Complement Levels at Admission Reflecting Progression to Severe Acute Kidney Injury (AKI) in Coronavirus Disease 2019 (COVID-19): A Multicenter Prospective Cohort Study

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Background: Dysregulation of complement system is thought to be a major player in development of multi-organ damage and adverse outcomes in patients with coronavirus disease 2019 (COVID-19). This study aimed to examine associations between complement system activity and development of severe acute kidney injury (AKI) among hospitalized COVID-19 patients.

Materials and Methods: In this multicenter, international study, complement as well as inflammatory and thrombotic parameters were analyzed in COVID-19 patients requiring hospitalization at one US and two Hungarian centers. The primary endpoint was development of severe AKI defined by KDIGO stage 2+3 criteria, while the secondary endpoint was need for renal replacement therapy (RRT). Complement markers with...
significant associations with endpoints were then correlated with a panel of inflammatory and thrombotic biomarkers and assessed for independent association with outcome measures using logistic regression.

**Results:** A total of 131 hospitalized COVID-19 patients (median age 66 [IQR, 54–75] years; 54.2% males) were enrolled, 33 from the US, and 98 from Hungary. There was a greater prevalence of complement over-activation and consumption in those who developed severe AKI and need for RRT during hospitalization. C3a/C3 ratio was increased in groups developing severe AKI (3.29 vs. 1.71; \( p < 0.001 \)) and requiring RRT (3.42 vs. 1.79; \( p < 0.001 \)) in each cohort. Decrease in alternative and classical pathway activity, and consumption of C4 below reference range, as well as elevation of complement activation marker C3a above the normal was more common in patients progressing to severe AKI. In the Hungarian cohort, each standard deviation increase in C3a (SD = 210.1) was independently associated with 89.7% increased odds of developing severe AKI (95% CI, 7.6–234.5%). Complement was extensively correlated with an array of inflammatory biomarkers and a prothrombotic state.

**Conclusion:** Consumption and dysregulation of complement system is associated with development of severe AKI in COVID-19 patients and could represent a promising therapeutic target for reducing thrombotic microangiopathy in SARS-CoV-2 infection.

**Keywords:** complement system, coronavirus disease 2019, SARS-CoV-2, acute kidney injury, renal replacement therapy (RRT)

**INTRODUCTION**

Immune dysregulation has been described as a pivotal factor in development of multi-organ injury and adverse outcomes in patients with coronavirus disease 2019 (COVID-19) (1, 2), and over activation of the complement system appears to be a significant element of this maladaptive host immune response (3–7). Several reports have found elevated levels of circulating C5a, a potent anaphylatoxin, in patients with severe COVID-19 (3, 5), while deposits of an array of complement constituents have been observed in lung biopsies of patients with evidence of microangiopathic organ injury as well as in those who died from the infection (4, 5). Holter et al. demonstrated that C4d and sC5b-9 independently predicted progression to respiratory failure over the course of hospitalization for COVID-19 (7).

Leading up to the present study, our groups have conducted two separate observational studies assessing the role of complement in COVID-19. In the first, we measured a panel of complement biomarkers in 52 COVID-19 patients in the US and found elevated complement dysregulation markers C3a, C3a/C3, and sC5b-9/C3 ratios (i.e., reflecting complement over-activation and consumption) in those with severe COVID-19 at admission. These findings suggested that complement levels reflect, and may in part mediate, acute disease status (6). Whilst in another study by our group from Hungary, we similarly measured complement in a cohort of 128 COVID-19 patients, and observed that higher C3a and C3a/C3 values were associated with increased risk of in-hospital mortality in COVID-19 patients (8).

The complement cascade has an important physiological role within the innate immune system in terms of clearing invading pathogens via its many constituents, including the opsonins (C3b and C4b), the anaphylatoxins (C3a and C5a), and finally the membrane attack complex (C5b-9, MAC) (9). However, uncontrolled complement hyper-activation has been reported to contribute to vascular thrombosis and organ injury in the setting of thrombotic microangiopathies (TMAs) such as atypical hemolytic uremic syndrome (10), as well as in systemic lupus nephritis (11), antineutrophil cytoplasmic antibodies (ANCA)-associated vasculitis (12), and recently in COVID-19 (4, 13). Due to the presence of a fenestrated endothelium, the kidneys are particularly vulnerable to complement mediated attack (14). A recent observational study has reported findings of secondary TMA-mediated acute kidney injury (AKI) among COVID-19 patients, particularly when accompanied by a low ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) to vWF:Ag (von Willebrand factor antigen) ratio (15). Moreover, a bidirectional relationship between vWF and complement components Factor H and C3b has been noted, such that the coagulation and complement systems can act in synergy for accelerating thrombosis (16, 17). In accordance with all these findings, it is plausible that complement is involved in the pathogenesis of microvascular thrombosis within the kidneys of COVID-19 patients leading to severe AKI.

Therefore, this prospective, international, multi-center cohort study aimed to examine the associations between complement and development of severe AKI among COVID-19 patients hospitalized in three different centers. We decided to combine the cohorts of our two individual studies, both to increase statistical power of results and account for the fact that incidence of severe COVID-19 associated AKI is highly variable between patients.
hospitals, and geographical regions. In a pooled analysis, the incidence of severe AKI (defined as Kidney Disease Improving Global Outcomes [KDIGO] Stages 2+3) in COVID-19 patients averaged to ~16% (18), ranging from as little as 2.6% in China (19), up to 65% in France (20). This heterogeneity may be attributed to differences in underlying patient characteristics, varying disease course, and even under-recognition of AKI in centers with less frequent assessment of kidney function (18).

MATERIALS AND METHODS

Study Design and Cohort

Adult patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection confirmed by reverse transcriptase polymerase chain reaction (RT-PCR) via nasopharyngeal swab, hospitalized at the University of Cincinnati Medical Center (UCMC) in the US and two tertiary hospitals in Budapest, Hungary between April 2020 and July 2020 were consecutively enrolled in this prospective cohort study. Exclusion criteria was the presence of stage 5 chronic kidney disease (CKD) on admission.

This study was approved by the Institutional Review Board (IRB) of the University of Cincinnati (IRB ID 2020-0278) and the Hungarian Ethical Review Agency (ETT-TUKEB; No. IV/4403-2/2020/EKU) and received a waiver of informed consent in the US center. In Hungarian centers, total classical pathway activity of alternative pathway, and levels of sC5b-9 and C3a, as in the US center. In Hungarian centers, total classical pathway activity was measured by hemolytic titration test based on Mayer’s method (22). Antigen concentration of C3 and C4 was measured by immunoturbidimetry (Beckman Coulter, Brea, CA). Radial immunodiffusion was performed for quantifying the antigen concentrations of Factor I and Factor B, using specific polyclonal antibodies (23). The levels of Factor H and C1q were determined by homemade ELISA (23, 24). Reference values and cut-offs were defined according to manufacturers’ guidelines.

Endpoints

The primary endpoint was development of severe AKI during hospitalization as defined by KDIGO Stage 2+3 serum creatinine (SCr) criteria (21). The secondary endpoint was need for renal replacement therapy (RRT). Patients were stratified for the purpose of analysis based on center of enrollment: patients from UCMC were stratified into the US cohort, while those from the two hospitals in Budapest were stratified into the Hungarian cohort.

Statistical Analysis

Categorical variables were described by absolute (n) and relative (%) frequencies, and differences between AKI and RRT groups were evaluated using Fisher’s exact test. For contingency tables with a number of rows or columns <2, the network algorithm developed by Mehta and Patel was used to perform the test (25). Continuous data were reported as median and interquartile range (IQR), and differences between AKI and RRT groups were analyzed using Mann-Whitney’s U test. Additionally, C3a/C3 ratio was calculated to express the extent of overactivation and consumption of the central component of all complement pathways. Since the reference ranges and measures are different between centers, complement measurements were treated categorically in the analysis, grouped as follows: below, within, or above reference range, as defined by the individual center of testing, to enable pooled analyses. Based on results of bivariate analysis, variables with $p < 0.010$ were initially included in multivariable logistic regression to identify complement variables independently associated with development of severe AKI after adjusting for age, sex, and comorbidities, with calculation of odds ratios (OR) and 95% confidence intervals (95% CI). In regression, complement parameters were standardized to have a mean of zero and a standard deviation (SD) of one, such that a one-unit increase would correspond to an increase of one SD. Variable selection was performed via step-by-step (forward
RESULTS

A total number of 135 adult hospitalized patients with RT-PCR confirmed SARS-CoV-2 infection were initially enrolled, though 4 were excluded from the analysis due to having stage 5 CKD present on admission. Thus, the final sample consisted of 131 patients: 33 in the US cohort and 98 in the Hungarian cohort. A study enrolment flow diagram for both cohorts can be found in Supplementary Figure 1.

At the time of sample collection, 38 patients (29%) required intensive care indicating a severe disease state, with 30 (22.9%) requiring invasive ventilation. Specifically, in the US Cohort (n = 33), 6 (18.2%) patients required intensive care at sampling, 3 (9.1%) of which required invasive ventilation. In the Hungarian cohort (n = 98), 32 (32.7%) patients required intensive care at sampling, 27 (27.6%) of which needed invasive ventilation. The number of patients requiring intensive care was comparable between groups (p = 0.13). However, there was a statistically significant difference in the need for invasive ventilation between groups (p = 0.03).

In the US cohort, the median (IQR) time between symptom onset and sampling was 7 (2.5–10) days. Whereas, for the Hungarian cohort, the median (IQR) time from symptom onset to sampling was 9 (6–21) days. Symptom onset date could not be determined for 16 patients in the Hungarian cohort.

Outcomes and Patient Characteristics

Baseline patient characteristics and comorbidities are presented in Table 1. Severe AKI developed in 17.6% (n = 23) of the combined cohort, with 12 (9.2%) needing RRT. Overall, the median age was 66 (IQR, 54–75) years, and males amounted to 54.2% (n = 71) of the cohort. There were no significant differences in sex distribution between those developing/not developing severe AKI, nor those needing/not needing RRT. However, patients who developed severe AKI were significantly older (70 vs. 65 years; p = 0.03), while no age difference was observed among those requiring/not requiring RRT (67 vs. 65 years, p = 0.54). Among comorbidities, hypertension (n = 89; 67.9%), heart disease (n = 50; 38.2%), and CKD (n = 53; 40.5%) were the most prevalent, and both hypertension (p = 0.05) and CKD (p = 0.04) were more prevalent in severe AKI patients. Body mass index was not recorded in the Hungarian Cohort but was elevated in all US patients (median 28.5 kg/m²; IQR, 24.9–33.7 kg/m²) and significantly lower among those developing severe AKI (24.5 vs. 29.5 kg/m²; p = 0.02).

Complement Profile

A summary of complement parameters in COVID-19 patients, with levels of complement defined as below, within, or above their reference range (defined by respective testing centers), in total and stratified according to cohort and severe AKI status is shown in Table 2. Continuous measurements of complement parameters are summarized in Supplementary Table 1.

When looking at complement levels as categorical variables in the combined cohort, a C3a level above reference range was more common in patients with severe AKI (73.9 vs. 39.8%; p = 0.005). Within the Hungarian cohort, severe AKI patients more frequently had below normal alternative pathway activity (63.6 vs. 23.0%; p = 0.03), classical pathway activity (36.4 vs. 9.2%; p = 0.04), as well as above normal C4 levels (27.3 vs. 4.6%; p = 0.04), and C3a levels (81.8 vs. 39.1%; p = 0.01). In the US cohort, Factor I elevation was less prevalent in severe AKI patients (8.3 vs. 47.6%; p = 0.03).

When looking at complement levels as continuous variables in the total cohort, the C3a/C3 ratio was higher in patients with severe AKI (3.29 vs. 1.71; p < 0.001) (Figure 1). Among the Hungarian cohort, those who developed severe AKI had lower alternative pathway activity (p = 0.008), classical pathway activity (p = 0.001), C3 (p = 0.002), and C4 (p = 0.002), while C3a (p = 0.005) and C3a/C3 ratio (p < 0.001) were significantly elevated in these patients. Among the US cohort, only C3a/C3 ratio was significantly elevated in severe AKI patients (p = 0.016) (Supplementary Table 1).

Patients who needed RRT had significantly greater prevalence of C3a levels above reference range compared with those who did not (100 vs. 53.8%; p = 0.001). When stratified, this pattern remained significant in the Hungarian cohort only (100 vs. 42.4%; p = 0.04). Additionally, C3a/C3 ratio was significantly higher in patients requiring RRT in the combined cohort (3.42 vs. 1.79; p < 0.001), as well as in the US (3.24 vs. 1.65; p = 0.002) and Hungarian (3.7 vs. 1.79; p = 0.01) cohorts separately (Figure 1 and Table 3).

In multivariate logistic regression, adjusted for age, and comorbidities, C3a measured at admission in the Hungarian cohort predicted the development of severe AKI during hospitalization (Table 4). Each standard deviation increase in C3a (SD = 210.1) was independently associated with 89.7% increased odds of developing severe AKI (95% CI, 7.6–234.5%).

Correlations and Associations With Inflammatory and Thrombotic Biomarkers

Correlation analysis was performed for complement parameters that had significant difference between groups, for the purpose of identifying interplays and associations with imbalances in other systems (Table 5). Independent associations were assessed through stepwise linear regression (Supplementary Table 2).

In the US cohort, Factor I correlated positively with ADAMTS13 (p = 0.03), fibrinogen (p = 0.006), and haptoglobin (p = 0.005); and negatively with admission creatinine (p = 0.003). C3a/C3 correlated positively with admission creatinine (p < 0.001), ferritin (p = 0.04), and IL-6 (p = 0.02); and inversely...
### TABLE 1 | Baseline characteristics (A) and comorbidities (B) in hospitalized COVID-19 patients in US and Hungary, stratified according to development of severe AKI.

|                         | Combined cohort | US cohort       | Hungarian cohort |
|-------------------------|-----------------|-----------------|-----------------|
|                         | Total (n = 131) | No Severe AKI   | Severe AKI      | Total (n = 33) | No Severe AKI | Severe AKI | Total (n = 98) | No Severe AKI | Severe AKI | p         |
|                         | (n = 108)       | (n = 23)        |                 | (n = 21)       | (n = 12)       |           | (n = 87)       | (n = 11)       |           |           |
| **A. Baseline characteristics** |                 |                 |                 |               |               |           |               |               |           |           |
| Age, years              | 66 (54–75)      | 65 (53–73)      | 70.5 (66–78)    | 0.03          | 65 (47–70)     | 64 (40–69) | 66 (56–70)    | 0.17          |           |           |
| BMI, kg/m$^2$           |                |                 |                 |               | 28.5 (24.9–33.7) | 29.5 (25.8–34.5) | 24.5       | 0.02          | (21.6–27.5)  |           |           |
| Gender                  |                |                 |                 |               |                |             |               |               |           |           |
| Male                    | 71 (54.2%)      | 58 (53.7%)      | 13 (56.5%)      | 0.82          | 18 (54.5%)     | 11 (52.4%) | 7 (58.3%)     | 0.9           |           |           |
| Female                  | 60 (45.8%)      | 50 (46.3%)      | 10 (43.5%)      |               | 15 (45.5%)     | 10 (47.6%) | 5 (41.7%)     |               |           |           |
| Obesity                 |                |                 |                 |               | 18 (54.5%)     | 16 (76.2%) | 2 (16.7%)     | 0.09          |           |           |
| Hypertension            | 89 (67.9%)      | 69 (63.9%)      | 20 (87.0%)      | 0.05          | 26 (78.8%)     | 15 (71.4%) | 11 (91.7%)    | 0.22          |           |           |
| Heart disease           | 50 (38.2%)      | 37 (34.3%)      | 13 (56.5%)      | 0.06          | 17 (51.5%)     | 6 (28.6%)  | 11 (91.7%)    | **0.001**     |           |           |
| Hyperlipidemia          |                |                 |                 |               | 15 (45.5%)     | 11 (52.4%) | 4 (33.3%)     | 0.73          |           |           |
| Diabetes                | 43 (32.8%)      | 34 (31.5%)      | 9 (39.1%)       | 0.47          | 21 (63.6%)     | 15 (71.4%) | 6 (50.0%)     | 0.74          |           |           |
| COPD                    | 30 (22.9%)      | 24 (22.2%)      | 6 (26.1%)       | 0.79          | 8 (24.2%)      | 4 (19.0%)  | 4 (33.3%)     | 0.08          |           |           |
| Chronic kidney disease  | 53 (40.5%)      | 39 (36.1%)      | 14 (60.9%)      | **0.04**      | 6 (18.2%)      | 1 (4.8%)   | 5 (41.7%)     | **0.002**     |           |           |
| Chronic liver disease   |                |                 |                 |               | 7 (21.2%)      | 3 (14.3%)  | 4 (33.3%)     | **0.04**      |           |           |

**All continuous data presented as median (IQR), with P-values calculated using Mann-Whitney’s U test. All categorical data presented as frequency (%), with P-values calculated using Fisher’s exact test. KDIGO, Kidney Disease Improving Global Outcomes criteria used to define AKI using serum creatinine. No severe AKI-KDIGO 0+1; Severe AKI- KDIGO 2+3. Data on BMI, obesity, hyperlipidemia, and chronic liver disease was not recorded in Hungarian cohort. Statistical significance denoted with bold text.**
TABLE 2 | Frequency of COVID-19 patients with levels of complement parameters below, within, or above their respective reference range, in total and stratified according to cohort and AKI group.

| Complement parameter | US cohort | Hungarian cohort |
|----------------------|-----------|------------------|
|                      | Total (n = 131) | No severe AKI (n = 108) | Severe AKI (n = 23) | p | Reference range | Total (n = 98) | No severe AKI (n = 87) | Severe AKI (n = 11) | p |
| Alternative pathway  |                       |                       |                   |   |                   |                       |                       |                   |   |
| sC5b-9               | Below 12            | 23 (21.3%)            | 9 (39.1%)         | 0.18 | >63% | Below 2          | 70–130%           | Below 12           | 2 (16.7%)         | 0.9 | 70–130%           |
|                      | Normal 97           | 83 (76.9%)            | 14 (60.9%)        | 0.06 | 101–300 CH50/mL  | Normal 28           | 18 (85.7%)         | Normal 2           | 2 (2.3%)          | 0.03 |
|                      | Above 2             | 2 (1.9%)              | 0 (0.0%)          |       |       | Above 12          | 12 (9.2%)          | Above 12           | 4 (36.4%)         |     |
| Classical pathway   | Below 19            | 12 (11.1%)            | 6 (26.1%)         | 0.06 | 101–300 CH50/mL  | Below 7             | 4 (19.0%)          | Below 12           | 8 (9.2%)          | 0.04 |
|                      | Normal 103          | 89 (82.4%)            | 14 (60.9%)        | 0.06 | 101–300 CH50/mL  | Normal 22           | 15 (71.4%)         | Normal 8           | 5 (5.7%)          |     |
|                      | Above 10            | 7 (6.5%)              | 3 (13.0%)         |       |       | Above 12          | 12 (11.6%)         | Above 12           | 4 (36.4%)         |     |
| C3                   | Below 16            | 12 (11.1%)            | 4 (17.4%)         | 0.34 | 71–150 mg/dL    | Below 2             | 1 (4.8%)           | Below 12           | 4 (36.4%)         |     |
|                      | Normal 99           | 84 (77.8%)            | 15 (65.2%)        | 0.34 | 71–150 mg/dL    | Normal 19           | 12 (57.1%)         | Normal 8           | 8 (72.7%)         |     |
|                      | Above 16            | 12 (11.1%)            | 4 (17.4%)         |       |       | Above 12          | 8 (38.1%)          | Above 12           | 4 (36.4%)         |     |
| C3a                  | Below –             |                      |                   | 0.005 | 30–250 ng/mL   | Below 12           | 3 (3.3%)           | Below 12           | 4 (4.6%)          |     |
|                      | Normal 69           | 63 (58.3%)            | 6 (26.1%)         | 0.30 | 70–270 ng/mL    | Normal 16           | 12 (57.1%)         | Normal 8           | 2 (18.2%)         |     |
|                      | Above 60            | 43 (39.8%)            | 17 (73.9%)        |       |       | Above 17          | 9 (42.9%)          | Above 12           | 9 (81.8%)         |     |
| C4                   | Below 8             | 5 (4.6%)              | 3 (13.0%)         | 0.27 | 15.7–47 mg/dL  | Below 1             | 1 (4.8%)           | Below 12           | 4 (36.4%)         |     |
|                      | Normal 107          | 90 (83.3%)            | 17 (73.9%)        | 0.27 | 15.7–47 mg/dL  | Normal 26           | 17 (81.0%)         | Normal 8           | 7 (63.6%)         |     |
|                      | Above 16            | 13 (12.0%)            | 3 (13.0%)         |       |       | Above 6           | 3 (14.3%)          | Above 12           | 0 (0.0%)          |     |
| C1q                  | Below 22            | 18 (16.7%)            | 4 (17.4%)         | 0.12 | 5.1–7.5 mg/dL  | Below 16           | 12 (57.1%)         | Below 12           | 4 (36.4%)         |     |
|                      | Normal 96           | 82 (75.9%)            | 16 (65.2%)        | 0.12 | 5.1–7.5 mg/dL  | Normal 10           | 6 (28.6%)          | Normal 8           | 10 (90.9%)        |     |
|                      | Above 13            | 8 (7.4%)              | 5 (21.7%)         |       |       | Above 7           | 3 (14.3%)          | Above 8            | 5 (5.7%)          |     |
| Factor B             | Below 3             | 3 (2.8%)              | 0 (0.0%)          | 0.80 | <244 ng/mL     | Below 14           | 10 (47.6%)         | Below 12           | 3 (3.4%)          | 0.82 |
|                      | Normal 54           | 43 (39.8%)            | 11 (47.8%)        | 0.80 | <244 ng/mL     | Normal 22           | 14 (68.7%)         | Normal 32           | 2 (18.2%)         |     |
|                      | Above 72            | 60 (55.6%)            | 12 (52.2%)        |       |       | Above 11          | 7 (33.3%)          | Above 61           | 8 (72.7%)         |     |
| Factor H             | Below 7             | 5 (4.6%)              | 2 (8.7%)          | 0.33 | 37.0–68.0 mg/dL | Below 2             | 2 (9.5%)           | Below 12           | 3 (3.4%)          | 0.14 |
|                      | Normal 77           | 66 (61.1%)            | 11 (47.8%)        | 0.33 | 37.0–68.0 mg/dL | Normal 15           | 10 (47.6%)         | Normal 62           | 6 (54.5%)         |     |
|                      | Above 47            | 37 (34.3%)            | 10 (43.5%)        |       |       | Above 16          | 9 (42.9%)          | Above 31           | 3 (27.3%)         |     |
| Factor I             | Below 12            | 9 (8.3%)              | 3 (13.0%)         | 0.46 | 2.4–4.9 mg/dL  | Below 2             | 11 (52.4%)         | Below 12           | 9 (10.3%)         | 0.20 |
|                      | Normal 91           | 74 (68.5%)            | 17 (73.9%)        | 0.46 | 2.4–4.9 mg/dL  | Normal 22           | 11 (52.4%)         | Normal 69           | 6 (54.5%)         |     |
|                      | Above 28            | 25 (23.1%)            | 3 (13.0%)         |       |       | Above 11          | 10 (47.6%)         | Above 17           | 15 (17.2%)        |     |

All data presented as frequencies (%). P-values calculated with Fisher’s exact test. KDIGO, Kidney Disease Improving Global Outcomes criteria used to define AKI using serum creatinine. No severe AKI-KDIGO 0+1; Severe AKI-KDIGO 2+3; CH50-50% hemolytic complement activity. Statistical significance denoted with bold text.
with ADAMTS13 ($p = 0.02$) and ADAMTS13/vWF:Ag ratio ($p = 0.02$).

Factor I maintained independent positive association with haptoglobin ($p = 0.048$) and negative association with admission creatinine ($p = 0.026$) in linear regression. C3a/C3 ratio remained independently and positively associated with admission creatinine ($p = 0.035$) and IL-6 ($p < 0.001$).

In the Hungarian cohort, alternative pathway activity and classical pathway activity both correlated positively with ADAMTS13 ($p < 0.001; p = 0.007$), ADAMTS13/vWF:Ag ratio ($p < 0.001; p = 0.02$), fibrinogen ($p = 0.01; p = 0.002$), and haptoglobin ($p = 0.044; p < 0.001$). Alternative pathway activity additionally correlated positively with lymphocyte count ($p = 0.02$) and inversely with neutrophil count ($p = 0.03$) and neutrophil-to-lymphocyte ratio (NLR) [$p = 0.006$]. C3a and C3a/C3 both correlated positively with vWF:Ag ($p < 0.001; p < 0.001$), C reactive protein (CRP) [$p < 0.001; p < 0.001$], ferritin ($p < 0.001; p = 0.003$), interleukin (IL)-6 ($p < 0.001; p = 0.003$), lactate dehydrogenase (LDH) [$p = 0.006; p = 0.002$], neutrophil count ($p = 0.04; p = 0.04$), and NLR ($p < 0.001; p < 0.001$); and negatively with ADAMTS13/vWF:Ag ratio ($p < 0.001; p < 0.001$), and lymphocyte count ($p = 0.004; p = 0.006$). C3a/C3 ratio also correlated with admission creatinine ($p = 0.006$), and ADAMTS13 ($p = 0.001$).

Alternative pathway activity remained independently and positively associated with ADAMTS13/vWF:Ag ratio and haptoglobin ($p = 0.011$ and $p = 0.009$, respectively), and negatively with NLR ($p = 0.007$). Classical pathway activity maintained independent association with increase in fibrinogen ($p = 0.019$) and haptoglobin ($p = 0.003$), as well as decrease in neutrophil count ($p < 0.001$). C3a maintained independently and positively associated with CRP ($p < 0.001$) and ferritin ($p = 0.016$), and negatively with fibrinogen ($p = 0.034$). C4 maintained positive independent association with fibrinogen ($p = 0.003$), and negative with LDH ($p = 0.009$) and neutrophil count ($p = 0.007$). Lastly, the C3a/C3 ratio remained independently associated with increase in CRP ($p < 0.001$), as well as decrease in LDH ($p < 0.001$) and neutrophil count ($p < 0.001$).

**DISCUSSION**

Overall, these findings show a greater prevalence of complement over-activation and consumption on admission in COVID-19 patients who developed severe AKI and required RRT during hospitalization. Specifically, elevations of complement activation marker and potent anaphylatoxin C3a were more frequently found in this patient population, such that each standard deviation increase in C3a was independently associated with 89.7% increased odds of developing severe AKI in logistic regression. Furthermore, C3a/C3 ratio, reflecting the magnitude of complement over-activation and consumption,
Complement, Severe AKI, and COVID-19

TABLE 3 | Complement levels on admission in patients with COVID-19 in combined cohort, and in US and Hungary separately, stratified by need for renal replacement therapy (RRT).

| Variable | Combined Cohort | US Cohort | Hungarian Cohort |
|----------|----------------|-----------|-----------------|
|          | p (n = 98)     | p (n = 33) | p (n = 94)      |
| Need for RRT |                  |           |                 |
| No need | 14 (13.9%) | 1 (4%)     | 0.9 | 0 (0%) | 0 (0%) | 0.0 (0%) | 0.0 (0%) | 0.06 (0.02–0.22) |
| Need for RRT | 104 (94%) | 74 (72%) | 0.9 | 0 (0%) | 0 (0%) | 0.0 (0%) | 0.0 (0%) | 0.03 (0.01–0.12) |
| BMI | 26 (24%) | 21 (33%) | 0.33 | 17 (20%) | 16 (19%) | 0.41 | 18 (22%) | 17 (22%) | 0.41 |
| Hypertension | 32 (29%) | 15 (21%) | 0.36 | 16 (19%) | 14 (16%) | 0.29 | 19 (23%) | 17 (21%) | 0.29 |
| C3 | Below 16 | 15 (12%) | 0.87 | 0.64 (0.37–1.09) | 0.43 | 0.37 (0.19–0.72) | 0.03 | 0.06 (0.03–0.13) | 0.2893 |
| Normal | 90 (79%) | 60 (78%) | 1.9 | 1.897 (1.076–3.345) | 1.79 | 1.851 (1.042–3.316) | 0.03 | 0.02 (0.00–0.06) | 0.1216 |
| Above | 14 (12%) | 6 (8%) | 0.05 | 0.064 (0.02–0.19) | 0.07 | 0.064 (0.02–0.19) | 0.28 | 0.058 | 0.36 |
| C3a | Below 0.001 | 2 (2%) | 0.001 | 0.001 (0.000–0.001) | 0.001 | 0.001 (0.000–0.001) | 0.001 | 0.001 (0.000–0.001) | 0.001 |
| Normal | 54 (48%) | 32 (44%) | 0.01 | 0.064 (0.02–0.19) | 0.064 (0.02–0.19) | 0.064 (0.02–0.19) | 0.064 (0.02–0.19) | 0.064 (0.02–0.19) | 0.064 (0.02–0.19) |
| Above | 75 (68%) | 22 (28%) | 0.01 | 0.064 (0.02–0.19) | 0.064 (0.02–0.19) | 0.064 (0.02–0.19) | 0.064 (0.02–0.19) | 0.064 (0.02–0.19) | 0.064 (0.02–0.19) |

All continuous data presented as median (IQR), with P-values calculated using Mann-Whitney’s U test. All categorical data presented as frequency (%), with P-values calculated using Fisher’s exact test. RRT, renal replacement therapy.

TABLE 4 | Multivariate logistic regression for development of severe AKI over course of hospitalization in the US cohort (A) and the Hungarian cohort (B).

| Variable | Coefficient | Standard Error | p | OR (95% CI) |
|----------|-------------|----------------|---|-------------|
| A. US cohort | BMI | −0.15967 | 0.15967 | 0.07682 | 0.04 | 0.852 (0.733–0.991) |
| Hypertension | 3.63947 | 1.23538 | 0.03 | 14.006 (1.244–157.71) |
| B. Hungarian cohort | Age | 0.10874 | 0.041809 | 0.009 | 1.115 (1.027–1.210) |
| C3a (standardized) | 0.6404 | 0.2893 | 0.03 | 1.897 (1.076–3.345) |

OR, odds ratio; 95%CI, 95% confidence interval; BMI, body mass index.

was consistently increased in those developing severe AKI and requiring RRT in each cohort.

C3a was the only complement parameter independently predicting progression to severe AKI. We suspect C3a may contribute to TMA-mediated renal injury (frequently seen in COVID-19-related AKI (26)), by inducing inflammation, activating the endothelium (27), and triggering platelet aggregation by binding of the C3a receptor (C3aR) on platelets (4, 28). Consistent with these findings, we found that decreased alternative and classical pathway activity, and consumption of C4, a lectin and classical pathway component, were more common in COVID-19 patients who progressed to severe AKI. Importantly, in a recent study combining transcriptomic, proteomic, and mechanistic studies, Georg and colleagues identified that C3a induces highly cytotoxic CD16+ T cells in patients with severe COVID-19 (29). These cells cause T-cell receptor independent degranulation, cytotoxicity, and microvascular injury particularly unique to COVID-19 (29). This finding provides further evidence linking elevations in C3a to adverse outcomes in COVID-19 patients.

AKI in COVID-19 patients is associated with significantly prolonged ICU stay and increased mortality, particularly in those with severe illness (30, 31). Complement levels on admission may hence be useful for risk-stratification of COVID-19 patients. Furthermore, interventions directed at suppressing a dysregulated complement cascade, including the administration of eculizumab (anti-C5 monoclonal antibody) (32), BDB-0001 (anti-C5a monoclonal antibody) (5), and AMY-101 (a compstatin-based C3 inhibitor) (33), have shown promising potential at attenuating the complement-mediated hyper-inflammatory response and severe outcomes in COVID-19 patients in several small case series, and should be investigated in the context of reducing TMA-mediated sequelae in the kidneys. More recent studies on anti-complement therapy have largely focused on eculizumab, underpinning its safety, and efficacy at improving respiratory function and survival in COVID-19 patients (34–37). Nonetheless, it is important to note that early complement inhibitors (i.e., AMY-101) appear to offer broader therapeutic control compared to complement inhibitors targeting later stages of the complement cascade (i.e., eculizumab) (38). However, large prospective trials involving anti-complement therapy are still underway (clinicaltrials.gov, NCT04288713, NCT04395456, NCT04787137, NCT04804847, NCT04805091).
TABLE 5 | Spearman’s Correlation coefficients between complement parameters and other circulating biomarkers measured on admission.

| Hungarian cohort | US cohort |
|------------------|-----------|
|                  | Alternative Pathway | Classical pathway | C3a | C4 | C3a/C3 | Factor I | C3a/C3 |
| ED Creatinine     | -0.079 (p = 0.44)   | -0.179 (p = 0.08) | 0.144 (p = 0.16) | -0.066 (p = 0.52) | 0.276 (p = 0.006) | -0.499 (p = 0.003) | 0.696 (p < 0.001) |
| ADAMTS13         | 0.491 (p < 0.001)   | 0.269 (p = 0.007) | -0.183 (p = 0.07) | 0.310 (p = 0.002) | -0.318 (p = 0.001) | 0.389 (p = 0.03) | -0.398 (p = 0.02) |
| vWF              | -0.164 (p = 0.12)   | -0.152 (p = 0.15) | 0.387 (p < 0.001) | -0.077 (p = 0.47) | 0.370 (p < 0.001) | 0.182 (p = 0.31) | 0.133 (p = 0.46) |
| ADAMTS13/vWF:Ag  | 0.395 (p < 0.001)   | 0.245 (p = 0.02)  | -0.353 (p < 0.001) | 0.259 (p = 0.01)  | -0.441 (p < 0.001) | 0.131 (p = 0.47) | -0.407 (p = 0.02) |
| CRP              | 0.034 (p = 0.74)    | 0.128 (p = 0.21)  | 0.618 (p < 0.001) | 0.160 (p = 0.12)  | 0.475 (p < 0.001) | 0.149 (p = 0.41) | 0.284 (p = 0.11) |
| Ferritin         | -0.037 (p = 0.73)   | 0.016 (p = 0.88)  | 0.392 (p < 0.001) | 0.032 (p = 0.76)  | 0.305 (p = 0.003) | -0.188 (p = 0.30) | 0.365 (p = 0.04) |
| IL-6             | -0.130 (p = 0.21)   | -0.028 (p = 0.79) | 0.340 (p < 0.001) | 0.067 (p = 0.51)  | 0.298 (p = 0.003) | -0.139 (p = 0.45) | 0.419 (p = 0.02) |
| Fibrinogen       | 0.305 (p = 0.01)    | 0.371 (p = 0.002) | 0.141 (p = 0.25)  | 0.260 (p = 0.03)  | -0.026 (p = 0.83) | 0.537 (p = 0.006) | 0.021 (p = 0.92) |
| LDH              | -0.119 (p = 0.26)   | -0.161 (p = 0.13) | 0.289 (p = 0.006) | -0.194 (p = 0.07) | 0.320 (p = 0.002) | -0.150 (p = 0.41) | 0.220 (p = 0.22) |
| Lymphocytes      | 0.230 (p = 0.02)    | -0.031 (p = 0.76) | -0.296 (p = 0.004) | -0.144 (p = 0.16) | -0.279 (p = 0.006) | 0.067 (p = 0.71) | -0.217 (p = 0.22) |
| Neutrophils      | -0.218 (p = 0.03)   | -0.188 (p = 0.07) | 0.215 (p = 0.04)  | -0.197 (p = 0.05) | 0.215 (p = 0.04) | 0.146 (p = 0.42) | 0.137 (p = 0.44) |
| NLR              | -0.276 (p = 0.006)  | -0.110 (p = 0.28) | 0.345 (p < 0.001) | -0.052 (p = 0.61) | 0.333 (p < 0.001) | 0.027 (p = 0.88) | 0.218 (p = 0.22) |
| Haptoglobin      | 0.204 (p = 0.04)    | 0.351 (p < 0.001) | 0.180 (p = 0.08)  | 0.335 (p < 0.001) | -0.059 (p = 0.56) | 0.478 (p = 0.005) | -0.066 (p = 0.72) |

ED Creatinine, emergency department creatinine; ADAMTS13, a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13; vWF, von Willebrand factor antigen; CRP, C reactive protein; IL-6, interleukin-6; LDH, lactate dehydrogenase; NLR, neutrophil to lymphocyte ratio; CH50, 50% hemolytic complement activity. Statistical significance denoted with bold text.

NCT04382755). Several recent studies identified variants of complement-related genes predisposing to more severe outcomes in COVID-19, suggesting that anti-complement therapy can be targeted at individuals with a more susceptible genotype (48–51).

Alternative and classical complement pathway activity, and particularly the complement marker C4, was found to positively correlate with ADAMTS13 and ADAMTS13/vWF:Ag ratio. Although, after multivariate regression, only alternative pathway activity maintained independent association with ADAMTS13/vWF:Ag ratio when the components of alternative and classical pathways were consumed, as in patients developing severe AKI, this would reflect a state characteristic of TMA (15, 39). Conversely, complement activation product C3a and the C3a/C3 ratio, which were elevated in these patients, displayed a parallel inverse, although not independent, correlation with these pathognomonic TMA markers. These findings not only corroborate emerging evidence of secondary TMA-mediated AKI in COVID-19 patients (6, 15), but also suggest a parallel role of complement in the process.

Similarly, in a study by Holter et al. complement dysregulation on admission was associated with organ dysfunction, albeit pulmonary, later on in the course of illness (7). Gao et al. observed complement deposits in pulmonary tissue of patients who succumbed to COVID-19, comprising mainly C3, C4a, C5b-9, mannose binding lectin (MBL), and mannose-binding protein-associated serine protease 2 (MASP-2) (5). Furthermore, Magro et al. demonstrated evidence of C4d, MASP-2, and terminal complement (C5d-9) deposits in lung and skin tissue biopsies in patients with confirmed microvascular thrombosis (4). The researchers concluded that complement may augment thrombotic microvascular injury through endothelial damage and resultant activation of coagulation (4). In keeping with this, it is conceivable that deposition of complement activation products in the kidneys may also mediate renal injury in COVID-19 patients. It should be noted, that complement may also induce formation of neutrophil extracellular traps (NETs) (40), extracellular chromatin-based fibers that have been implicated in various thrombotic pathologies (41–43). NETs in turn provide a scaffolding on which further complement may be activated (44). This process has been demonstrated in vitro, wherein incubation of NETs with serum led to consumption of complement components and generation of complement activation products (45). However, NETosis was not measured in our cohorts, and its role in complement mediated renal injury in COVID-19 should be further investigated.

Lastly, C3a and C3a/C3 both positively correlated with an array of inflammatory biomarkers, including CRP, ferritin, and IL-6. The importance of this interplay is strengthened by the efficacy of anti-complement therapy to dampen a dysregulated inflammatory response as reported in several recent studies on COVID-19 (33, 38, 46). Furthermore, negative correlation with the lymphocyte count, and positive correlation with the neutrophil count as well as with NLR, points to a state of lymphopenia and neutrophilia in patients developing severe AKI. In a recent meta-analysis by Henry and colleagues, this pattern at admission has been associated with increased odds of severe disease and mortality in patients with SARS-CoV-2 infection (47).

There are a number of sources of limitations in this pooled analysis. First, with only 33 hospitalized patients, the US cohort was considerably smaller than the Hungarian cohort. However, this is largely explained by the absence of a significant first wave where our US center is located. Due to the emergent nature of the pandemic, we did not perform a priori sample size calculations. The paucity of significant findings in the US cohort could be type II error on the basis of low statistical power. Conversely, cohorts of different geographic settings may have been impacted by confounders such as distinct inflammatory patterns, underlying
patient demographics, such as slightly more advanced age in the Hungarian cohort (especially among those with severe AKI) and the higher prevalence of cardiovascular comorbidities in the US cohort, as well as differences in dominant circulating viral strains (52). Similarly, due to sample limitations, we did not explore the impact of racial or ethnic background on the obtained results. Furthermore, despite the same classification, there was a difference in the incidence of underlying CKD and development of severe AKI during illness between countries in our study, with less CKD but more frequent severe AKI in the US cohort, which may be partially attributed to small cohort sizes, as well as the aforementioned confounders. Further research should employ a larger sample to reduce influence of interindividual differences within and between cohorts and increase generalizability of results beyond the study. The laboratory methods for measurement of certain complement parameters were unique to each testing center, and each center used their own cut-off points that have been pre-established on the basis of their population. To account for this, we resorted to treating complement measurements as categorical variables, relative to their respective reference range, except in the case of the C3a/C3 ratio for which a reference range is unavailable. Finally, we only measured complement levels at time of hospital admission, which provides no indication of changes in complement profile over the course of illness or the impact of variable disease course and sample collection timing on complement parameters.

In conclusion, we found evidence of an association between complement consumption and dysregulation on admission, and progression to severe AKI and need for RRT in COVID-19 patients in our multicenter observational study. We hence suggest that C3a/C3 ratio and C3a levels upon admission could be considered potential early predictors of the risk to develop severe AKI and a potential therapeutic target in this patient population. Further research would be needed to elucidate the mechanisms and role of complement derangement in COVID-19.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board (IRB) of the University of Cincinnati (IRB ID 2020-0278) and the Hungarian Ethical Review Agency (ETT-TUKEB; No. IV/4403-2/2020/EKU). Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

BH, GS, DCs, EF, GL, VM, ZI, JG, MR, LG, PR, BS, BL, JS, IB, ZiP, TM, IV-N, and ZoP: research idea and study design. BH, JB, SB, NP-S, BM, ZF, DCs, LH, EK, LC, and PK: sample collection and running experiments. JB, MO, DCs, GS, MR, VM, ZI, JG, LG, PR, BS, BL, JS, IB, ZiP, TM, and ZoP: data acquisition. BH, IS, MO, GS, ZiP, and ZoP: data analysis. BH, GS, IS, GL, JB, DCs, EF, NP-S, SB, DCo, VM, ZI, JG, MR, LG, PR, BS, BL, JS, IB, ZiP, ZF, LH, EK, LC, BM, PK, TM, and ZoP: data interpretation. IS: preparation of first draft. All authors contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual's own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

FUNDING

This study was funded by the University of Cincinnati College of Medicine Special Coronavirus (COVID-19) Research Pilot Grant Program, as well as the Higher Education Institutional Excellence Program of the Ministry of Human Capacities in Hungary, within the framework of the molecular biology thematic program of the Semmelweis University, and by the National Office for Innovation and Research (KH130355 to ZoP, and 2020-1.1.6-JOVO-2021-00013 to ZoP), and by the MSCA-ITN (Horizon 2020) “CORVOS” (Grant 860044 to ZoP and LH). DCs was supported by a grant of the Premium Postdoctoral Fellowship Program of the Hungarian Academy of Sciences (PPD2018-016/2018).

ACKNOWLEDGMENTS

We acknowledge the technical assistance of Mártí Kókai, Éva Zsuzsanna Szendrei, Lászlóné Kertész, Edina Szabó, and Beáta Takács with many thanks.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2022.796109/full#supplementary-material

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