RESEARCH ARTICLE

Complement levels at admission as a reflection of coronavirus disease 2019 (COVID-19) severity state

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Abstract

Complement system hyperactivation has been proposed as a potential driver of adverse outcomes in severe acute respiratory syndrome coronavirus 2 infected patients, given prior research of complement deposits found in tissue and blood samples, as well as evidence of clinical improvement with anticomplement therapy. Its role in augmenting thrombotic microangiopathy mediated organ damage has also been implicated in coronavirus disease 2019 (COVID-19). This study aimed to examine associations between complement parameters and progression to severe COVID-19 illness, as well as correlations with other systems. Blood samples of COVID-19 patients presenting to the emergency department (ED) were analyzed for a wide panel of complement and inflammatory biomarkers. The primary outcome was COVID-19 severity at index ED visit, while the secondary outcome was peak disease severity over the course of illness. Fifty-two COVID-19 patients were enrolled. C3a ($p = 0.018$), C3a/C3 ratio ($p = 0.002$), and sC5b-9/C3 ratio ($p = 0.021$) were significantly elevated in with severe disease at ED presentation. Over the course of illness, C3a ($p = 0.028$) and C3a/C3 ratio ($p = 0.003$) were highest in the moderate severity group. In multivariate regression controlled for confounders, complement hyperactivation failed to predict progression to severe disease. C3a, C3a/C3 ratio, and sC5b-9/C3 ratio were correlated positively with numerous inflammatory biomarkers, fibrinogen, and VWF:Ag, and negatively with plasminogen and ADAMTS13 activity. We found evidence of complement hyperactivation in COVID-19, associated with hyperinflammation and thrombotic microangiopathy. Complement inhibition should be further investigated for potential benefit in patients displaying a hyperinflammatory and microangiopathic phenotype.

KEYWORDS
complement system, coronavirus disease 2019, SARS-CoV-2 hyperinflammation, thrombotic microangiopathy

1 | INTRODUCTION

Evidence has emerged that multiorgan injury from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is propagated by a maladaptive, dysregulated host immune response, including complement hyperactivation.[1–3] The complement system plays a central role within the innate immune system, responding to invading pathogens via the classical, alternative, and lectin pathways, which all converge at component 3 (C3), a core constituent in this pathway.[4] This common product results in enzymatically-driven

formation of many activation products (C3a, C5a), and culminates in formation of the membrane attack complex (C5b-9), which kills invading pathogens via generation of membrane pores.[4] Complement not only plays a key role in the first line of defense against infectious agents, but also acts to bridge innate and adaptive immune responses through activation of T and B cells, and creation of immunologic memory.[5]

While complement is essential in defense against viral infections, its hyperactivation and dysregulation can result in widespread systemic multiorgan damage.[6] Moreover, complement mediates vascular injury in various thrombotic microangiopathies (TMAs), such as atypical hemolytic uremic syndrome and antiphospholipid syndrome.[7,8] In keeping with this previous evidence, complement has been implicated in the pathogenesis of microvascular thrombosis frequently observed in patients with coronavirus disease 2019 (COVID-19).[9]

In five COVID-19 patients with pulmonary and dermatologic signs of microvascular thrombosis, Magro et al. identified terminal complement (C5d-9), C4d, and mannose-binding protein-associated serine protease 2 (MASP-2) deposits in biopsies of lung and skin tissue, thus highlighting a potential contribution of the complement pathway to the process of thrombotic microvascular injury via endothelial damage and subsequent activation of the coagulation cascade.[9] Gao et al.[10] performed lung biopsies of patients who died from COVID-19, and also reported evidence of complement activation, including deposits of mannose binding lectin, MASP-2, C4a, C3, and C5b-9. Additionally, patients with severe COVID-19 illness were found to have significantly increased serum C5a, a potent anaphylatoxin which triggers the release of a myriad of inflammatory cytokines from leukocytes.[2,10] Holter and colleagues observed increased values of all markers of complement early in the course of SARS-CoV-2 infection, as well as an association of C4d and C5b-9 with respiratory failure and systemic inflammation.[11] In accordance with these findings, small case series using anti-C5a and anti-C5 monoclonal antibodies (BDB-0001 and eculizumab) in patients with COVID-19 reported decreases in systemic inflammation and improvement in pulmonary function.[10,12]

Hence, in this study we have carried out an assessment of a complete complement panel in patients with COVID-19, using blood samples collected at initial presentation at the Emergency Department (ED). Our aim was to analyze the relationship between complement parameters and COVID-19 severity, as well as explore correlations between the complement cascade and other systems, to help elucidate the pathophysiology of SARS-CoV-2 infection.

2 | MATERIALS AND METHODS

Adult patients with reverse transcriptase polymerase chain reaction confirmed SARS-CoV-2 infection presenting at the ED of the University of Cincinnati Medical Center\ between April and May 2020 were enrolled in this prospective, observational study. Blood samples were taken as part of routine blood draws in the ED, and analyzed at the Clinical Nephrology Lab of the Cincinnati Children's Hospital Medical Center, a national referral center for complement testing. This study was approved by the Institutional Review Board of the University of Cincinnati and received a waiver of informed consent. This study was conducted in compliance with the Declaration of Helsinki, under the terms of relevant local and national legislation.

Serum levels of 50% hemolytic complement activity (CH50, representing total complement activity, MicroVue; Quidel Corporation), alternative pathway activity and lectin pathway activity (Wieslab, SVAR) were assessed using enzyme-linked immunosorbent assays (ELISA). Quidel MicroVue ELISA kits were also used to measure complement components, including C3a, C4a, C5a, sC5b-9, Bb, and C1 inhibitor activity. C3, C4, and C1 inhibitor antigen were measured by immunonephelometry on a Behring Nephelometer II (BNII; Siemens Medical Solutions USA). Radioimmunoassays were performed using both institutionally developed (Factor I: Cincinnati Children's Hospital Medical Center) and commercially available antisera (Factor B, Factor H, C1q: Complement Technology, Inc.).

Inflammatory markers evaluated by immunonephelometry included C reactive protein (CRP), ferritin, haptoglobin, myoglobin, fibrinogen, and plasminogen (BNII System; Siemens Medical Solutions USA). Interleukin (IL)-6, IL-8, IL-10, and tumor necrosis factor-α (TNF-α) were run on a multi-analyte immunoassay (MesoScale Diagnostics LLC). PAI-1 and von Willebrand Factor antigen (VWF:Ag) were assayed with ELISA (Technoclone), whilst ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) activity was quantified using a fluorescence resonance energy transfer (FRET) assay (Immucor, Inc.). Lactate dehydrogenase (LDH) was measured on RxL Integrated Chemistry System (Siemens Medical Solutions USA). Complete blood cell count and differential was carried out on Beckman Coulter UniCel DxH 800 Cellular Analysis System (Brea). All assays were run according to manufacturer instructions; ELISA assays were run on either the DS2 or DSX automated ELISA processing systems (DYNEX Technologies). The primary outcome was COVID-19 disease severity at index ED visit, while the secondary outcome was peak disease severity over the course of illness. Patients were stratified into three severity level classes, according to the World Health Organization R&D Blueprint for COVID-19 severity scale,[13] as follows: mild (ambulatory), moderate (hospitalized), severe (requiring intensive care unit admission).

Categorical variables were reported as frequencies (%), whilst continuous data was described using median and interquartile ranges (IQR). Statistically significant differences in laboratory values among groups were identified using the Kruskal–Wallis test, followed by Dunn test for multiple comparisons (when necessary). The relationship between complement parameters and inflammatory biomarkers was tested using Spearman's correlation coefficient. C3a/C3 and sC5b-9/C3 ratios were calculated to assess potential alternative pathway and terminal pathway hyperactivation, respectively. Multivariable logistic regression was used to identify complement variables independently predicting disease severity after adjusting for age, sex, and comorbidities, with calculation of odds ratios and their 95% confidence intervals (95% confidence interval). Variable

TABLE 1 Patient demographics, comorbidities, and outcomes

Demographics	
Males, n (%)	31 (59.6%)
Age, median (IQR)	50.5 (41.5–66.0) years
Race, n (%)	
Black	21 (40.4%)
Hispanic	19 (36.5%)
White	9 (17.3%)
Other	3 (5.8%)
Comorbidities, n (%)	
Hypertension	26 (50%)
Coronary artery disease	8 (15.4%)
Heart failure	9 (17.3%)
Hyperlipidemia	15 (28.8%)
Diabetes	21 (40.4%)
COPD	8 (15.4%)
Chronic kidney disease	6 (11.5%)
Chronic liver disease	7 (13.5%)
Emergency department disease status, n (%)	
Mild	19 (36.5%)
Moderate	27 (51.9%)
Severe	6 (11.5%)
Peak disease status, n (%)	
Mild	19 (36.5%)
Moderate	17 (31.7%)
Severe	16 (30.8%)

Abbreviations: COPD, chronic obstructive lung disease; IQR, interquartile range.

selection was based on univariate analysis and Stepwise algorithm. All statistical analyses were performed using R software (version 4.0.2; R Foundation for Statistical Computing), with $p < 0.05$ being considered statistically significant.

3 | RESULTS

A total number of 52 adult patients with laboratory-confirmed SARS-CoV-2 infection were enrolled in the study. The median age was 50.5 (IQR: 39.3–66.0) years and 30 (57.7%) were males. Patient demographics and outcomes are summarized in Table 1. At ED presentation, 6 patients (12%) were severe, while over the course of illness 16 (31%) patients reached a peak severe disease status.

The values of complement parameters stratified according to COVID-19 disease severity at ED presentation and peak are summarized in Table 2. Statistically significant elevations were observed in C3a ($p = 0.018$) over the normal range, as well as C3a/C3 ratio ($p = 0.002$) and sC5b-9/C3 ratio ($p = 0.021$) in those with severe disease at ED presentation (Figure 1). For peak disease, only C3a ($p = 0.028$) and the C3a/C3 ratio ($p = 0.003$) were significantly different between severity groups, with moderate group displaying the highest values. C5a and sC5b-9 had nonsignificant trends toward higher levels those with more severe disease both at ED presentation and at peak, although levels were relatively within normal range. When adjusted for age and comorbidities, no complement variable measured at ED presentation was predictive of progression to severe disease during illness.

Spearman's correlation coefficients representing association between C3a, C3a/C3, as well as sC5b-9/C3 and a panel of inflammatory markers are presented in Table 3. Levels of the individual inflammatory markers measured at ED presentation are summarized in Table S1. Increased C3a was positively and significantly correlated with CRP ($r = 0.630$), ferritin ($r = 0.485$), haptoglobin ($r = 0.437$), IL-6 ($r = 0.626$), IL-8 ($r = 0.470$), IL-10 ($r = 0.522$), LDH ($r = 0.432$), TNF-α ($r = 0.514$), myoglobin ($r = 0.492$), fibrinogen ($r = 0.487$), as well as with VWF:Ag ($r = 0.484$), all at a $p \leq 0.001$. C3a levels were negatively associated with ADAMTS13 activity ($r = -0.416$, $p = 0.002$) and ratio of ADAMTS13/VWF:Ag ($r = -0.630$, $p < 0.001$). Increased C3a/C3 was positively and significantly correlated with CRP ($r = 0.478$, $p < 0.001$), ferritin ($r = .389$, $p = 0.04$), IL-6 ($r = 0.631$, $p < 0.001$), IL-8 ($r = 0.573$, $p < 0.001$), IL-10 ($r = 0.505$, $p < 0.001$), LDH ($r = 0.430$, $p = 0.001$), TNF-α ($r = 0.632$, $p < 0.001$), myoglobin ($r = 0.540$, $p < 0.001$), and VWF:Ag ($r = 0.405$, $p = 0.003$). C3a/C3 levels were negatively associated with plasminogen ($r = -0.305$, $p = 0.029$), ADAMTS13 activity ($r = -0.552$, $p < 0.001$) and ratio of ADAMTS13/VWF:Ag ($r = -0.640$, $p < 0.001$). Finally, the ratio of sC5b-9/C3 had a statistically significant positive correlation with CRP ($r = 0.458$, $p < 0.001$), ferritin ($r = 0.350$, $p = 0.012$), haptoglobin ($r = 0.289$, $p < 0.039$), IL-6 ($r = 0.540$, $p < 0.001$), IL-8 ($r = 0.333$, $p = 0.018$), IL-10 ($r = 0.405$, $p = 0.003$), LDH ($r = 0.300$, $p = 0.032$), TNF-α ($r = 0.305$, $p = 0.031$), myoglobin ($r = 0.307$, $p = 0.028$), VWF:Ag ($r = 0.466$, $p < 0.001$), and a negative association with ADAMTS13 ($r = -0.354$, $p = 0.011$), and ADAMTS13/VWF:Ag ($r = -0.520$, $p < 0.001$). None of the complement markers were correlated significantly with PAI-1, D-dimer, white leukocyte or lymphocyte counts.

4 | DISCUSSION

In this prospective study, we observed evidence of complement hyperactivation in patients presenting to the ED with severe disease, as reflected by significant increase in C3a above the norm, as well as elevations in the C3a/C3 and sC5b-9/C3 ratios, which are suggestive of alternative and terminal pathway hyperactivation, respectively.

C3a is an anaphylatoxin that induces inflammation, endothelial activation,[14] as well as coagulation via binding of the C3a receptor (C3aR) on platelets,[9,15] in turn aggravating microthrombosis and contributing to a TMA-like phenomenon.[16] Interestingly though, emerging evidence also proposes an

TABLE 2 Complement levels measured on admission in patients with COVID-19 stratified by disease severity upon emergency department (ED) presentation and at peak during hospitalization

Lab variable	Reference range	COVID-19 severity at ED presentation				Peak COVID-19 severity			
		Mild (n = 19)	Moderate (n = 27)	Severe (n = 6)	p value	Mild (n = 19)	Moderate (n = 17)	Severe (n = 16)	p value
Alternative pathway, %	>63%	103.4 (90.1–108.9)	89.7 (77.7–102.0)	92.5 (81.0–100.0)	0.102	103.4 (90.1–108.9)	89.3 (82.0–102.3)	94.6 (77.1–100.6)	0.099
Lectin pathway, %	>11%	88.1 (1.6–120)	23.2 (0.6–99.9)	51.7 (1.2–106.9)	0.516	88.1 (1.6–120)	20.2 (0.1–103.6)	37.0 (3.8–101.5)	0.398
Classical pathway, CH50/ml	101–300 units	211.8 (162.7–280.8)	196.2 (142–257.6)	169.6 (138.8–232.1)	0.457	211.8 (162.7–280.8)	240.3 (148.2–306.0)	159.8 (127.6–195.2)	0.075
C3, mg/dl	71–150 mg/dl	151.0 (120.5–169.0)	140.0 (119.0–153.5)	134.5 (100.3–166.2)	0.723	151.0 (120.5–169.0)	143.0 (121.0–158.0)	132.5 (112.0–149.5)	0.501
C3a, ng/ml	25–88.2 ng/ml	205.4 (138.1–218.0)	244.0 (174.6–392.0)	389.1 (226.9–499.2)	**0.018**	205.4 (138.1–218.0)	299.6 (179.2–397.4)	229.8 (195.1–482.0)	**0.028**
C4, mg/dl	15.7–47 mg/dl	33.5 (28.0–36.1)	31.5 (26.4–39.9)	37.0 (25.4–45.3)	0.868	33.5 (28.0–36.1)	33.5 (25.9–42.3)	30.9 (25.9–38.2)	0.787
C4a, ng/ml	383.5–8168.2 ng/ml	445.7 (276.9–692.5)	498.5 (352.6–1154.5)	585.8 (386.8–815.8)	0.347	445.7 (276.9–692.5)	709.7 (476.6–1198.9)	420.4 (334.4–847.5)	0.098
C5a, ng/ml	2.7–16.3 ng/ml	8.7 (6.9–14.1)	16.8 (8.1–23.8)	16 (9.1–24.2)	0.150	8.7 (6.9–14.1)	23.3 (7.2–25)	12.5 (9.4–20)	0.115
C1q, mg/dl	5.1–7.5 mg/dl	4.8 (3.8–5.9)	5.1 (3.3–6.9)	4.8 (1.7–5.7)	0.909	4.83 (3.8–5.9)	5.7 (3.1–6.9)	4.8 (3.0–6.0)	0.865
Factor B, mg/dl	13.3–31.5 mg/dl	15.6 (12.0–19.6)	15.2 (10.4–18.6)	13.2 (8.3–18.8)	0.785	15.6 (12.0–19.6)	15.6 (10.4–18.6)	13.2 (10.3–19.9)	0.821
Factor Bb, µg/ml	0.49–1.42 µg/ml	1.24 (0.96–1.92)	1.75 (1.32–2.58)	2.16 (1.25–2.68)	0.192	1.24 (0.96–1.92)	1.69 (1.29–2.37)	2.07 (1.26–2.62)	0.185
sC5b-9, ng/ml	<244 ng/ml	125.3 (86.3–238.8)	199.6 (144.4–297.5)	238.5 (207.2–273.3)	0.094	125.3 (86.3–238.8)	203.1 (139.6–314.1)	213.4 (178.5–258.6)	0.166
C1-inhibitor antigen, mg/dl	21.0–39.0 mg/dl	37.6 (33.4–48.0)	44.8 (36.1–54.2)	43.2 (42.9–51.3)	0.255	37.6 (33.4–48.0)	44.3 (35.9–54.5)	44.0 (42.3–54.0)	0.242
C1-inhibitor activity, %	>67%	92.0 (88.1–96.6)	95.5 (89.8–97.3)	91.7 (90.4–94.4)	0.647	92.0 (88.1–96.6)	95.5 (89.6–97.5)	94.2 (90.2–96.6)	0.840
Factor H, mg/dl	37.0–68.0 mg/dl	70.6 (58.0–84.4)	68.7 (52.7–82.5)	52.0 (42.8–66.9)	0.283	70.6 (58.0–84.4)	72.4 (55.4–79.9)	59.4 (45.9–76.2)	0.431
Factor I, mg/d	2.4–4.9 mg/d	4.45 (3.98–5.51)	4.6 (3.7–5.4)	4.1 (3.4–4.8)	0.742	4.4 (4.0–5.5)	4.8 (4.3–5.4)	4.3 (3.3–5.0)	0.568
C3a/C3	–	1.1 (1.1–1.7)		1.7 (1.5–2.8)	**0.002**	1.1 (1.1–1.7)	2.4 (1.5–3.0)	1.9 (1.5–2.9)	**0.003**
SC5b-9/C3	–	1.11 (0.60–1.64)	1.5 (1.0–2.1)	2.0 (1.5–2.9)	**0.021**	1.1 (0.6–1.6)	1.6 (1.0–2.1)	1.6 (1.2–2.4)	0.059

Note: Bold p values indicate p < 0.05. All data presented as median (IQR), p Values calculated with Kruskal–Wallis test. CH50=50% hemolytic complement activity.
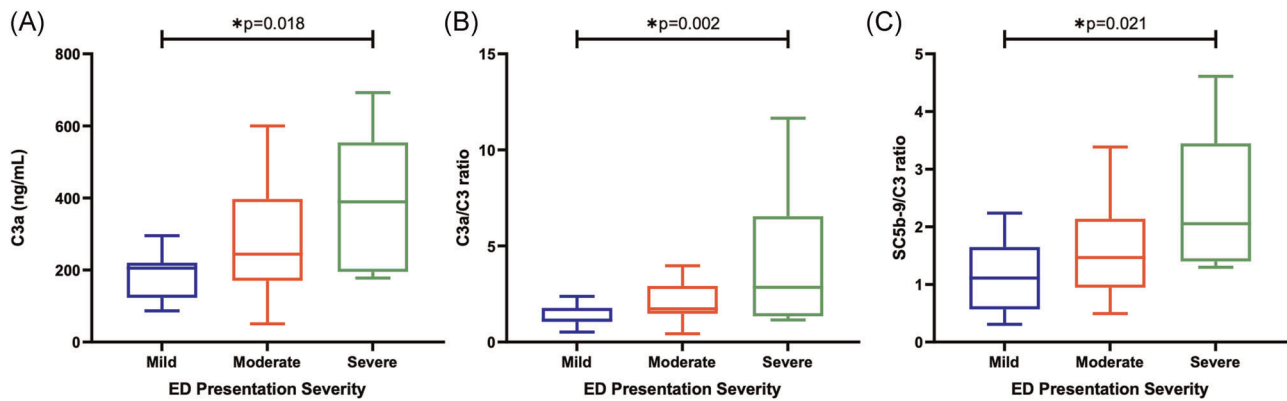
Abbreviation: COVID-19, coronavirus disease 2019.

FIGURE 1 (A) C3a levels, (B) C3a/C3 ratio, and (C) sC5b-9/C3 ratio measured at emergency department (ED) presentation according to COVID-19 severity at time of presentation. COVID-19, coronavirus disease 2019

TABLE 3 Spearman rank correlation coefficients between C3a, C3a/C3, and sC5b-9/C3 and inflammatory biomarkers

Lab variable	Spearman's correlation coefficient (p value)		
	C3a	C3a/C3	sC5b-9/C3
CRP	0.6299 (p < 0.001)	0.478 (p < 0.001)	0.458 (p < 0.001)
Ferritin	0.485 (p < 0.001)	0.389 (p = 0.004)	0.350 (p = 0.012)
Haptoglobin	0.437 (p = 0.001)	0.213 (p = 0.133)	0.289 (p = 0.039)
IL-6	0.626 (p < 0.001)	0.631 (p < 0.001)	0.540 (p < 0.001)
IL-8	0.470 (p < 0.001)	0.573 (p < 0.001)	0.333 (p = 0.018)
IL-10	0.522 (p < 0.001)	0.505 (p < 0.001)	0.405 (p = 0.003)
LDH	0.432 (p = 0.001)	0.430 (p = 0.001)	0.300 (p = 0.032)
TNF-α	0.514 (p < 0.001)	0.632 (p < 0.001)	0.305 (p = 0.031)
Myoglobin	0.492 (p < 0.001)	0.540 (p < 0.001)	0.307 (p = 0.028)
Fibrinogen	0.487 (p = 0.001)	0.271 (p = 0.090)	0.117 (p = 0.470)
Plasminogen	−0.026 (p = 0.852)	−0.305 (p = 0.029)	−0.080 (p = 0.577)
PAI-1	0.244 (p = 0.084)	0.185 (p = 0.194)	0.199 (p = 0.161)
D-Dimer	0.147 (p = 0.483)	0.245 (p = 0.238)	0.182 (p = 0.384)
WBC	0.169 (p = 0.246)	0.109 (p = 0.456)	0.249 (p = 0.084)
Lymphocytes	−0.169 (p = 0.249)	−0.232 (p = 0.112)	−0.148 (p = 0.313)
ADAMTS13 activity	−0.416 (p = 0.002)	−0.552 (p < 0.001)	−0.354 (p = 0.011)
VWF:Ag	0.484 (p < 0.001)	0.405 (p = 0.003)	0.466 (p < 0.001)
ADAMTS13/VWF:Ag	−0.630 (p < 0.001)	−0.640 (p < 0.001)	−0.520 (p < 0.001)

Note: Levels of inflammatory biomarkers are summarized in Table S1.

Abbreviations: ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; CRP, C reactive protein; IL-6, interleukin-6; LDH, lactate dehydrogenase; TNF-α, tumor necrosis factor-α; VWF:Ag, von Willebrand Factor antigen; WBC, white blood cells.

anti-inflammatory role for C3a, with potential ability to antagonize the potent anaphylatoxin and chemoattractant C5a,[17] which may potentially explain the observation of lower admission levels of C3a among the group that developed highest severity later in the course of hospitalization. This dichotomy in the function of C3a may even contribute to immune dysregulation, now a distinguishing feature of COVID-19.[3]

Finding elevations above the normal range of only C3a may seem surprising in light of several other studies, such as that by Cugno et al.,[18] as well as Carvelli et al.,[19] that observed considerable elevations in other complement cascade components implicated in inflammation and tissue damage in COVID-19, namely C5a and sC5b-9, and with levels corresponding to disease severity. Nonetheless, despite being within normal range in most of our patients

measured at time of ED presentation, C5a and sC5b-9 displayed a nonsignificant trend toward higher levels among patients with moderate and severe disease severity both in the ED and at peak during hospitalization. Moreover, the trend for sC5b-9 is in part supported by significant elevations in the sC5b-9/C3 ratio in patients with severe disease on admission. Absence of such considerable elevations in C5a and sC5b-9 in our study may be attributed to the differences in severity of patients at time of sampling and the non-significance may be explained by differences in sample sizes, whereby the smaller cohort in our study compared to the study by Cugno et al. may have limited the statistical power to identify these differences, or by heterogeneity related to underlying baseline characteristics of included patients in both studies.[20] Additionally, C5a has a much shorter half-life than relatively more stable C3a, as it quickly binds to high affinity C5a receptors (C5aR) on neutrophils,[21] complicating the ability to detect accurate levels of C5a in blood samples.

In multivariable regression, complement hyperactivation did not predict progression to severe disease in patients with mild or moderate illness at ED presentation. This suggests that complement levels may only reflect acute disease status, becoming hyperactivated following other insults during progression to more critical illness. This finding is in opposition to a recent report suggesting that complement overactivation on admission was associated with development of respiratory failure.[11] We suspect that this heterogeneity between studies may also be attributed to the differences in severity of patients at time of sampling.

Gralinski et al. earlier reported that SARS-CoV infected mice bearing a deficient C3 gene had significantly less respiratory dysfunction than those with an intact complement system, despite equivalent viral loads.[22] In COVID-19, Ramlall et al. noted that none of the patients with complement deficiency who would normally be at higher risk of developing severe infection required invasive oxygen support or died.[23] Moreover, the role of complement in driving COVID-19 severity is reflected in small clinical trials demonstrating reduced system inflammation and lung injury with anti-C5, anti-C5a, and anti-C3 monoclonal antibodies.[10,12,24]

Our results also show that C3a and the C3a/C3 and sC5b-9/C3 ratios were correlated with an array of inflammatory biomarkers, thus suggesting a close interplay between different systems. Elevated C3a was correlated with fibrinogen levels, which is in accordance with extensive cross-talk between complement and coagulation systems.[25] C3a/C3 was inversely correlated with plasminogen, in agreement with reports of lower plasminogen levels in COVID-19 patients requiring ICU admission, and thus suggesting fibrinolytic system disruption occurring in concert with complement hyperactivation.[26] Fibrinolysis disturbance due to increased PAI-1,[1] however, did not appear to be linked to complement hyperactivity, therefore suggesting that plasminogen depletion may be the result of consumptive fibrinolysis.

Positive correlations of C3a, C3a/C3, and sC5b-9/C3 with VWF:Ag, with parallel negative correlations with ADAMTS13 activity and ADAMTS13 activity/VWF:Ag ratio is strongly suggestive of a TMA-like phenomenon in COVID-19. Henry et al. provided evidence suggesting multiorgan injury in COVID-19 is driven by a secondary TMA, with low ADAMTS13 activity/VWF:Ag ratio associated with severe illness and acute kidney injury.[16] Bazzan et al. found lower ADAMTS13 activity and higher VWF:Ag to be highly predictive of fatal outcome in COVID-19.[27] Recently, by demonstrating an association between sC5b-9 and VWF, Cugno et al.[18] provided evidence suggesting a role of complement in bridging endotheliopathy and VWF-mediated hypercoagulability. Adverse outcomes in other TMAs, such as thrombotic thrombocytopenic purpura and hemolytic uremic syndrome have likewise been associated with reduced ADAMTS13 activity and elevated VWF, notably in the setting of complement activation.[28] This association may explain in part the high complement levels observed in COVID-19 patients, given that the microthrombotic process triggered by ADAMTS13 deficiency has been shown to activate complement.[29] The result is a vicious cycle, wherein complement activation results in further endothelial damage and hemostasis activation.[9] Evidence of these associations not only substantiates the use of anticoagulants and/or antiplatelet agents in hospitalized COVID-19 patients, but also warrants further investigation of the potential benefits of using anticomplement therapy.

Findings of significant correlations between complement end products and inflammatory mediators, such as CRP, IL-6, IL-8, and TNF-α corroborate the favorable effects that complement inhibitors may have in dampening the hyperinflammatory response in patients with severe COVID-19.[12,30,31] However, in light of the strong associations previously observed between anti-inflammatory IL-10 and disease progression in COVID-19 patients, our findings of positive correlations of complement with IL-10 give further evidence for a picture of immune dysregulation in COVID-19, stressing the fact that immunomodulatory therapy must be fitted to the particular patient immunophenotype.[3] Furthermore, close correlations call for further research to test whether inflammatory biomarkers may serve as a reliable proxy for the state of the complement system. This is important in light of the fact that complement biomarkers are fairly unstable, and thus may be unreliable for identification of disease severity.[6]

This study was limited by the relatively modest sample size of 52 patients, such that certain differences in complement may have been missed. Additionally, variance in findings with the limited other complement studies in patients with COVID-19 may stem from significant differences in inflammatory laboratory values at admission that have been reported in other cohorts, especially across different geographic regions.[20] As such, these values could be impacted by several confounders, such as different viral strains or underlying patient demographics.[20] These findings should thus be corroborated by additional multi-national studies. However, finding statistically significant differences in C3a level, and the C3a/C3 and sC5b-9/C3 ratios in a design with high probability of a false negative error, reinforces our confidence that the results are valid. Additionally, our study is strengthened by many multi-system biomarkers analyzed, enabling assessment of potential interaction between complement

and other systems. Finally, as this study was exploratory, we did not control for multiple comparisons.

In conclusion, our results suggest that complement hyper-activation may be associated with hyperinflammation and thrombotic microangiopathy in COVID-19. As complement activation may propagate immune-mediated and thrombotic organ damage, further studies should be planned to address whether complement inhibition may have potential therapeutic benefits in selected categories of patients with a hyperinflammatory and microangiopathic phenotype.

ACKNOWLEDGMENTS

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

AUTHOR CONTRIBUTIONS

Brandon Michael Henry, Jens Vikse, and Giuseppe Lippi conceived and designed the study. Brandon Michael Henry, Justin L. Benoit, and Stefanie W. Benoit collected samples and ran experiments. Justin L. Benoit and Maria Helena Santos de Oliveira performed data acquisition and collection. Brandon Michael Henry, Ivan Szergyuk, and Maria Helena Santos de Oliveira did data analysis. Brandon Michael Henry, Ivan Szergyuk, Giuseppe Lippi, Justin L. Benoit, Jens Vikse, and Stefanie W. Benoit interpreted the data. Ivan Szergyuk prepared the first draft. Brandon Michael Henry, Maria Helena Santos de Oliveira, Giuseppe Lippi, Justin L. Benoit, Jens Vikse, and Stefanie W. Benoit critically revised the manuscript for important intellectual content. All authors have approved the final article.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1002/jmv.27077

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

This study was approved by the Institutional Review Board (IRB) of the University of Cincinnati and received a waiver of informed consent on the basis of no more than minimal risk.

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SUPPORTING INFORMATION