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RESEARCH LETTER

Platelet and Vascular Biomarkers Associate With Thrombosis and Death in Coronavirus Disease

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The SARS-CoV-2 (SARS-CoV-2 severe acute respiratory syndrome coronavirus 2) coronavirus disease (COVID-19) is a global pandemic. Laboratory testing suggests a coagulopathy with up to 30% of hospitalized patients with COVID-19 developing thrombotic events.[1] Platelets are central protagonists in both arterial and venous thrombosis, and virus-platelet interactions contribute to thrombotic risk, promoting an overall procoagulant and inflammatory states during viral infection.[2] Furthermore, recent studies report platelets to be hyperactivated in subjects with COVID-19.[3]

We speculated that in COVID-19, biomarkers of platelet activation are associated with incident thrombosis or death. Thus, we investigated in vivo surrogate biomarkers of platelet activation and vascular inflammation collected in the early phase of COVID-19 hospitalization. Plasma levels of soluble CD40 ligand (sCD40L), P-selectin, the metabolite of thromboxane A_2, thromboxane B_2 (TxB_2), and mean platelet volume (MPV) were assessd. Venous blood samples were collected within 24 hours of hospital admission to NYU Langone Health between March 15 and April 20, 2020, in accordance with the policies of the NYU Langone Health Institutional Review Board. Plasma was collected by centrifugation of PST tubes; MPV was measured during routine care from EDTA tubes on a SYSMEX analyzer.

Among 100 randomly selected hospitalized patients with COVID-19, median age was 65 years, 39 were female, 48 were White, 53 had hypertension, and at admission, 27 were on antiplatelet therapy, of whom 24 (89%) were on aspirin. Thrombosis or death occurred in 32 subjects (24 deaths and 14 thrombotic events [8 VTE (venous thromboembolism), 5 myocardial infarction, and 1 VTE and myocardial infarction]), 6 subjects experienced a thrombotic event and subsequently died. Patients with thrombosis or death were older and more likely to have chronic obstructive pulmonary disease than those without an event. There was no significant difference in platelet count or the use of antiplatelet therapy at presentation between groups (Table 1).

We analyzed banked samples collected on the day of COVID-19 diagnosis to investigate in vivo platelet activity and vascular health biomarkers. Following adjustment for age, sex, race/ethnicity, antiplatelet therapy, platelet count, and chronic obstructive pulmonary disease, TxB_2 (P=0.006), P-selectin (P=0.005), sCD40L (P=0.016), and MPV (P=0.012) were independently associated with the composite of thrombosis or death. Of the 14 patients who experienced a thrombotic event, only TxB_2 was associated with thrombosis after multivariable adjustment (P=0.013). Of the 24 patients who died TxB_2 (P=0.006), P-selectin (P=0.005), sCD40L (P=0.016), and MPV (P=0.012) were associated with all-cause mortality after multivariable adjustment (Table 2). Aspirin was associated with a lower TxB_2 (P<0.001; data not shown) and was not associated with other measured biomarkers. In a sensitivity analysis, the association between TxB_2 and thrombosis or death remained robust (P=0.012) after excluding subjects on aspirin.

Patients hospitalized with COVID-19 are at increased risk for thrombosis, and autopsy data from our group and others demonstrate micro- and macro-thrombi across vascular beds in patients with and without clinical thrombosis.[4,5] Randomized trials are ongoing testing dosing strategies of anticoagulation. However, emerging data suggest that patients continue to accrue thrombotic events even on high-dose anticoagulation.

Key Words: COVID-19 ■ pandemics ■ platelet activation ■ thrombosis ■ thromboxane

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Table 1. Baseline Characteristics of Patients With COVID-19 Stratified by the Incidence of Thrombosis or Death

	No Death or Thrombosis (N=68)	Death or Thrombosis (N=32)	*P* Value
Age, y, median [IQR]*	63.50 [48.50–73.00]	69.50 [63.00–80.00]	0.002
Female, n (%)†	27 (39.7)	12 (37.5)	1
Race, n (White, %)†	30 (46.2)	18 (58.1)	0.383
BMI, median [IQR], kg/m^2*	27.62 [24.77–31.45]	28.88 [25.42–33.36]	0.483
Smoker, n (current/former, %)†	13 (20.3)	8 (25.8)	0.733
Hypertension, n (%)†	33 (48.5)	20 (62.5)	0.275
Hyperlipidemia, n (%)†	17 (25.0)	12 (37.5)	0.294
Diabetes mellitus, n (%)†	13 (19.1)	8 (25.0)	0.681
Coronary artery disease, n (%)†	8 (11.8)	5 (15.6)	0.828
Stroke or TIA, n (%)‖	3 (4.4)	0 (0.0)	0.549
COPD, n (%)‡	0 (0.0)	5 (15.6)	0.003
Chronic kidney disease, n (%)†	6 (8.8)	4 (12.5)	0.722
Cancer, n (%)‡	3 (4.4)	4 (12.5)	0.206
Atrial fibrillation, n (%)‡	2 (2.9)	3 (9.4)	0.324
Antiplatelet therapy, n (%)†	18 (26.5)	9 (28.1)	1
Aspirin, n (%)†	16 (23.5)	8 (25.0)	1
Clinical labs			
WBC, median [IQR] cells/L*	7.0 [5.2–8.9]	8.1 [6.1–11.8]	0.080
Lymphocyte, median [IQR] cells/L*	11.0 [8.0–14.3]	10.50 [7.0–14.3]	0.859
Platelet count, median [IQR] 10^9/L*	205.0 [164.8–253.8]	187.5 [147.5–257.5]	0.385
Mean platelet volume, median [IQR] fL*	10.55 [10.1–11.2]	11.00 [10.5–11.9]	0.022
Hemoglobin, median [IQR] g/dL*	13.15 [11.8–14.3]	13.40 [11.8–14.4]	0.894

Two-sided $P<0.05$ were considered statistically significant. n=100 for all analyses. BMI indicates body mass index; COPD, chronic obstructive pulmonary disease; and IQR, interquartile range. TIA indicates transient ischemic attack; and WBC, white blood cell.

*Continuous variables were compared with Mann-Whitney test.

†Categorical variables were compared with χ^2 test.

‡Categorical variables were compared with Fisher exact test.

We report for the first time that biomarkers of platelet activity and vascular health, are significantly associated with the composite outcome of thrombosis or death in hospitalized patients with COVID-19. A composite outcome was used due to a competing risk of death and likely underdiagnosing of thrombotic events in hospitalized patients with COVID-19. These findings suggest that multiple platelet-related processes contribute to thrombosis and mortality in patients with COVID-19. Increased plasma TxB_2 levels indicate the activation of platelets via COX-1. Moreover, both platelet P-selectin and sCD40L contribute to thrombosis by supporting platelet-myeloid heteroaggregate formation and thrombi stability by interaction with PSGL-1 and αIIbβ3, respectively. Our current study does not characterize the cellular source of measured biomarkers, thus in addition to platelets, plasma P-selectin and sCD40L may originate from alternate sources including endothelial cells and T

Table 2. Multivariable Regression Models Predicting Thrombosis or All-Cause Mortality, Thrombosis, and All-Cause Mortality

	Thrombosis or All-Cause Mortality		Thrombosis		All-Cause Mortality	
Parameter	OR [95% CI]	*P* Value	OR [95% CI]	*P* Value	OR [95% CI]	*P* Value
TxB_2	2.59 [1.37–5.43]	0.006	2.96 [1.36–7.75]	0.013	2.04 [1.07–4.11]	0.036
MPV	2.17 [1.22–4.15]	0.012	2.05 [0.98–4.71]	0.066	2.33 [1.27–4.67]	0.010
sCD40L	1.94 [1.15–3.43]	0.016	1.18 [0.55–2.33]	0.639	1.93 [1.1–3.43]	0.019
P-selectin	2.36 [1.36–4.56]	0.005	1.36 [0.73–2.51]	0.312	2.22 [1.29–4.19]	0.007

OR from logistic regression analysis per SD increase for biomarker levels adjusted for age, sex, race, antiplatelet therapy, platelet count, and COPD. Plasma was collected in PST tubes, TxB_2 levels were measured by ELISA (Cayman Chemical; 1:100 dilution), P-Selectin and sCD40L were measured by LEGENDplex bead-based immunoassay (1:50 dilution). MPV was measured via automated hemogram (Sysmex, Japan) from EDTA blood collection tubes. Multivariate logistic regression was performed with all-cause mortality, thrombosis, and death/thrombosis as dependent outcomes. Candidate covariates were chosen because of their known association with the outcome of interest as well as statistical differences on univariate testing. ORs and 95% CIs were reported. Two-sided $P<0.05$ were considered statistically significant. n=100 for all analyses. COPD indicates chronic obstructive pulmonary disease; MPV, mean platelet volume; OR, odds ratio; sCD40L, soluble CD40 ligand; and TxB_2, thromboxane B_2.

cells, respectively. However, consistent with our surrogate biomarkers of platelet activity is the similar association obtained with the platelet-specific marker, MPV, a biomarker of platelet hyperactivity.

Our findings are consistent with recent reports of platelet hyperactivity in patients with COVID-19.[3] We extend those finding and demonstrate that biomarkers of platelet activation are associated with thrombosis or death in patients hospitalized with COVID-19. Our findings suggest platelet activation mechanisms may contribute to adverse events and warrant further investigation into the mechanistic role of platelets in COVID-19 pathogenesis and highlight the potential role of antiplatelet therapy.

ARTICLE INFORMATION

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Disclosures

None.

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