the cohort was intubated, requiring ventilation, and the remainder either required vasopressor support or high-flow oxygen support. Written informed consent was obtained from the patient or their legally authorized representative if they lacked decision-making capacity. Patients were ineligible for enrollment if they were enrolled in a blood conservation program or were incarcerated. Citrated whole blood was collected on days 1, 3, 7, 14, and 21 after enrollment or until discharge from ICU or death. The average time from entry to ICU to day 1 of study was 6.9 (SD, 5.3) days. These samples were centrifuged and stored at –80°C for analysis. All patients received prophylactic low-molecular-weight heparin (0.5 mg/kg twice daily) or unfractionated heparin (5,000 IU every 8 h) dosing for venous thromboembolic prophylaxis excluding ECMO patients who received full dose titrated unfractionated heparin to a partial thromboplastin time goal of 50 to 60 s. The study was approved by the academic center's institutional review board and is reported as per the Strengthening the Reporting of Observational Studies in Epidemiology guidelines (Supplemental Digital Content 1 table S1, http://links.lww.com/ALN/C849).

Clinical Data Extraction

The clinical data were extracted from the electronic medical record. Vital signs; laboratory data; codes for comorbidity analysis from the International Statistical Classification of Diseases, Tenth Revision; and demographic data were extracted from the Oracle electronic medical record database across the cohort's ICU encounters using SQL queries.

Association of ARDS Severity with Laboratory Values

The ratio of arterial partial pressure of oxygen (P_{AO_2}) to fraction of inspired oxygen (F_{IO_2}) was calculated from ventilator and clinical laboratory data at each episode of blood collection for each patient. P_{AO_2}/F_{IO_2} thresholds of 300 to 200, 199 to 100, and less than 100 were classified as mild, moderate, and severe ARDS, respectively, per the Berlin criteria.[16] In addition, stratification of disease severity based on a patient's lowest P_{AO_2}/F_{IO_2} during their ICU course was completed,

and an outcomes analysis was performed. All patients requiring ECMO were classified as having severe ARDS as the Pao_2/Fio_2 ratios for ECMO patients fail to take into account the additional oxygenation provided by the ECMO circuit itself, resulting in a Pao_2/Fio_2 ratio that does not reflect the severity of the patient's respiratory distress. All laboratory values were associated with the patient's Pao_2/Fio_2 severity level at the same date and time of blood collection.

Enzyme-linked Immunosorbent Assays and Activity Assays

The following activity assays and enzyme-linked immunosorbent assays (ELISAs) were performed per the manufacturers' instructions using citrated plasma at dilutions indicated for each assay: Factor VIII Chromogenix Coamatic activity assay (K822585, Diapharma, USA), 1:800 dilution; microparticle-bound tissue factor (Zymuphen 521196), undiluted plasma; A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13; ab234559, Abcam, United Kingdom), 1:1,000; vWF (ab223864, Abcam), 1:8,000; Factor IX (ab188393, Abcam), 1:1,000; tissue factor pathway inhibitor (ab274392, Abcam), 1:100; and plasminogen activator inhibitor 1 (ab269373, Abcam), 1:20. All plates were read on a 96-well plate reader (Spectral Max i3; Molecular Devices, USA).

Population Means and Assay Validation with Healthy Controls

Healthy population means are depicted by dotted lines in figures 1 to 3 and were determined from clinical laboratory normal ranges for D-dimer and fibrinogen and from previous reports for vWF,[17] ADAMTS13,[18] tissue factor pathway inhibitor,[19] and plasminogen activator inhibitor 1.[20] Healthy mean values for factor VIII activity were determined by assessment of plasma from eight healthy young adult volunteers. This was in part due to a lack of consistent reported health population means. Validation of ELISA data from COVID-19 patients was performed *via* within-assay comparison to healthy plasma from donors matched for age, race, ethnicity, and body mass index of COVID-19 patients purchased from Innovative Research (USA).

Rotational Thromboelastometry

All assays were performed on a clinical rotational thromboelastometry (ROTEM) machine (ROTEM *delta*; Tem Innovations, Germany) according to the manufacturer's instructions including fibrin-based extrinsically activated test with tissue factor and the platelet inhibitor cytochalasin D (FIBTEM), extrinsically activated test with tissue factor (EXTEM), intrinsically activated test using ellagic acid (INTEM), and INTEM assay performed in the presence of heparinase (HEPTEM). EXTEM and FIBTEM were performed for all patients, and either INTEM or HEPTEM was performed depending on patient's heparization status, with HEPTEM was performed if the patient was therapeutically heparinized. The ROTEM machine was calibrated daily, and reagents were calibrated weekly.

Data Visualization and Statistical Analysis

There were no missing data within the study period, which included the total duration of the ICU stay, after enrollment in the study. Before accessing the data, a statistical analysis plan was formulated. The clinical data were analyzed using R code (R Foundation for Statistical Computing, Austria). Assuming an event rate of 25%, power analysis demonstrated that with an α of 0.05 and 80% power, a sample size of 52 would be sufficient to detect a 50% difference in biomarker levels between survivors and nonsurvivors. Primary outcomes included mortality, major thrombotic events (deep vein thrombosis, pulmonary embolism, cerebrovascular accident), and ARDS severity per the Berlin criteria mild (Pao_2/Fio_2 200 to 300), moderate (Pao_2/Fio_2 100 to 199), and severe (Pao_2/Fio_2 less than 100).[16] The lowest Pao_2/Fio_2 ratio during a patient's hospitalization was used to determine the severity of their ARDS. This excluded patients on ECMO. Explanatory variables included coagulopathy biomarkers and thromboelastometric measures. Laboratory values were measured multiple times each day during the ICU hospitalization. A maximum, minimum, or average of these measurements was used to represent the worst effect the variable had on clinical outcome in each ICU. Furthermore, Wilcoxcon rank sum tests were used to associate each $\log_2$-transformed biomarker on ICU day 1, study day 1, or ICU day before a clinical event of interest with each outcome. A P value less than 0.05 was regarded as statistically significant. Logistic regression models were used to assess the association between each biomarker and each clinical outcome. Two-way analysis of variance (ANOVA) and mean $\pm$ SD of laboratory values were analyzed with GraphPad Prism 9 software for ECMO *versus* non-ECMO analysis. Only laboratory data and biomarkers from the first day of the ICU stay were used to avoid repeated measures in the analysis of the ECMO data. Potential confounding variables including age, race, and comorbidities were assessed *via* stratification analysis and reported in tables for each outcome measure. Homogeneity of variance for each continuous measure was assessed *via* residual plot, and normality of distribution was assessed *via* Q-Q plot. Dotted lines are used to indicate the mean of normal clinical values, and gray is shading used to indicate the range of normal clinical values where applicable.

Results

Clinical Characteristics

The median age was 57 yr (SD 6), and 14 of 55 (25%) of the cohort were female (table 1). Thirty percent of the cohort identified as Black, and 14 of 55 (25%) identified as Latino. The most common comorbidities were obesity (31 of 55

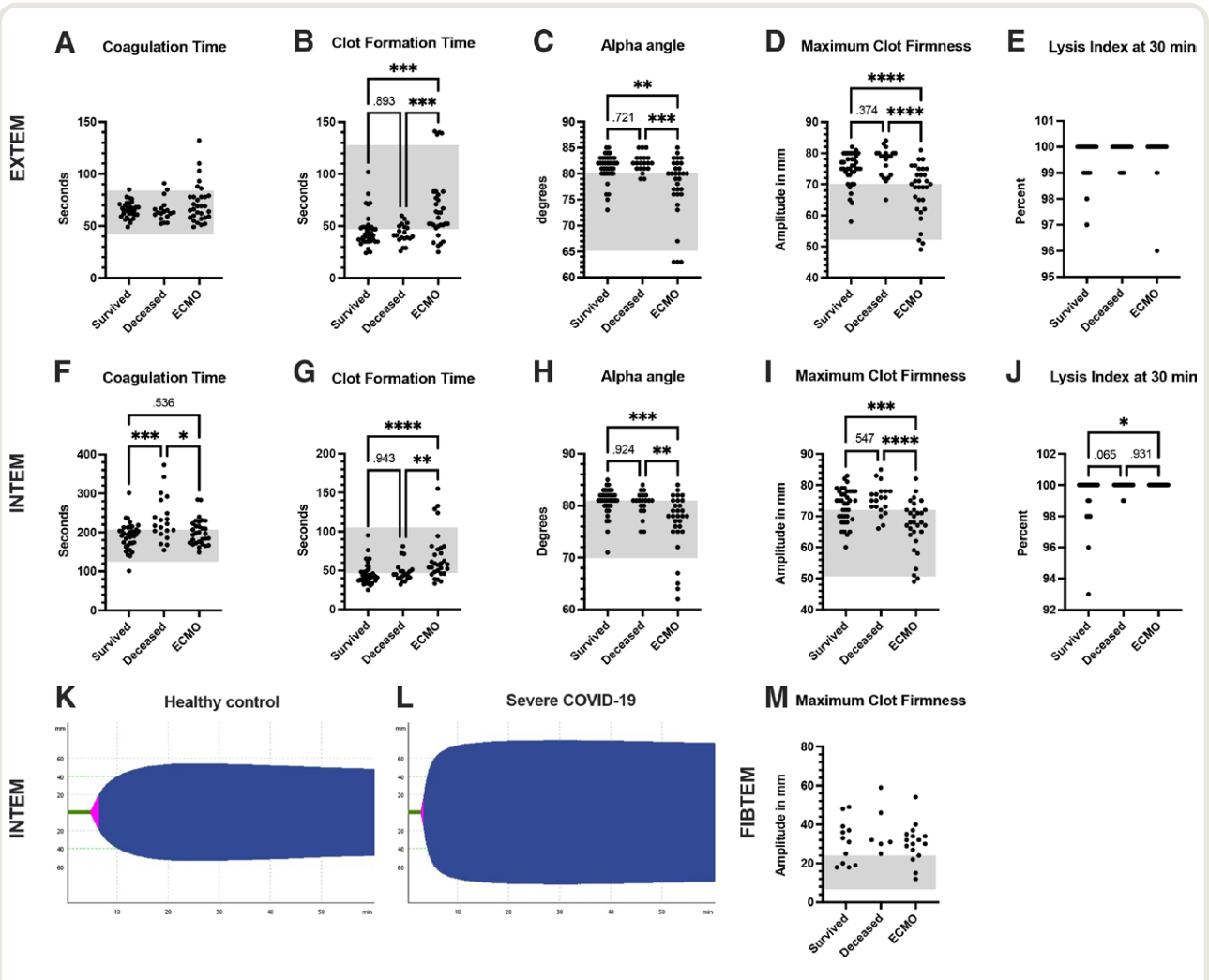

Fig. 1. Patients with severe COVID-19 demonstrate procoagulant profile on rotational thromboelastometry (ROTEM), marked by shortened clot formation time (*B*, *G*), increased α angle (*C*, *H*), and reduced lysis at 30 min after clotting time (*E*, *J*) on extrinsically activated test with tissue factor (EXTEM) and intrinsically activated test using ellagic acid (INTEM) and elevated maximum clot firmness on EXTEM, INTEM, and fibrin-based extrinsically activated test with tissue factor and the platelet inhibitor cytochalasin D (FIBTEM; *D*, *I*, *M*). Extracorporeal membrane oxygenation (ECMO) alters coagulative phenotype of COVID-19 patients, increasing average clot formation time (*B*, *G*) and lowering α angle (*C*, *H*) and maximum clot firmness (*D*, *I*) into the normal range. Extrinsic coagulation time was normal (*A*), although drifting was longer in INTEM due to anticoagulation with low-molecular-weight heparin (*F*). *Gray shading* indicates the range of normal clinical values. Each *dot* represents a single patient time point. Significant one-way ANOVAs were followed by Tukey's *post hoc* comparisons shown in figures. $P < 0.05$ was considered significant. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. Nonsignificant *P* values are written out. The values are plotted as means ± SD. Representative INTEM tracings from a healthy donor (*K*) and an intensive care unit patient (*L*) are included for reference, with the *green line* representing coagulation time, the *pink triangle* representing clot formation time, the angle of incidence of the clot formation time as the α angle, the thickest aspect of the tracing as the maximum clot firmness, and the residual clot firmness amplitude in percentage of maximum clot firmness at 30 min after clotting time as the lysis index at 30 min.

[55%]), hypertension (23 of 55 [41%]), type 2 diabetes mellitus (20 of 55 [36%]), and cardiovascular disease (11 of 55 [20%]). The average time from the first polymerase chain reaction positive test to day 1 of study was 8 days. Average time from entry to ICU to day 1 of study was 3 days. Of the cohort, 53 of 55 (96%) of the cohort required mechanical ventilation, and 9 of 55 (16%) required ECMO. A total of 13 of 55 (23%) died during the index hospitalization, 27 of 55 (48%) of patients developed ventilator associated pneumonia, 10 of 55 (17.8%) of patients required continuous renal replacement therapy, and 3 of 55 (5.3%) of patients developed heart failure due to myocarditis. Mean duration of mechanical ventilation was 21 days; 9 of 55 (16%) of patients developed major hemorrhage, most of whom were on ECMO support; and 14 (25%) of patients developed major thrombosis defined as deep vein thrombosis in upper and/or lower extremities, pulmonary embolism, and/or stroke (Supplemental Digital Content 2 table S2, http://links.lww.

Table 1. General Clinical Summary of Patients Including Comorbidities

Characteristics	Total Population (n = 55)	Survivors (n = 35)	Nonsurvivors (n = 11)	ECMO (n = 9)
Age, mean	57	55	63	51
Sex, female (%)	14 (25)	10 (28)	2 (18)	2 (22)
Race/ethnicity, n (%)				
Black	21 (38)	15 (42)	4 (36)	1 (11)
White	17 (30)	12 (33)	3 (27)	3 (27)
Latino	14 (25)	7 (19)	4 (36)	4 (44)
Other/unknown	4 (16)	2 (5)	0	1 (11)
Comorbidities, n (%)				
Any condition	50 (89)	32 (89)	10 (91)	7 (78)
Body mass index > 30	31 (55)	22 (61)	3 (27)	6 (67)
Hypertension	23 (41)	16 (44)	6 (55)	5 (56)
Diabetes	20 (36)	14 (39)	4 (36)	2 (22)
Cardiovascular disease	11 (20)	7 (19)	4 (36)	0
Chronic lung disease	10 (18)	6 (17)	4 (36)	0
Renal disease	8 (14)	3 (8)	4 (36)	0
Cancer	8 (14)	3 (8)	3 (27)	1 (11)
Previous stroke	6 (11)	5 (14)	1 (9)	0
COVID-19 treatments, n (%)				
Remdesivir	45 (80)	29 (81)	8 (73)	90 (100)
Dexamethasone	29 (52)	19 (53)	6 (55)	5 (56)
Convalescent plasma	12 (21)	7 (19)	2 (18)	3 (33)

ECMO, extracorporeal membrane oxygenation.

com/ALN/C850). Analysis of clinical characteristics and comorbidities between survivors, nonsurvivors, and ECMO patients demonstrated that patients on ECMO were on average younger with fewer comorbidities, which correlates with criteria for clinical ECMO eligibility. The nonsurvivor group had less obesity, as well as less cardiovascular disease and chronic lung disease compared to the survivor group in contrast to previous studies.[21] The rates of diabetes and renal disease were similar across survivor and nonsurvivor groups but decreased in ECMO patients. Nonsurvivors and ECMO patients had more hypertension compared to survivors.

Procoagulant Profile of Severe COVID-19

ECMO-naive COVID-19 patients requiring ICU care had hypercoagulability on viscoelastic testing with ROTEM compared to the normal reference range (Supplemental Digital Content 2 table S2, http://links.lww.com/ALN/C850; fig. 1) characterized by shortened clot formation time and increased α angle on EXTEM and INTEM, indicative of increased activity of both clotting factors and platelets (fig. 1, B, C, G, and H).[22,23] Our cohort also demonstrated elevated maximum clot firmness on FIBTEM, EXTEM, and INTEM, aligning with the hyperfibrinogenemic state of severe COVID-19 (fig. 1, D, I, and M). Furthermore, lysis indices at 30 min of 100% despite elevated D-dimer levels are consistent with fibrinolysis inhibition (fig. 1, E and J). The median lysis index at 45 min was 100%, and the mean was 99%, providing further evidence for fibrinolytic suppression.

There was no difference in ROTEM tracings of ECMO-naive survivors *versus* nonsurvivors in this cohort (Supplemental Digital Content 2 table S2, http://links.lww.com/ALN/C850). In contrast, COVID-19 patients placed on ECMO support did not consistently manifest the typical procoagulant phenotypes of severe COVID-19 as assessed by ROTEM analyses, with significantly increased clot formation time and reduced α angle and maximum clot firmness compared to ECMO naïve patients (Supplemental Digital Content 2 table S2, http://links.lww.com/ALN/C850; fig. 1). When measured over time, some patients placed on ECMO lost the procoagulant pattern characteristic of this ROTEM tracing but regained the phenotype after decannulation (Supplemental Digital Content 3 fig. S1, http://links.lww.com/ALN/C851). Others were shown to become more procoagulable over time. These observations demonstrate variability in coagulation changes in patients on ECMO. The loss of procoagulant phenotype seen in some ECMO persisted even after discontinuation of heparin for hemorrhage. Platelet counts were analyzed over time, and there was no significant change in platelet count corresponding to changes in maximum clot firmness in ROTEM. Given these findings, we separated ECMO-naïve patients from the nine ECMO-requiring patients for analysis.

Survivors Have Less Severe Procoagulant Profile Compared to Nonsurvivors

Patients who survived their hospitalization were less likely to have higher levels of procoagulant acute phase reactants

including microparticle-bound tissue factor (odds ratio, 0.14 [0.02, 0.99]; $P = 0.049$). Survivors were also noted to have lower vWF levels compared to nonsurvivors, although this result did not meet significance in a $\log_2$-transformed analysis with Wilcoxon rank sum test (survivors, 5.4 ± 0.04 *vs.* nonsurvivors, 6.2 ± 0.4; $P = 0.063$). Furthermore, patients who did not experience major bleeding events had significantly smaller changes in ADAMTS13 levels, an enzyme that shortens large procoagulant vWF multimers, compared to patients who did not have major bleeding events during their hospitalization (odds ratio, 0.05 [0.7]; $P = 0.026$).

Elevations in Plasminogen Activator Inhibitor 1, vWF, D-Dimer, and Factor VIII Are Associated with Major Thrombotic Events Independent of ECMO

Twenty-five percent of patients had clinically significant thrombotic events, including deep vein thrombosis in upper and/or lower extremities, pulmonary embolism, and stroke. Patients requiring ECMO had a higher frequency of thrombotic events (7 of 9 [78%]) compared to non-ECMO patients (7 of 47 [18%]). We next explored which coagulation factors were associated with thrombotic events, separating the ECMO-naïve from ECMO-requiring given the ECMO-associated differences in coagulative profile described above (Supplemental Digital Content 4 table S3, http://links.lww.com/ALN/C852). Non-ECMO patients who experienced a thrombotic event were more likely to have significantly elevated D-dimer and plasminogen activator inhibitor 1 levels on day 1 of ICU hospitalization compared to those without (odds ratio, 1.95 [1.21, 3.14]; $P = 0.006$; and odds ratio, 3.52 [0.99, 12.48]; $P = 0.05$, respectively).

A two-way ANOVA of coagulation parameters, also measured on day 1 of a patient's ICU stay, for clotting × ECMO was performed. A significant amount of variation in D-dimer levels was associated with clotting (fig. 2B; $P_{clot} < 0.001$, mean for no clot, 5,301 ± 12,239 ng/ml *vs.* clot, 16,540 ± 21,233 ng/ml). However, this effect was largely driven by elevation in D-dimer values within the ECMO population, as illustrated in figure 2B (ECMO ($P_{ecmo} < 0.001$) and ECMO × clot ($P_{clot\ x\ ecmo} = 0.001$; means for ECMO-naive, 5,835 ± 11,270 ng/ml *vs.* ECMO, 23,491 ± 25,806 ng/ml).

Plasminogen activator inhibitor 1 levels accounted for a significant source of variation in thrombotic events (fig. 2C; $P_{clot} = 0.003$; mean no clot, 26.3 ± 17.8 ng/ml *vs.* clot, 38.8 ± 15.2 ng/ml), as well as factor VIII activity (fig. 2D; $P_{clot} = 0.003$; mean no clot, 1.15 ± 0.28 OD650 *vs.* clot, 1.42 ± 0.31 OD650) and vWF levels (fig. 2E; $P_{clot} = 0.096$; mean no clot, 36.9 ± 22.5 μg/ml *vs.* clot, 45.2 ± 22.1 μg/ml). This suggests that fibrinolytic suppression and endothelial damage–associated factor release contribute to clinically significant thrombotic events regardless of ECMO status. In non-ECMO patients, ADAMTS13 levels showed an inverse association with vWF levels (mean no clot, 486 ± 184 ng/ml *vs.* clot, 399 ± 139 ng/ml), although this pattern was not observed in patients requiring ECMO (fig. 2E). Changes in fibrinogen levels were not associated with thrombotic events (fig. 2A). Supplemental Digital Content 4 table S3 (http://links.lww.com/ALN/C852) further reports ECMO and non-ECMO patient characterization with and without thrombotic events. Patients with thrombotic events had a higher incidence of ventilator-associated pneumonia, dialysis, and mortality independent of ECMO status.

Elevated Plasminogen Activator Inhibitor 1 Levels Are Associated with Severe ARDS

Table 2 summarizes the means of laboratory values of all patient time points stratified by the Berlin Criteria for ARDS severity at time of blood collection.[16] In Supplemental Digital Content 5 table S4 (http://links.lww.com/ALN/C853), patients who at one point during their hospitalization met criteria for severe ARDS had higher mortality and morbidity, including elevated rates of ventilator-associated PNA, major hemorrhagic events, and major thrombotic events, as previously described, compared to patients who remained in either mild or moderate ARDS throughout their ICU course. As seen in table 2, plasminogen activator inhibitor 1 levels were significantly elevated during periods of severe ARDS compared to mild and moderate ARDS (severe, 44.2 ± 14.9 ng/ml *vs.* mild, 31.8 ± 14.7 ng/ml and moderate, 33.1 ± 15.9 ng/ml; $P = 0.029$ and 0.039, respectively; fig. 3). Elevation of microparticle-bound tissue factor, a marker of endothelial damage and thromboimmune activity, was also observed in severe ARDS, although this did not reach significance (severe, 1.8 ± 1.5 pg/ml; moderate, 1.2 ± 1.0 pg/ml; mild, 1.2 ± 0.8 pg/ml; $P = 0.116$; table 2; fig. 3). Additional nonsignificant differences associated with worsening Pao_2/Fio_2 ratios include elevation of tissue factor pathway inhibitor and vWF along with a reduction of ADAMTS13 (table 2).

Discussion

We evaluated critically ill ICU patients with ARDS from SARS-CoV-2 infection to further characterize COVID-19-associated coagulopathy. While our cohort size is small, a stratified comorbidities analysis demonstrates that survivors and nonsurvivors had similar rates of cardiovascular disease, chronic lung injury, kidney disease, and diabetes. Interestingly, the survivor cohort had higher body mass indices compared to nonsurvivors. Obesity is associated with worse coagulopathy in the literature, presumably through baseline endothelial inflammation.[21,24,25] Our cohort did not demonstrate this association, as survivors had higher body mass indices than nonsurvivors, likely due to the small sample size. This suggests that the coagulopathy and the associated ARDS severity we describe are less dependent on the presence of higher body mass index or other identified chronic diseases.

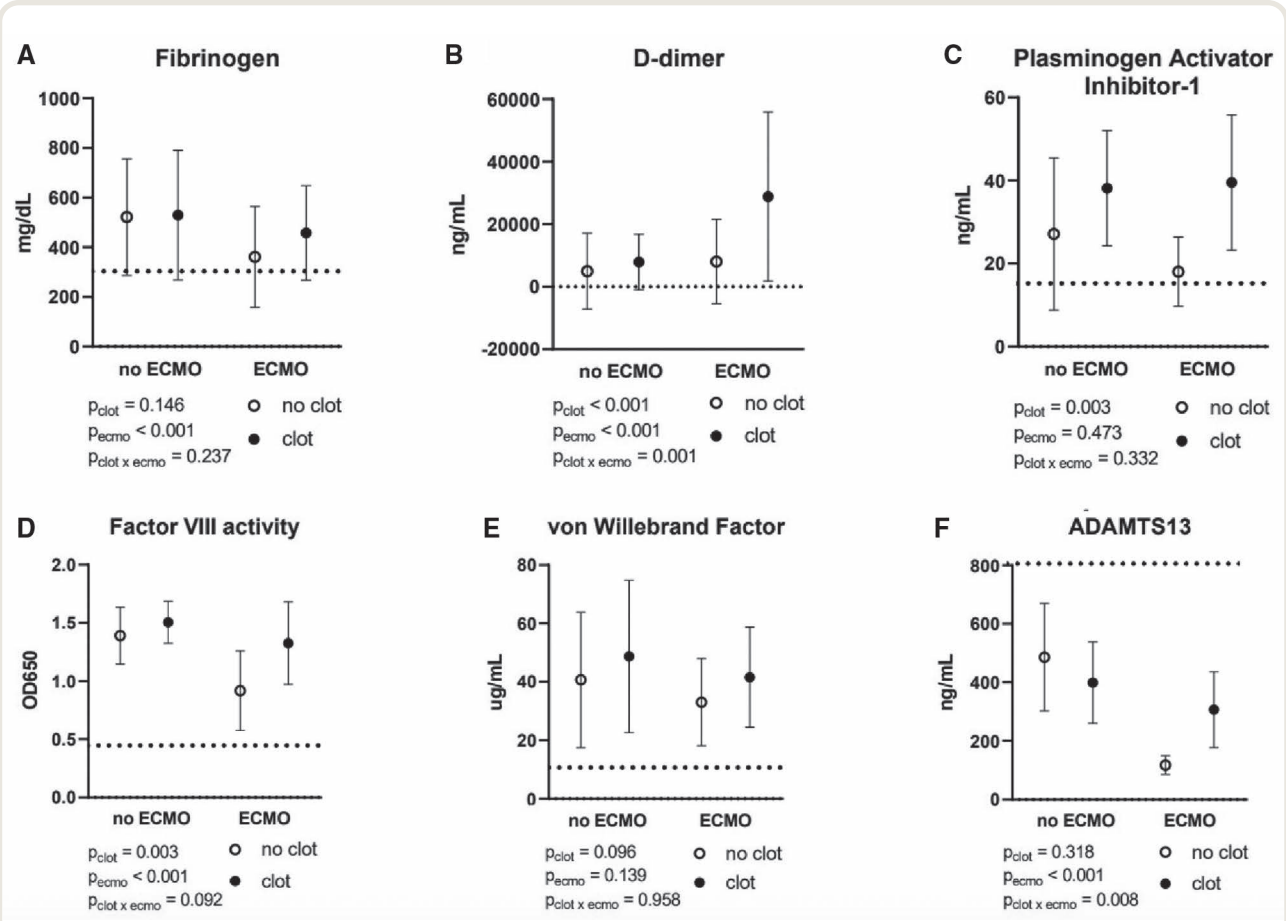

Fig. 2. Coagulative parameters for patients with and without significant thrombotic events (clot) during hospitalization, separated by extracorporeal membrane oxygenation (ECMO) status. Regardless of ECMO status, elevations in plasminogen activator inhibitor 1 (*C*), von Willebrand Factor (*E*), and factor VIII (*D*) were associated with thrombotic events. Neither fibrinogen (*A*) nor ADAMTS13 levels (*F*) had variance accounted for by thrombotic events, although the levels of A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13), were lower in non-ECMO patients who had a thrombotic event (*F*). D-Dimer levels were markedly elevated in ECMO patients who had a thrombotic event (*B*), which drives the significant interaction term of clot × ECMO (*B*). The *P* values shown on graphs from *top* to *bottom* are the *P* values for variance accounted for by clotting term (P_{clot}), ECMO term (P_{ecmo}), and clotting × ECMO term ($P_{clot \times ecmo}$), respectively, in a two-way ANOVA for clot × ECMO. *Dotted lines* indicate the means of normal healthy values for swift comparison. The values are plotted as means ± SD.

Severe COVID-19 coagulopathy is associated with macrothrombotic events such as deep vein thrombosis, pulmonary embolism, and cerebrovascular accident leading to increased morbidity and mortality in this patient population. Fibrinogen, D-dimer, and vWF levels and reduced ADAMTS13 levels (an enzyme that shortens vWF long multimers) characterize the hypercoagulable state in COVID-19 patients with ARDS. Our study redemonstrates that patients with COVID-19 coagulopathy have shortened clot formation time and increased maximal clot firmness on viscoelastic testing.[26] We also found that levels of fibrinolysis -inhibiting plasminogen activator inhibitor 1 were elevated with no evidence of clot lysis on ROTEM (LI30 and LI45), a finding consistent with fibrinolytic suppression in COVID-19. Furthermore, patients experiencing a clinically significant thrombotic event during their hospital course had elevated plasminogen activator inhibitor 1 levels, vWF levels, and factor VIII activity compared to those that did not experience such events despite prophylactic anticoagulation (fig. 2).[27,28] These findings are consistent with recent studies of ICU patients with COVID-19 that have identified plasminogen activator inhibitor 1 and inhibition of fibrinolysis as predictors of mortality.[15]

Elevations in vWF, tissue factor pathway inhibitor, microparticle-bound tissue factor, and plasminogen activator inhibitor 1 were also observed in patients with severe ARDS, revealing an association between coagulopathy and ARDS severity in COVID-19 (fig. 3; table 2). Although previous studies have commented on the association between fibrinolytic inhibition and major thrombotic events in

Table 2. Means and Standard Deviations of Laboratory Values by ARDS Severity

Measure	Mild ARDS (PaO_2/FiO_2 300 to 200)	Moderate ARDS (PaO_2/FiO_2 100 to 200)	Severe ARDS (PaO_2/FiO_2 < 100)
Total patients	1	14	31
PaO_2/FiO_2 (SD)	263	147 (27.7)	82 (14.2)
Aspartate transaminase, U/l (SD)	59	55 (86)	255 (1,864)
Alanine aminotransferase, U/l (SD)	64	42 (47)	108 (621)
D-Dimer, ng/ml (SD)	4641	8,937 (16,546)	9,744 (16,996)
Hematocrit, % (SD)	27	28 (5)	28 (6)
Platelets, $\times 10^9$/l (SD)	265	220 (127)	192 (115)
White blood cell count, $\times 10^3$/ml (SD)	11.9	13.1 (7.0)	14.7 (8.3)
C-reactive protein, mg/l (SD)	7.6	12.6 (10.2)	15.2 (10.0)
Fibrinogen, mg/dl (SD)	460	458 (245)	417 (240)
Lactate, mmol/l (SD)	1.92	1.63 (1.52)	3.20 (4.19)
Lactate dehydrogenase, U/l (SD)	438	455 (290)	407 (228)
pH (SD)	7.39	7.37 (0.08)	7.33 (0.12)
INR (SD)	1.20	1.27 (0.43)	1.23 (0.36)
PTT, s (SD)	48.3	44.0 (20.9)	45.0 (19.8)
Creatinine, mg/dl (SD)	2.11	1.54 (1.59)	1.62 (1.36)
von Willebrand factor, μg/ml (SD)	36.9	45.4 (20.5)	47.4 (25.1)
ADAMTS13, ng/ml (SD)	459	332 (184)	397 (169)
Plasminogen activator inhibitor 1, ng/ml (SD)	31.8	33.1 (15.9)	44.2 (14.9)
Tissue factor pathway inhibitor, ng/ml (SD)	408	444 (174)	513 (251)
Microparticle-bound tissue factor, pg/ml (SD)	1.2	1.2 (1.0)	1.8 (1.5)
Factor VIII activity, optical density at 650 nm (SD)	1.41	1.32 (0.34)	1.48 (0.27)
Anti-factor Xa activity, IU/ml (SD)	0.14	0.16 (0.09)	0.17 (0.08)

The means were calculated using laboratory values during at the same time PaO_2/FiO_2 was determined. Extracorporeal membrane oxygenation patients are not included in this table.

ADAMTS13, A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; ARDS, acute respiratory distress syndrome; INR, international normalized ratio; PaO_2/FiO_2, ratio of partial pressure of oxygen (PaO_2) to fraction of inspired oxygen (FiO_2); PTT, partial thromboplastin time.

COVID-19,[29–34] our report links biomarkers, viscoelastic determination of hypercoagulability, and fibrinolytic suppression to the severity of hypoxemia in these patients. Our results describe this mechanism through elevations in endotheliopathic biomarkers and fibrinolytic inhibition, which may cause the extensive pulmonary microcirculatory thrombosis seen in ARDS secondary to COVID-19 leading to severe hypoxemia.[7,8]

While these microthrombotic changes and increased D-dimer levels are also seen in patients with disseminated intravascular coagulation (DIC), patients with severe COVID-19 present with normal platelet counts and prothrombin times (Supplemental Digital Content 2 table S2, http://links.lww.com/ALN/C850) unlike patients with DIC.[34] COVID-19 patients also lack the systemic vasoplegia and consumptive coagulopathy seen with DIC, likely due to the initial localized endothelial injury and subsequent thrombosis within the pulmonary microcirculation caused by SARS-CoV-2 infection.[7,35] When accompanied by upregulation of acute-phase reactants such as fibrinogen and vWF, COVID-19–associated coagulopathy most aligns with a thrombotic microangiopathic disorder originating at the alveolar–capillary interface, subsequently engendering the patients' hypoxic state.

We found that nonsurvivors had higher levels of vWF and microparticle-bound tissue factor. Patients experiencing a major bleeding event had lower levels of ADAMTS13 compared to patients who did not. This demonstrates that markers consistent with severe endotheliopathy and thromboinflammatory response can distinguish outcomes even among the sickest patients.[36] While increased levels of vWF can reflect a normal host inflammatory response to infection, elevated levels can demonstrate endothelial injury. Elevated vWF is known to increase prothrombotic effects through multiple mechanisms including platelet activation and aggregation, as well as stabilization of factor VIII, the only intrinsic pathway factor produced by endothelium. Notably, the immediate upstream intrinsic clotting factor IX, produced by the liver, was not elevated in these patients. The decrease of ADAMTS13 results in larger procoagulant vWF multimers, which further exacerbates this endothelial-driven hypercoagulability. The degree of these changes can distinguish between differing severity of disease and survivors from nonsurvivors in critically ill patients.[37–40]

As a potential therapeutic strategy, lytic therapy is currently being investigated.[41] Specific inhibition of plasminogen activator inhibitor 1 may also have utility in this disease process, and future studies from our group will explore the impact of an aptamer that inhibits the antiproteolytic activity of plasminogen activator inhibitor 1 in COVID-19.[42,43] These approaches are important as current studies involving therapeutic dose anticoagulation in the ICU have stopped due to futility based on the Accelerating COVID-19 Therapeutic Interventions and Vaccines studies.[44]

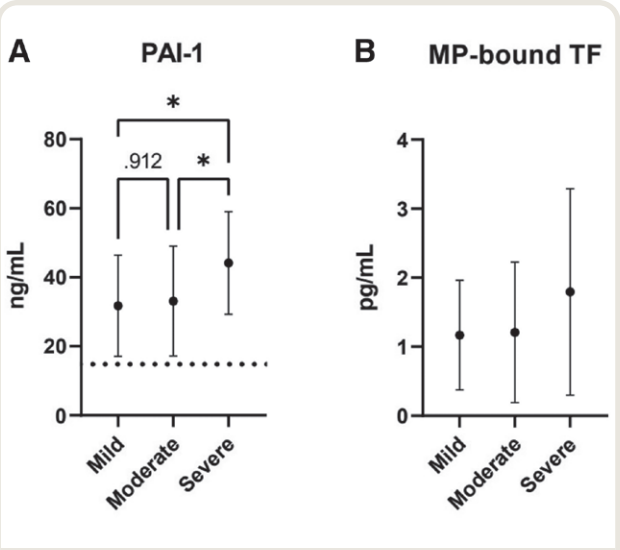

Fig. 3. Markers of endothelial damage like plasminogen activator inhibitor 1 and microparticle-bound tissue factor are elevated in severe acute respiratory distress syndrome (ARDS) compared to mild and moderate ARDS. Mild ARDS is defined as Pa_{O_2}/Fi_{O_2} between 300 and 200, moderate ARDS is defined as Pa_{O_2}/Fi_{O_2} between 100 and 200, and severe ARDS is defined as Pa_{O_2}/Fi_{O_2} of less than 100. The values are plotted as means ± SD. The *dotted line* indicates the mean of healthy normal, where available. Significant one-way ANOVA for plasminogen activator inhibitor 1 was followed by Tukey's *post hoc* comparison between groups displayed in the figure. $P < 0.05$ was considered significant. *$P < 0.05$. Nonsignificant P values are written out.

We conclude that COVID-19–associated coagulopathy in ECMO patients should be considered separately from ECMO-naïve patients. Patients requiring ECMO have severe refractory hypoxemia resulting from pulmonary microcirculatory injury with subsequent higher risk of thrombotic and hemorrhagic events.[40] Rates of major thrombotic events were 7 of 9 (78%) in ECMO patients compared to 4 of 11 (36%) and 3 of 36 (8.3%) in non-ECMO nonsurvivors and survivors, respectively. Significant hemostatic changes observed on ROTEM viscoelastic testing demonstrated variability including the loss of the characteristic procoagulant profile of hemostatic biomarkers in some ECMO patients when compared to non-ECMO COVID-19 patients with severe ARDS (fig. 1; Supplemental Digital Content 3 fig. S1, http://links.lww.com/ALN/C851). Studies using ROTEM on COVID-19 patient samples describe a high rate of prothrombotic events for ECMO (90%) compared to non-ECMO (46%) patients but did not separate these cohorts when generating models predictive of thrombotic events.[26] Although ECMO patients demonstrated a loss in the procoagulant profile from a biomarker perspective, they have increased thrombotic outcomes, likely due to the addition of ECMO circulation, which provides a nonendothelial interface for contact activation of the coagulation cascade despite heparin anticoagulation.[45–47] This prothrombotic interface exacerbates the thromboinflammatory response observed in COVID-19, increasing risk for thrombotic and bleeding events through subsequent consumptive coagulopathy. This ECMO difference is reflected by procoagulant biomarkers through relative reduction of fibrinogen and vWF levels and apparent normalization of ROTEM tracings.[44] The tissue factor pathway inhibitor levels observed in our cohort's ECMO patients are also higher than the tissue factor pathway inhibitor levels reported in the non–COVID-19 ECMO cohort by Mazzeffi *et al.*[48] (Supplemental Digital Content 2 table S2, http://links.lww.com/ALN/C850). This difference is likely due to widespread endotheliopathy associated with severe COVID-19 and tissue factor pathway inhibitor's primary location on the endothelial surface and indicates the compounding coagulopathic insults of ECMO and SARS-CoV-2 infection.

The therapeutic heparinization required to maintain ECMO circuit patency in this hypercoagulable disease state increases risk of hemorrhagic events in these patients over their ECMO-naive counterparts, who generally receive prophylactic doses of low-molecular-weight heparin. Of note, 54 of 55 (96.4%) patients observed received either prophylactic or therapeutic anticoagulation with heparin products during hospitalization. Investigation into the combined effects of ECMO and COVID-19 *versus* other viral infections is needed to understand each prothrombotic stimuli and its contribution to the associated pathophysiology.

Despite having a large amount of data for analyses, this study was limited to the relatively small cohort size of this observational study. Due to inconsistent data across time points, we were unable to perform temporal analyses. To reduce bias through repeated measures, biomarker and laboratory data were either used on ICU day 1 or collapsed into maximum, minimum, and means during a patient's ICU hospitalization. Possible selection bias could be present due to the limited sample size of participants. Larger population studies are needed to further characterize COVID-19 coagulopathy in ICU patients. This small cohort size also precludes detailed longitudinal analysis, which will be a valuable approach for future studies. Assessment of fibrinolytic inhibition would have been more robust with LI45 and LI60 data for all patients in the study, although the calculated mean difference of 1% between LI30 and LI45 indicates minimal clot dissolution between the two time points. Other groups have shown that fibrinolytic suppression measured by LI60 predicts thromboembolic complications in COVID-19 patients,[26,31,33] with even stronger predictive value if D-dimer levels are included.[31] These findings demonstrate the clinical utility of ROTEM and the importance of these later time points in assessment of fibrinolytic abnormalities. Coagulation protein data were limited compared to clinical electronic health record data due to comparatively fewer time points for biorepository sampling and laboratory processing constraints. Additionally, an

established link exists between obesity, poorer outcomes in COVID-19,[24,25] and the association between obesity and elevated markers of endothelial damage, including plasminogen activator inhibitor 1.[27,28] Future studies with larger patient cohorts will include the impact of body mass index on COVID-19 coagulopathy on ARDS severity given the elevated average of body mass index in our cohort.

We found that procoagulant biomarkers including those characteristic of endothelial injury are elevated in ICU patients with COVID-19 in association with severe hypoxemic respiratory failure. We note that ECMO-requiring COVID-19 patients need to be considered separately from the broader ICU cohort for analysis of coagulopathy given the consumptive coagulopathy engendered by the dual insult of SARS-CoV-2 infection and the ECMO circuit itself. We found evidence of fibrinolytic suppression on viscoelastic testing with elevated plasminogen activator inhibitor 1 levels that is more apparent in COVID-19 patients with severe disease who develop major thrombotic events. Coupled with markedly elevated D-dimer levels, these findings indicate that increased fibrin deposition occurs with decreased elimination in the microcirculation.[22] These findings warrant further investigation and encourage the development of potential therapeutic approaches limiting endothelial injury or working to counteract the pathologic sequela resulting from both the micro- and macrothrombotic pathology of severe COVID-19.

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Competing Interests

Dr. Kraft declares the following financial relationships: Boehringer Ingelheim (Ingelheim, Germany), Shinonogi Inc. (Osaka, Japan), Paratek Pharmaceuticals (Boston, Massachusetts), La Jolla Pharmaceutical Company (La Jolla, California), Savara Pharmaceuticals (West Lake Hills, Texas), and American College of Chest Physicians (Northbrook, Illinois). Dr. Levy delares the following financial relationships: Instrumentation Labs (Bedford, Massachusetts), Merck (Darmstadt, Germany), and Octapharma (Lachen, Switzerland). The other authors declare no competing interests.

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