Critical Care Explorations

Thromboembolism, Hypercoagulopathy, and Antiphospholipid Antibodies in Critically Ill Coronavirus Disease 2019 Patients: A Before and After Study of Enhanced Anticoagulation

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Objectives: To determine the prevalence of thrombotic events, functional coagulation tests, inflammatory biomarkers, and antiphospholipid antibodies before and after enhanced anticoagulation in critically ill coronavirus disease 2019 patients.

Design: Retrospective.

Setting: Tertiary intensive care unit.

Patients: Two cross-sectional cohorts of ICU-treated coronavirus disease 2019 patients were included before (cohort 1, $n = 12$) and after (cohort 2, $n = 14$) enhanced prophylactic anticoagulation strategy.

Interventions: Before and after study of enhanced anticoagulation.

Measurements and Main Results: Thromboelastometry point-of-care coagulation tests were performed by thromboelastography (Tem International GmbH, Munich, Germany), standard blood tests were extracted from patient charts, and presence of antiphospholipid antibodies in plasma was measured. All patients were males on mechanical ventilation. In cohort 1 (low-molecular-weight heparin dose: 129 ± 53 U/kg/24 hr), 50% had pulmonary embolism, and thromboelastography analysis revealed hypercoagulation in a majority of patients and greater than 80% had detectable antiphospholipid antibodies. In the second cohort (enhanced low-molecular-weight heparin dose: 200 ± 82 U/kg/24 hr; $p = 0.04$ vs cohort 1), we found a nonsignificantly lower prevalence of pulmonary embolism (21%; $p = 0.22$), lower fibrinogen (6.3 ± 2.5 vs 8.7 ± 2.0; $p = 0.02$), reduced fibrinogen-dependent thromboelastography ($p < 0.001$), and lower inflammatory markers.

Conclusions: In these two cross-sectional cohorts of ICU-treated coronavirus disease 2019 patients, thromboembolic complications, hypercoagulation, and antiphospholipid antibodies were common. A more aggressive anticoagulation regime was associated with a reduction in inflammatory biomarkers including plasma fibrinogen and a reduction in fibrinogen-dependent hypercoagulation, as indicated by thromboelastography analyses.

Key Words: coagulopathy; coronavirus disease 2019; thrombosis

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A major and serious manifestation of the coronavirus disease 2019 (COVID-19) pandemic is hypercoagulation and subsequent thromboembolism, which is especially evident in critically ill patients on mechanical ventilation. A high prevalence of pulmonary embolism (PE) and other thrombotic events has been reported in several studies (1–3) and postmortem findings indicate widespread thrombus formation including microembolism in several organs (4). The latter finding may be part of the COVID-19 pulmonary pathophysiology characterized by marked deterioration of gas-exchange with preserved pulmonary compliance (5). Prophylactic anticoagulation treatment has recently been associated with decreased mortality in COVID-19

TABLE 1. Comparison of Demographics, Comorbidities, Treatment and Blood Tests Between the Two Study Cohorts

Variable	Cohort 1 (*n* = 12)	Cohort 2 (*n* = 14)	*p*
Demographics			
Age (yr)	54 ± 9	59 ± 8	0.16
Male sex	12 (100)	14 (100)	1.00
Body weight (kg)	91 ± 15	81 ± 11	0.06
Body mass index (kg/m^2)	30.3 ± 5.6	28.2 ± 4.2	0.28
ICU treatment duration at thromboelastography analysis (d)	13 (7–16)	18 (13–29)	0.01
Comorbidities			
Hypertension	5 (42)	6 (43)	1.00
History of smoking	3 (25)	2 (14)	0.63
Pulmonary fibrosis	1 (8)	1 (7)	1.00
Asthma	3 (25)	2 (14)	0.63
Diabetes mellitus	5 (42)	2 (14)	0.19
Cardiovascular disease	1 (8)	4 (29)	0.33
ICU treatment			
Mechanical ventilation	12 (100)	14 (100)	1.00
Antibiotics	12 (100)	14 (100)	1.00
LMWH treatment	9 (75)	11 (79)	1.00
LMWH dose (U/kg/24 hr)	129 ± 53	200 ± 82	0.04
Heparin infusion	3 (25)	3 (21)	1.00
Heparin dose (1,000 U/24 hr)	40 ± 5	44 ± 8	0.57
Thrombolysis	2 (17)	1 (7)	0.58
Acetylsalicylic acid 75 mg daily	5 (42)	5 (36)	1.00
Dialysis	6 (50)	8 (57)	1.00
Blood tests (reference range)			
Platelet count (× 10^9/L) (145–348)	393 ± 151	320 ± 93	0.14
WBC count (× 10^9/L) (3.5–8.8)	13.7 ± 4.8	16.5 ± 7.8	0.29
International normalized ratio (< 1.2)	1.1 ± 0.11	1.2 ± 0.28	0.12
Activated partial thromboplastin time (s) (20–30)	31 (24–40)	26 (24–33)	0.16
Fibrinogen > 4.2 g/L	12 (100)	12 (86)	0.48
D-dimer (mg/L) (< 0.54)	6.9 (5.7–10.0)	3.9 (2.2–6.8)	0.06
C-reactive protein (mg/L) (< 3)	258 (135–348)	57 (37–137)	0.005
Ferritin (ug/L) (30–350)	1,055 (929–2,428)	1006 (755–1,514)	0.60
Procalcitonin (ug/L) (< 0.5)	2.4 (0.4–6.5)	0.7 (0.3–2.7)	0.35
Creatinine (umol/L) (< 100)	137 (53–219)	119 (63–210)	0.97
Estimated glomerular filtration rate $_{\text{Cystatine C}}$ (mL/min/1.73 m^2)	40 ± 28	27 ± 14	0.14
Interleukin-6 (ng/L) (< 7)	122 (99–352)	64 (21–246)	0.26
Tumor necrosis factor-α (ng/L) (< 12)	17 (10–28)	20 (12–31)	0.48
Erythrocyte sedimentation rate (mm) (< 10)	133 (126–140)	116 (81–130)	0.007

LMWH = low-molecular-weight heparin.

Data are presented as *n* (%) for categorical variables and mean ± SD for continuous variables with normal distribution and median (interquartile range) for continuous variables with skewed distribution.

patients (6, 7). In addition, presence of antiphospholipid antibodies may contribute to clot propensity and has been reported in COVID-19 patients prone to thromboembolism (8).

As these manifestations became apparent during the course of the pandemic, we changed our clinical routine in our hospital to an enhanced anticoagulation strategy as of mid-April 2020. The aim of this study was to determine the prevalence of thrombotic events, hypercoagulation as determined by functional coagulation tests, increased inflammatory biomarkers, and antiphospholipid antibodies before and after this change in practice. We hypothesized that circulating antiphospholipid antibodies would be common and that enhanced anticoagulation regime would be associated with a reduction in thromboembolism, hypercoagulation, and inflammatory biomarkers in critically ill ICU- treated COVID-19 patients.

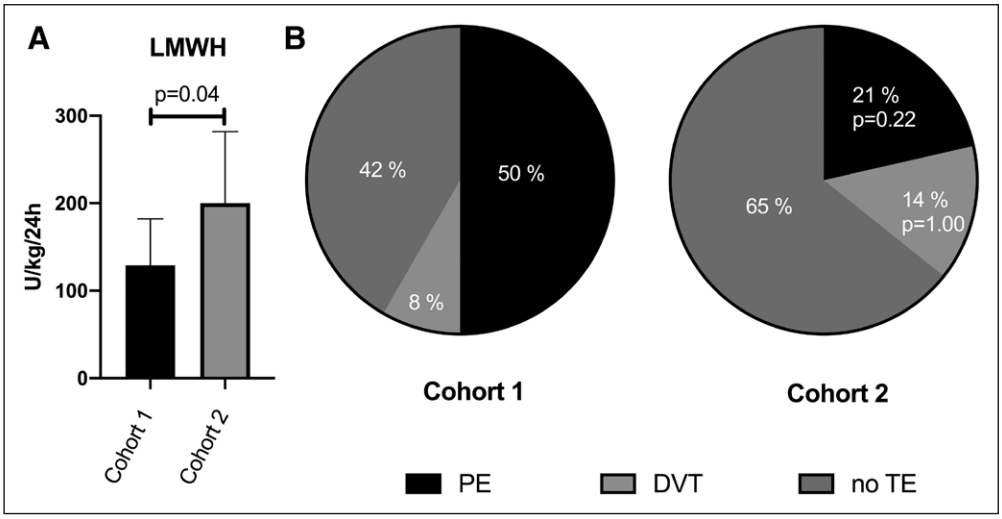

Figure 1. Low molecular weight heparin dosing and prevalence of pulmonary embolism and deep vein thrombosis in the two study cohorts. **A**, Significantly higher low-molecular-weight heparin (LMWH) in cohort 2 after changing to an enhanced anticoagulation strategy. **B**, High prevalence of pulmonary embolism (PE) and deep vein thrombosis (DVT) in cohort 1 that decreased nonsignificantly after introducing a more aggressive anticoagulation regime. TE = thrombotic event.

MATERIAL AND METHODS

We performed two cross-sectional analyses (before and after enhanced anticoagulation) of critically ill COVID-19 patients in the cardiothoracic ICU at Karolinska University Hospital. The first cohort consisted of 12 patients and the second cohort (included 3 wk later) consisted of 14 patients (excluding patients from the first cohort). Except the recommendation to enhance anticoagulation, there were no institutional changes in patient management during the study period. Low-molecular-weight heparin (LMWH) was used prophylactic or therapeutic either once daily or twice daily according to discretion of treating physician. Diagnoses of

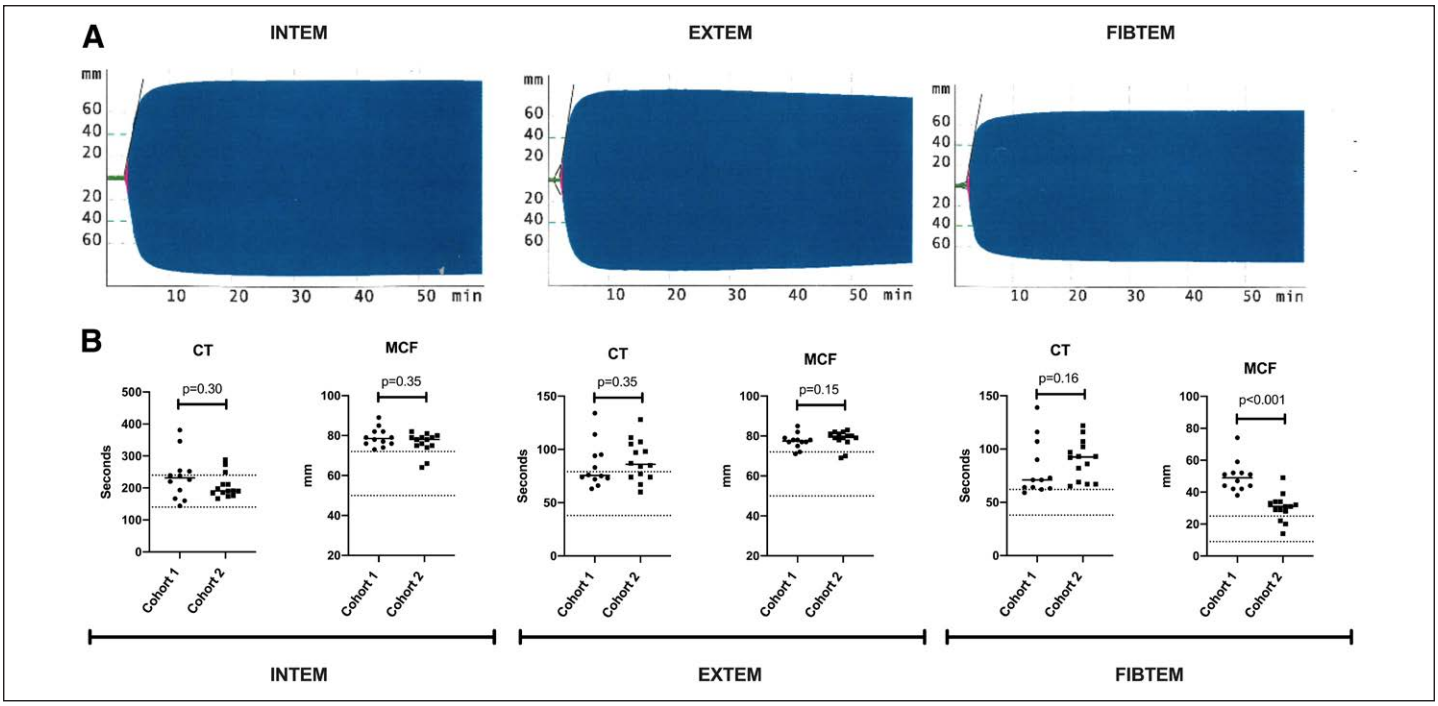

Figure 2. Thromboelastometry point-of-care coagulation test (ROTEM) was markedly increased in ICU-treated coronavirus disease 2019 (COVID-19) patients, but fibrinogen-dependent maximum clot firmness (MCF) was significantly reduced in cohort 2. **A**, Representative ROTEM analysis of intrinsic thromboelastometry (INTEM), extrinsic thromboelastometry (EXTEM), and fibrinogen-dependent thromboelastometry (FIBTEM) clot formation from one COVID-19 patient. **B**, Individual (*dots and squares*) and median (*solid black line*) coagulation time (CT) and MCF in INTEM, EXTEM, and FIBTEM. *Dashed lines* denote the reference range. ROTEM = thromboelastography.

PE and deep vein thrombosis (DVT) were based on standard chest computed tomography or ultrasound, respectively, according at the discretion of attending physicians. Standard blood tests (from the day of thromboelastography [ROTEM] test) and clinical data were extracted from the patient's charts. Thromboelastometry point-of-care coagulation tests were performed by ROTEM (Tem International GmbH, Munich, Germany) once in each patient. This test provides functional evaluation of coagulation. Extrinsic thromboelastometry (EXTEM) and intrinsic thromboelastometry (INTEM) coagulation cascades were studied and the influence of fibrinogen on clot firmness was estimated with the platelet-inactivated fibrinogen-dependent thromboelastometry (FIBTEM) test. Clotting time (CT) is defined as the time to onset of clot formation. Clot firmness is defined as the maximum amplitude of the thromboelastogram (maximum clot firmness [MCF]) and a high MCF indicates a hypercoagulable state. CT and MCF were compared with the normal reference range (upper and lower 99th percentile). Presence of antiphospholipid antibodies in plasma was evaluated using multiplex immunoassay in nine patients from cohort 1 and 14 patients in cohort 2. Since the duration of ICU treatment at the time of ROTEM analysis was significantly longer in the second cohort and may be an important confounder, we performed a sensitivity analysis of the key findings in this study where patients from the two cohorts were matched based on ICU treatment duration. The Swedish Ethical Review Authority approved this study (ethical permit Dnr: 2020-01302). Written informed consent from patients or relatives was waived as only retrospectively collected deidentified data from clinical records were used. Shapiro-Wilk test was used to test for normal distribution and data are presented as mean ± SD for data with normal distribution and median (interquartile range) for variables with skewed distribution. For continuous variables, significance testing for differences between the two cohorts was performed with unpaired *t* tests or Mann-Whitney *U* tests, as appropriate. Fisher exact test was used for categorical variables. All statistical analyses were performed using GraphPad Prism Version 8.3.0 (GraphPad Software, La Jolla, CA). A *p* value of less than 0.05 was determined as statistically significant and a *p* value of 0.05–0.10 was determined a trend.

RESULTS

Table 1 outlines the patient characteristics. The patients were all male with a high proportion of comorbidity. All patients were on mechanical ventilation and approximately half on renal replacement therapy. As expected, a higher background dose of LMWH was seen in the second cohort (200 ± 82 U/kg/24 hr) compared with the first cohort (129 ± 53 U/kg/24 hr; $p = 0.04$) (**Fig. 1*A***). Major clinical thromboembolic events were common (**Fig. 1*B***): PE was seen in 50% of patients in cohort 1 and 21% in cohort 2 ($p = 0.22$). **Figure 2** displays a representative ROTEM analysis and individual patient data. Most patients in both cohorts displayed INTEM and EXTEM CT within the normal reference range, whereas FIBTEM was prolonged. INTEM, EXTEM, and FIBTEM MCF were all elevated above the upper normal reference limit in greater than or equal to 10 of 12 patients in cohort 1, indicating hypercoagulation. In the second cohort, INTEM and EXTEM MCF were similarly elevated, whereas fibrinogen-dependent (FIBTEM) MCF was significantly lower than the first cohort ($p < 0.01$). The lower FIBTEM MCF in cohort 2 was accompanied by significantly lower plasma fibrinogen, a trend toward lower D-dimer (**Fig. 3*A***) and significantly reduced inflammatory biomarkers (**Fig. 3*B***). Detectable antiphospholipid antibodies (≥ 2 U/mL) were highly prevalent in both cohorts, especially IgA-type antibodies (**Fig. 4**).

In the sensitivity analysis, ICU stay at the time of ROTEM analysis was similar in cohort 1 (13 d [7–16 d]; $n = 12$) and cohort 2 (14 d [12–24]; $n = 11$; $p = 0.10$). As in the main analysis, LMWH-dosing was higher ($p = 0.09$), whereas the prevalence of PE ($p = 0.03$) and plasma fibrinogen ($p = 0.048$) and FIBTEM-MCF (< 0.001) were lower in the second cohort. Similarly, markers of inflammation were lower in cohort 2 ($p = 0.02$ for C-reactive protein (CRP) and $p = 0.01$ for erythrocyte sedimentation rate). The

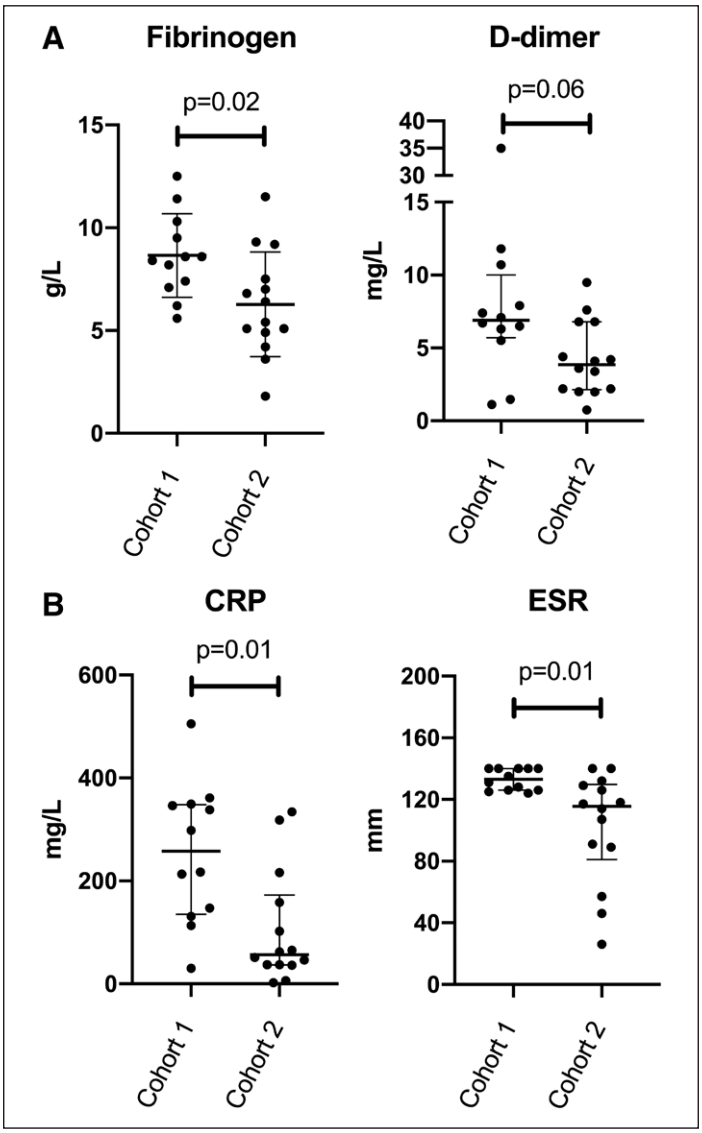

Figure 3. Blood markers of cloth formation propensity and inflammation in the two study cohorts. The reduction in FIBTEM MCF between the two cohorts was associated with a significant reduction in (**A**) plasma levels of fibrinogen and a trend toward decrease in D-dimer as well as (**B**) inflammation parameters. CRP = C-reactive protein, ESR = erythrocyte sedimentation rate.

results are detailed in **Supplemental Table 1** (http://links.lww.com/CCX/A467).

DISCUSSION

In this study, we report a high prevalence of hypercoagulation, thrombotic events, and antiphospholipid antibodies in critically ill COVID-19 patients. The enhanced anticoagulation strategy used in the second cohort was associated with a reduction in fibrinogen, FIBTEM-MCF, and inflammatory biomarkers. No differences were seen in INTEM or EXTEM coagulation parameters or the prevalence of antiphospholipid antibodies.

Thrombotic complications have been reported in 31–43% of ICU-treated COVID-19 patients (1–3), and histological examinations of COVID-19 patients have revealed thrombosis in small lung vessels and extra pulmonary organs (4). Pulmonary thrombosis may limit perfusion and impair pulmonary gas-exchange independent of the viral pneumonia. Hypercoagulation as indicated by elevated INTEM, EXTEM, and FIBTEM MCF, as well as increased fibrinogen and D-dimer in all our patients strengthen the hypothesis that the atypical respiratory distress features of COVID-19 may partly be caused by thromboembolism impairing lung perfusion. Indeed, a total of 9/26 study patients (35%) presented with PE and three more (3/26, 12%) had DVT. Importantly, as D-dimer, a fibrin-degradation product, will only be elevated after blood clot formation (9), MCF may indicate hypercoagulation at an earlier stage of critical illness when treatment with anticoagulants may be more effective in preventing clot formation. Indeed, an early Chinese retrospective study by Tang et al (6) indicated that early prophylaxis with LMWH may decrease mortality in a selected group of patients. In addition, a recent study from New York by Paranjpe et al (7), involving approximately 3,000 patients, showed that the change of practice to start general low-dose LMWH prophylaxis in hospitalized patients decreased mortality. In multivariate analysis, longer anticoagulation duration was associated with a 14% lower adjusted risk of inhospital death (hazard ratio, 0.86 per day; 95% CI, 0.82–0.89; $p < 0.001$) without a significant increase in bleeding. However, in that study, only 28% received prophylactic anticoagulation. The results were particularly conspicuous among ICU patients requiring mechanical ventilation, in whom inhospital mortality fell from 63% to 29% and median survival increased from 9 to 21 days. In contrast, all our patients received prophylactic anticoagulation. Still, the high INTEM-MCF values despite usual thrombus prophylaxis with LMWH may indicate that higher doses are needed. In addition, if individuals at risk of developing COVID-19-related thrombosis are identified at an early stage before needing ICU care, high-dose prophylaxis may decrease morbidity and mortality of COVID-19 patients.

The findings in this study are supported by a recently published Italian study, also reporting a high prevalence of thromboembolic events and hypercoagulation as evaluated by ROTEM, although their results are somewhat difficult to interpret as background treatment with LMWH was not reported (10). In analogy with the current study, they described a reduction in fibrinogen at day 10 compared with that at admission in consecutively drawn blood samples. In addition, another recently published study from the United States found similar high prevalence of hypercoagulation using ROTEM (44–70%, depending on parameter) and thromoboembolic events (31%) in critically ill COVID-19 patients treated in the ICU (11).

Increased dosing of LMWH is known to affect ROTEM per se in terms of increased CT time, at least with extreme dosing, but there are no reports of reduced FIBTEM-MCF solely attributed to enhanced anticoagulation (12), but the lack of scientific evidence that increased anticoagulation reduces FIBTEM-MCF does not preclude such an effect.

The finding in this study that fibrinogen, CRP, D-dimer, and FIBTEM-MCF were all reduced in the high LMWH cohort may suggest interplay between COVID-19 infection, reduced inflammation, and increased LMWH as a mechanism for FIBTEM-MCF reduction rather than increased LMWH alone. However, further studies are needed to elucidate the mechanisms responsible for the association between COVID-19 disease, anticoagulation, inflammation, and FIBTEM-MCF association or if a direct effect on LMWH on FIBTEM-MCF exists.

Intriguingly, the high prevalence of detectable antiphospholipid antibody titers in our patients indicates that COVID-19 may trigger antiphospholipid antibody production that may also play a role in explaining the

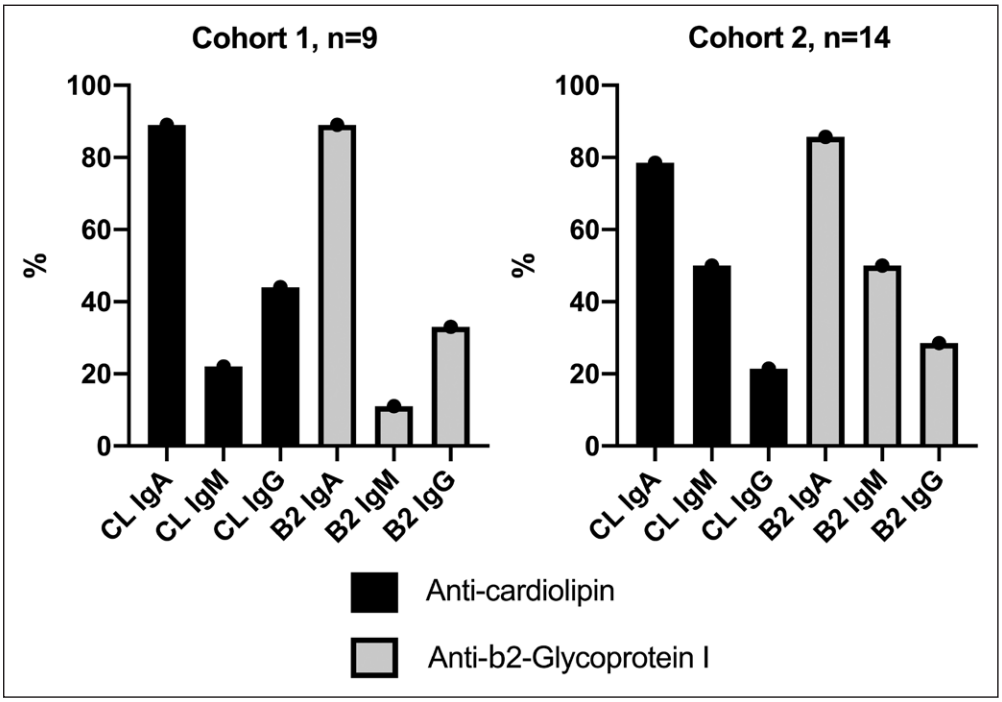

Figure 4. High prevalence of detectable (≥ 2 U/mL) anticardiolipin and anti-β2-glycoprotein I antibodies in critically ill coronavirus disease 2019 patients.

increased prevalence of clot formation. These data conform to the recent findings of antiphospholipid antibodies in three COVID-19 patients with multiple thrombotic events (8). A recently published larger study of COVID-19 acute respiratory distress syndrome patients also reported an 88% prevalence of lupus antibodies, supporting our finding of high presence of antiphospholipid antibodies in critically ill COVID-19 patients (1).

Importantly, this study was conducted in a cardiothoracic ICU that was transformed to a COVID-19 ICU as a pandemic overflow response and some patients were referred to the cardiothoracic ICU from other ICUs due to proximity to ECMO and high capacity for dialysis. Therefore, this patient cohort more commonly displayed multiorgan failure and on average had a more critical COVID-19 disease state than other ICU COVID-19 cohort.

Since this is not randomized controlled clinical trial, reported differences between the two cohorts must be interpreted with caution. One major confounder may be the difference in length of ICU stay at the time of ROTEM analysis between the cohorts, which may reflect different disease states. However, the sensitivity analysis with matched subjects from the two cohorts based on the duration of ICU stay showed similar results as in the main analysis, which support our findings. Nevertheless, we cannot rule out that other confounders may have influenced the results and a causal relationship between increased LMWH and observed reduction in PE occurrence, inflammation, fibrinogen, and fibrinogen-dependent ROTEM cannot be concluded from this study. Another limitation in this study is that the decision to refer patients for a computed tomography scan or ultrasound for PE or DVT diagnosis was left to the discretion of the treating physician and not according to a standardized protocol.

CONCLUSIONS

In these two cross-sectional cohorts of ICU-admitted COVID-19 patients, thromboembolic complications, hypercoagulation, and antiphospholipid antibodies were common. A more aggressive anticoagulation regime was associated with a reduction in inflammatory biomarkers including fibrinogen and a reduction in fibrinogen-dependent hypercoagulation (FIBTEM).

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