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Persistence of procoagulable thromboelastography results in hospitalized COVID-19 patients despite clinical improvement

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Abstract. – OBJECTIVE: COVID-19 patients have been shown to be hypercoagulable, increasing the risk for thromboembolic events. The kinetics of the blood coagulation process were monitored daily throughout hospitalization in COVID-19 positive patients.

PATIENTS AND METHODS: Thromboelastography (TEG) was used to assess blood coagulation in 48 adult patients hospitalized for COVID-19 in this prospective cohort study. Clinical risk was assessed *via* National Early Warning Scores (NEWS) for each day of hospitalization.

RESULTS: During hospitalization, 98% of patients had one or more procoagulable TEG result. Thromboelastography results remained prothrombotic upon discharge in 80% of patients. NEWS significantly decreased by discharge compared to the peak scores.

CONCLUSIONS: Overall, patients were discharged from the hospital with significant clinical improvement, but without abnormal TEG results returning to a normal range. All patients in our study survived and few had thromboembolic events, so if and for how long these patients remain at risk for future complications warrants further investigation.

Key Words:
COVID-19, Thromboelastography, Blood coagulation disorders, Thromboembolism, Early warning score, Blood coagulation tests.

Introduction

The COVID-19 pandemic has resulted in over 6 million deaths worldwide. Both abnormal coagulation and the related high prevalence of thromboembolic events have been reported[1-3]. Thromboemobolic events, such as microvascular thrombosis and venous thromboembolism (VTE), are associated with higher morbidity, mortality, and complicated therapeutic regimens.

COVID-19 patients have been shown to be hypercoagulable by viscoelastic measurements of blood including thromboelastography (TEG) and rotational thromboelastometry (ROTEM)[4,5]. Unlike conventional coagulation tests, viscoelastic blood tests assess blood coagulation kinetics from clot formation through clot dissolution[6]. However, TEG and ROTEM are not widely used clinically, and there is limited or low-quality clinical data supporting use in infectious diseases in general[7].

Published TEG or ROTEM studies in COVID-19 patients often report data limited to one-to-three-time points[4,5,8]. We hypothesized that daily TEG measurements could provide insight into the course of hemostatic derangements experienced by patients hospitalized with COVID-19. In this descriptive study, daily coagulation status *via* TEG and clinical risk *via* the National Early Warning Score (NEWS) characterized COVID-19 patients admitted to the hospital for COVID-19 symptoms.

Patients and Methods

Patient Population

This prospective cohort study was approved by the IRB at Naval Medical Center, Portsmouth, Virginia in compliance with all applicable Federal regulations governing the protection of human subjects. All patients included in this study were hospitalized for COVID-19 symptoms, at least 18 years old, and confirmed positive for COVID-19 *via* polymerase chain reaction. Enrollment size

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Table I. Patient Demographics and NEWS by sex. Age is represented as mean (standard deviation); length of stay, day of illness and NEWS are represented as median (interquartile range). *p*-values presented for male to female comparisons. †, *t*-test; §, Mann-Whitney; χ^2, chi-squared.

	Total	Male	Female	*p*-value
Patient Count	48	30	18	
Age	48.4 (18.5)	46.3 (17.0)	51.8 (20.8)	0.33†
Length of Stay	5.0 (3.0-7.0)	5.0 (1.0-8.3)	4.0 (2.8-7.0)	0.25§
Day of Illness on Admission	6.0 (5.0-7.0)	6.0 (5.0-7.5)	6.0 (4.0-7.0)	0.56§
Comorbidities				
Hypertension	44%	43%	44%	>0.99$^{\chi}$
Obesity	17%	17%	17%	>0.99 $^{\chi}$
Hyperlipidemia	17%	13%	22%	0.45$^{\chi}$
Diabetes	13%	6%	17%	0.39$^{\chi}$
COPD/asthma	10%	0%	28%	**0.005**$^{\chi}$
Cardiovascular Disease	6%	3%	11%	0.55$^{\chi}$
Treatments				
Oxygen	75%	83%	61%	0.09$^{\chi}$
Anticoagulants	94%	100%	83%	**0.047**$^{\chi}$
Antiviral	58%	70%	39%	0.07$^{\chi}$
Steroids	52%	60%	39%	0.23$^{\chi}$
Antibiotics	33%	20%	56%	**0.02**$^{\chi}$
NEWS				
Admission	2.0 (1.0-5.0)	2.0 (1.0-6.0)	2.5 (0-3.0)	0.18§
Peak	4.0 (1.0-7.0)	5.0 (2.5-8.0)	3.0 (1.0-6.0)	0.052§
Discharge	1.0 (0-3.0)	2.0 (0-2.5)	1.0 (0-3.3)	0.99§

was based on the total number of eligible, consenting patients over a 6-months, from June to November 2020. Patient demographics are shown in Table I.

Thromboelastography

Blood was collected in citrated tubes for native TEG analysis from all consented patients each day of hospitalization. All samples were tested within 2 hours of collection on the TEG 5000 Thrombelastograph Hemostasis Analyzer system (Haemonetics, Boston, MA, USA). Reference ranges for TEG came from the manufacturer. Results of TEG were for research purposes only and were not shared with clinical staff during the study.

National Early Warning Score (NEWS)

NEWS is a validated clinical decision tool to identify patients with a high risk of decompensation based on vital signs, mental status, and oxygen requirements[9,10]. A score of 0 to 4 indicates low clinical risk, a score of 5 or 6 indicates moderate clinical risk and a score ≥ 7 indicates high clinical risk. NEWS was calculated for every day of each patient's admission.

Statistical Analysis

Continuous data from all laboratory tests were assessed for normality. Spearman's correlation was used to determine correlation coefficients for peak NEWS compared to length of hospital stay. Within subjects mixed effects analysis with a Greenhouse-Geisser correction was used to determine how TEG data and platelet counts varied over the first 10 hospitalization days for each patient. Beyond day 10 was not included in the analysis due to limited number of subjects hospitalized for >10 days. Wilcoxon matched pairs signed rank test was used to compare peak and final NEWS values. Results were considered significant at $p < 0.05$. Aggregate data are presented in graphs because no differences were found between sex or age of patients (Tables I and II). All statistics were performed using GraphPad Prism version 9 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Moderately Ill Patients with Clinical Improvement by Discharge

The cohort of admitted patients included very few critically ill, with only 3 cases of VTE (a lower incidence rate than reported[11,12]), a single patient requiring mechanical ventilation and no deaths. Treatments during hospitalization are shown in Table I. Nearly all were prophylactical-

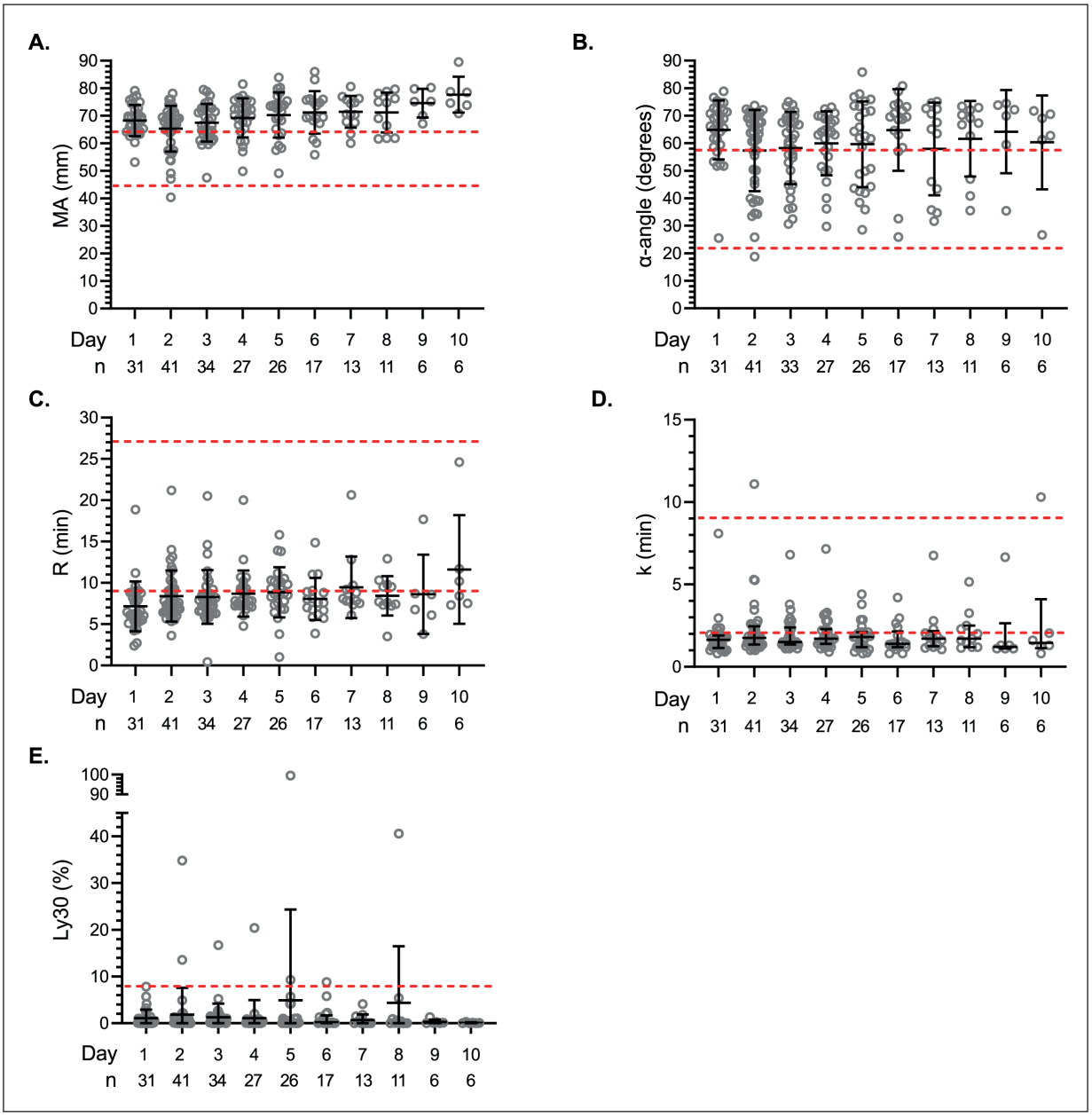

Figure 1. Coagulation parameters throughout hospitalization. Thromboelastography measures, MA (**A**), α-angle (**B**), R (**C**) and k (**D**) are plotted over each day of hospitalization, where red dashed lines represent the reference range according to the manufacturer. Ly30 (**E**) is often described as normal under 8%, represented by the single dashed line. Individual points represent a patient result with overlaid error bars representing mean and standard deviation for data with an approximately normal distribution in **A**-**C**. For non-normally distributed data in **D**, **E**, error bars represent median and interquartile range.

ly anticoagulated on admission with all but four receiving enoxaparin. On admission, 75% of patients required oxygen supplementation.

The day of illness was estimated based on patient reported symptom onset and was a median of 6 days (IQR: 5-7) upon admission. Patients remained in the hospital for a median of 5 days (IQR: 3-7). The score peaked at a median of 4 (IQR: 1-7). Peak NEWS correlated positively with length of hospitalization ($r = 0.75$, 95% CI, 0.59-0.85, $p < 0.0001$), validating its use in our study. Upon discharge, most patients had significantly decreased NEWS compared to peak NEWS ($p < 0.0001$), with a median score of 1 (IQR: 0-3). All but 5 patients hospitalized for more than 24 hours (42/47) discharged with a score ≤ 4, the lowest risk category.

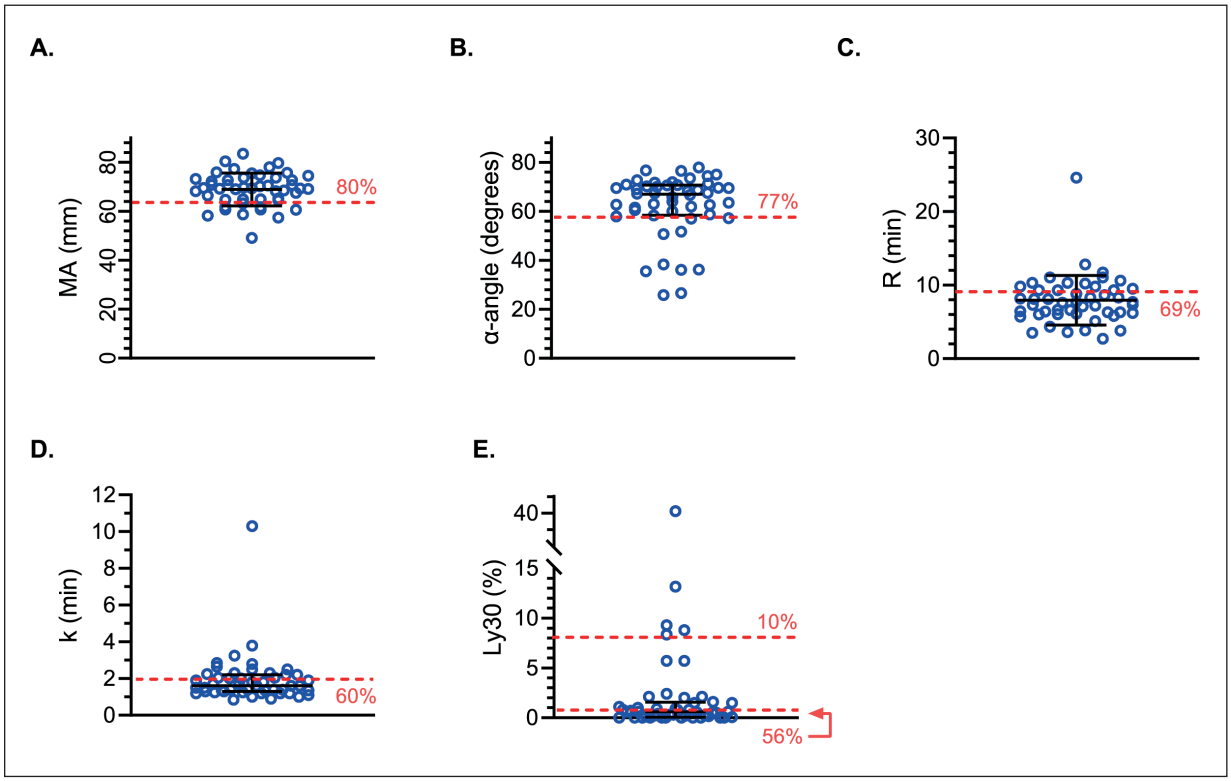

Figure 2. Thromboelastography on the day of patient discharge. Each point represents an individual patient result. MA (**A**) and α-angle (**B**) were procoagulable for points falling above the red dashed line, which represents the upper limit of the reference range. R, (**C**) and k (**D**), points falling below the dashed line are procoagulable, with the line representing the lower limit of the reference range. For Ly30 (**E**) points falling below the upper line represent potentially normal results, while points falling below the lower dashed line represent potentially hypofibrinolytic results. Error bars represent mean and standard deviation for the normally distributed data in **A**, and **C**. Error bars represent median and interquartile range for non-normally distributed data in **B**, **D**, **E**. **A**-**E**; n = 48.

Persistent Hypercoagulable Thromboelastography Profiles

Thromboelastography profiles were plotted by hospitalization day (Figure 1A-E). Within-subjects analysis showed no significant change to TEG parameters over hospitalization, except for maximum amplitude (MA) which increased slightly o ver time (p = 0.012). As reported by others[4,8,13], MA, a measure of clot firmness, was the parameter most frequently outside of the reference range in COVID-19 positive patients. At discharge, 80% of patients had an elevated MA (Figure 2A). For 21 patients, α-angle was found to remain abnormal (>58°) throughout the entirety of their hospitalization. In total 46 patients had one or more tests result with an α-angle >58°, while 37 patients (77%) had an abnormally high α-angle on discharge (Figure 2B).

Clot reaction time (R), remained abnormally short (< 9 min) throughout the entirety of 16 patient's hospitalization, while 36 patients had at least one R value < 9 min. At discharge, 33 patients (69%) had an R value < 9 min (Figure 2C). Similarly, k, a measure of clot formation kinetics, remained abnormally short (< 2 min) for 22 patients, while 43 patients had at least one shortened k value and 2 patients had one prolonged k value each (> 9 min). At discharge, 31 patients (60%) had a k value < 2 min (Figure 2D). TEG values at discharge were not significantly different between males and females (Table II). TEG values at discharge were also not significantly different between subjects 18 to 44 years old compared to subject's 45 years or older (Table II).

Hypofibrinolysis and Elevated d-dimer

The degree of fibrinolysis measured by TEG as the change in clot amplitude after 30 min (Ly30) is shown in Figure 1E. There are conflicting definitions of abnormal fibrinolysis. One standard for normal fibrinolysis is Ly30 < 8%, from the TEG manufacturer. Fibrinolytic shutdown has been defined as an elevated d-dimer paired with a Ly30 < 0.8%[14]. We found Ly30 was consistently < 8%

Table II. Thromboelastography values by Age and Sex. TEG results on the day of patient discharge from the hospital were not different between sex or age. Values are represented as mean (standard deviation). †, *t*-test.

		Thromboelastography Measure			
	n	MA (mm)	α-angle(degrees)	R (min)	k (min)
Reference Range		44-64	22-58	9-27	2-9
Overall	48	68.9 (6.7)	62.4 (12.9)	7.9 (3.4)	1.9 (1.4)
By Sex					
Male	30	69.1 (7.4)	60.6 (12.6)	8.5 (3.7)	2.1 (1.6)
Female	18	68.8 (5.6)	65.4 (13.2)	6.9 (2.6)	1.7 (0.8)
p-value		0.86†	0.21†	0.11†	0.29†
By Age					
18-44	21	68.6 (7.0)	61.3 (13.4)	8.0 (2.5)	1.9 (0.7)
≥45	27	69.2 (6.6)	63.2 (12.7)	7.9 (4.0)	2.0 (1.8)
p-value		0.73†	0.61†	0.98†	0.74†

for 83% of patients (40/48) and < 0.8% for 44% of patients. At discharge, Ly30 was < 0.8% for 27 patients (56%) (Figure 2E). We have incomplete results for serial d-dimer tests, but all 34 patients with at least one d-dimer test had a minimum of one elevated result [>0.5 μg/mL fibrinogen equivalent units (FEU)] and 5 patients had results >2.6 μg/mL FEU. Of the patients with both d-dimer and Ly30 results, 62% had an elevated d-dimer (> 0.5 μg/mL FEU) and Ly30 <0.8% on the same day or within one day of each other.

Discussion

Our data confirm that COVID-19 infection is associated with procoagulable thromboelastography results. Interestingly, the patients in our study were only moderately ill, improved clinically and were ultimately discharged from the hospital. Our cohort of 48 patients had a lower incidence of thrombotic events or death than previously reported. Despite the clinical improvement, TEG results remained prothrombotic at discharge.

Others have noted a similar prothrombotic state *via* TEG and ROTEM[13]. Despite the high prevalence of thrombotic events associate with COVID-19 infection, these studies have largely been unable to discriminate between patients with or without thrombotic events by TEG or ROTEM. One small study of 21 critically ill COVID-19 patients found that the 10 patients with at least 2 thrombotic events had significantly higher MA compared to the remaining 11 patients with 2 or less thrombotic events[8]. Conversely, at least two other studies of COVID-19 patients admitted to intensive care units have demonstrated hypercoagulability by TEG but were also unable to discriminate between those with and without thrombotic complications[15,16]. Although our prothrombotic TEG results are consistent with the literature, the implications remain unclear.

Impaired fibrinolysis has been suggested as a key mechanism in COVID-19 coagulopathy detectable by TEG and ROTEM[17-21]. In one study of critically ill COVID-19 patients, patients with 0% Ly30 and d-dimer >2,600 ng/ml had a VTE rate of 50% compared to 0% for those without either risk factor[19]. In our study, only one patient met this criterion, and this was one of the 3 patients to suffer a thromboembolic event. Of the remaining 2 patients with VTE, one had 0% Ly30, but d-dimer levels were not tested. The final VTE patient had a maximum d-dimer of 2.4 μg/mL FEU upon admission and a minimum Ly30 of 0.68% 2 days later. However, five other patients had similar results with d-dimer >2.4 μg/mL FEU and Ly30 < 0.8% that did not suffer VTE during their hospitalization, so the predictive value remains unclear.

Finally, nearly all patients in our study were treated with potentially confounding drugs. All but 3 patients received anti-coagulation treatment, however, nearly all patients remained prothrombotic by TEG at discharge. Because all patients were prophylactically anticoagulated, it is unclear if this, or any other treatments, significantly altered markers of hypercoagulability such as d-dimer or TEG. It has been shown that while thromboprophylaxis with nadroparin reduced some patients' d-dimer levels, fibrinogen and clot firmness, they primarily remained above normal[22]. Similarly, COVID-19 patients' R values displayed an inadequate pharmacodynamic response to prophylactic doses of enoxaparin[23]. These studies support that a) the hypercoagulable state observed in our study was not likely drastically altered by prophylactic doses of

anti-coagulants and b) TEG could be valuable to guide more effective anti-coagulant dosing.

Conclusions

In this descriptive study, we noted the sustainment of prothrombotic TEG parameters despite clinical improvement and ultimate discharge from the hospital. The duration beyond clinical recovery at which these patients potentially remain at risk for thromboembolic events is unknown. Our data suggests these abnormal coagulation profiles may persist even in less severe COVID-19 cases and for longer than previously thought. The impact of this persistent state warrants further investigation given the propensity of thromboembolic events in COVID-19 patients. More work is required before specific TEG parameters and ranges can be used as a tool to tailor and monitor treatments for COVID-19 patients.

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Disclaimers

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Authors' Statement

SS and EF contributed to study conception, design, and interpretation of data. EF contributed to data analysis, critical writing and revising the intellectual content. CT and TL contributed to data collection and interpretation of data. PM contributed to data analysis. All authors contributed to the final approval of the version to be published.

Conflict of Interest

The authors declare that they have no conflict of interest.

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