Complement activation fragments are increased in critically ill pediatric patients with severe acute kidney injury

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Key Points:
* Multiple urine and plasma complement fragments increase as severity of acute kidney injury increases in critically ill children.
* Complement fragments may identify patients at risk of requiring dialysis or developing major adverse kidney event outcomes.
* Future studies evaluating this association in a larger cohort of children, as well as studying complement inhibition, are warranted.

Abstract:
Background: Critically ill children with acute kidney injury (AKI) suffer from high morbidity and mortality rates, and lack treatment options. Emerging evidence implicates the role of complement activation in AKI pathogenesis, which could potentially be treated with complement inhibitors. The purpose of this study is to evaluate the association between complement activation fragments and severity of AKI in critically ill children. Methods: A biorepository of critically ill children from a prior multi-site study was leveraged to identify children with stage 3 AKI and matched to patients without AKI based on PELOD-2 (illness severity) scores. Specimens were analyzed for plasma and urine complement activation fragments of factor B, C3a, C4a, and sC5b-9. The primary outcomes were MAKE30 and severe AKI rates. Results: 14 patients with stage 3 AKI (5 requiring renal replacement therapy [RRT]) were matched to 14 patients without AKI. Urine factor Ba and plasma C4a levels increased stepwise as severity of AKI increased, from no AKI to stage 3 AKI, to stage 3 AKI with RRT need. Plasma C4a levels were independently associated with increased risk of MAKE30 outcomes (OR 3.2; IQR 1.1-8.9), and urine Ba and plasma Bb, C4a, and C3a were independently associated with risk of severe stage 2-3 AKI on day 3 of admission. Conclusions: Multiple complement fragments increase as magnitude of AKI severity increases. Very high levels of urine Ba or plasma C4a may identify patients at risk for severe AKI, hemodialysis, and MAKE30 outcomes. The fragments may be useful as a functional biomarker of complement activation and may identify those patients to study complement inhibition to treat or prevent AKI in critically ill children. These findings suggest the need for further specific investigations of the role of complement activation in critically ill children at risk of AKI.

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Complement activation fragments are increased in critically ill pediatric patients with severe acute kidney injury

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**Methods:** A biorepository of samples from critically ill children from a prior multi-site study was leveraged to identify children with stage 3 AKI and matched to patients without AKI based on PELOD-2 (illness severity) scores. Specimens were analyzed for plasma and urine complement activation fragments of factor B, C3a, C4a, and sC5b-9. The primary outcomes were MAKE30 and severe AKI rates.

**Results:** 14 patients with stage 3 AKI (5 requiring renal replacement therapy [RRT]) were matched to 14 patients without AKI. Urine factor Ba and plasma C4a levels increased stepwise as severity of AKI increased, from no AKI to stage 3 AKI, to stage 3 AKI with RRT need. Plasma C4a levels were independently associated with increased risk of MAKE30 outcomes (OR 3.2; IQR 1.1-8.9), and urine Ba and plasma Bb, C4a, and C3a were independently associated with risk of severe stage 2-3 AKI on day 3 of admission.

**Conclusions:** Multiple complement fragments increase as magnitude of AKI severity increases. Very high levels of urine Ba or plasma C4a may identify patients at risk for severe AKI, hemodialysis, and MAKE30 outcomes. The fragments may be useful as a functional biomarker
of complement activation and may identify those patients to study complement inhibition to treat or prevent AKI in critically ill children. These findings suggest the need for further specific investigations of the role of complement activation in critically ill children at risk of AKI.
**Introduction:**

Among children admitted to the pediatric intensive care unit (PICU) with critical illness, acute kidney injury (AKI) is independently associated with increased morbidity and mortality[1]. AKI occurs in roughly 25% of PICU patients, with severe AKI affecting roughly 12%[1]. AKI has also been independently associated with longer mechanical ventilation requirements, longer length of stay[1], and high risk for chronic kidney disease [2, 3]. Despite this prevalence and burden of disease from AKI, there are no effective treatment options other than supportive care.

The etiology of AKI in critically ill children is likely multifactorial and results from hemodynamic-induced renal ischemia, nephrotoxin exposure, and inflammatory dysregulation, which limits the impact of potential therapies that target a single component. The relative contributions of these injury mechanisms remain unclear and may influence the onset, severity, duration, and long-term outcomes of AKI. The complement system, which is a part of the innate immune system, is involved in these three injury mechanisms, and thus could present a unifying therapeutic target in the pathophysiology of AKI development in critically ill children.

The complement cascade is made up of 3 activation pathways: classical pathway, alternative pathway, and lectin pathway. These 3 pathways feed into the “terminal complement cascade”. These pathways generate protein fragments that can be quantified to determine the level of complement activation in each pathway in a given disease: C4a (classical and lectin pathways), factors Ba and Bb (alternative pathway), C3a (all three pathways) and sC5b-9 (terminal pathway). The kidney is particularly susceptible to complement activation as complement proteins are concentrated in the kidney via glomerular filtration and activation is promoted via the kidney’s acidic environment and ammonia synthesis[4]. Complement factors have been shown to be instrumental in the pathogenesis of AKI in basic science models of sepsis and ischemia reperfusion injury [5, 6].

All three pathways have been implicated in the pathogenesis of a wide variety of kidney diseases[4, 7] and complement inhibitors have been used to safely and effectively treat these
patients [8-12]. There is also evidence in adults showing that complement activation fragments are associated with AKI severity after cardiac surgery [13]. Finally, complement activation in atypical hemolytic uremic syndrome has been effectively and safely treated with complement inhibitors [13-15].

Despite this growing evidence, the role of complement activation in the pathogenesis of AKI has not been explored in critically ill children. Importantly, since there are complement inhibitors already in clinical use, there are potential treatment modalities [15] which may either halt the damage to the kidney or be able to reverse already apparent damage, which has been shown in animal studies [16, 17]. The purpose of this study is to evaluate the association between complement activation fragments and severity of AKI in critically ill children. We hypothesized that urinary complement activation fragments from the alternative complement pathway would correlate with severity of AKI. To test this hypothesis, we measured activation fragments from urine and plasma biospecimens obtained from critically ill children who required prolonged mechanical ventilation.
Materials and Methods:

Biorepository identification: An existing database of >450 critically ill children prospectively enrolled through the Collaborative Pediatric Critical Care Research Network (CPCCRN) was leveraged to identify suitable patients. Patients were enrolled at one of 8 pediatric intensive care units (PICUs) throughout the U.S. The study was conducted in critically ill children requiring invasive mechanical ventilation for at least 72 hours to identify microbiome related risk factors of ventilator-associated pneumonia [18]. This study was approved by the University of Utah central Institutional Review Board and parents/guardians of enrolled patients provided informed consent.

Patient Identification: Patients were included in this study if they met the following inclusion criteria: 1) age upon mechanical ventilation initiation between ≥31 days and <18 years; 2) expected to require mechanical ventilation via endotracheal tube (ET) for >72 hours; 3) urine and plasma samples available within 96 hours after initiation of invasive mechanical ventilation; 4) urine and plasma samples obtained within 30 hours of each other. Patients were excluded if they had any of the following exclusion criteria: 1) research team failure to obtain an ET aspirate within 24 hours of intubation; 2) presence of a tracheostomy tube or plans to place one; 3) any condition in which deep tracheal suctioning is contraindicated; 4) previous episode of mechanical ventilation during hospitalization; 5) family/team lack of commitment to aggressive intensive care as indicated by do not resuscitate orders and/or other limitations of care; 6) diagnosis of chronic kidney disease (KDIGO CKD stages III-V); 7) kidney transplant in preceding 90 days; 8) known atypical hemolytic uremic syndrome; 9) primary or chronic diagnosis of a rheumatologic or immunologic disorder; or 10) known pregnancy.

AKI Diagnosis and Matching: We identified patients with stage 3 AKI within the first 7 days of admission, which was defined using serum creatinine (sCr) KDIGO guidelines (sCr ≥3x
baseline; sCr ≥4 mg/dl; acute increase of 0.5mg/dl; or need for renal replacement therapy )[19].

Patients with stage 3 AKI were then separated into those who required renal replacement therapy (RRT) and those who did not. If patients did not have a baseline sCr, one was calculated using patient’s height with an estimated GFR of 120ml/min/1.73m$^2$ based on previous validated studies [1, 20, 21]. Stage 3 AKI patients were matched to those who did not have AKI for the 28 days of the study period.

_Sample Acquisition and Handling:_ Urine was obtained at a single timepoint within the first 96 hours after study enrollment. Blood was obtained at two timepoints on days 1 and 3 of mechanical ventilation. Blood was collected into EDTA tube, immediately centrifuged, aliquoted, and frozen at -80C within 30 minutes of collection. Urine was collected from an indwelling foley catheter, straight catheter, bed pan, or cotton balls within a diaper. Urine was aliquoted and placed on ice within 15 minutes of collection and subsequently frozen at -80C. Urine and plasma specimens were stored at -80C until analysis. The blood specimen that was obtained closest to the time of urine specimen obtainment was chosen to analyze for complement factors as described below.

_Complement Measurements:_ We measured the following factors in both urine and plasma samples: C3a, C4a, sC5b-9, and factor B fragments (Ba and Bb). Complement levels were measured at Exsera Biolabs, a CLIA-certified laboratory with robust experience analyzing complement levels. Complement activation fragments were detected using commercially available ELISAs validated for clinical diagnostics (Quidel, San Diego, CA). In settings of impaired renal elimination low molecular weight proteins such as factor Ba and factor D may cause elevation of plasma Ba levels, as factor D is the activating enzyme in the alternative pathway[7, 22]. Therefore, plasma Ba fragment levels may be interpreted purely as a marker of
reduced GFR and not necessarily a mechanistic mediator. In comparison, plasma Bb fragment levels are not as influenced by impaired renal filtration[22].

**Outcomes:** The primary outcome of interest was incidence of MAKE30 outcomes. MAKE30 was defined as persistent elevation of sCr >2x baseline, RRT requirement, or death by 30 days. Secondary outcomes of interest included severe AKI (defined as stage 2 or 3 AKI on day 3 of PICU admission), length of stay and duration of mechanical ventilation. Stage 2 AKI on day 3 was diagnosed based on KDIGO definition of sCr ≥2x and <3x baseline.

**Statistical Methods:**
Patients with stage 3 AKI within 7 days of the start of ventilation were matched 1:1 with patients with no AKI within 28 days of ventilation start. Patients were matched on PELOD-2 (without the serum creatinine component) on the day of urine and plasma samples. If urine and plasma samples were obtained on different calendar days, then the PELOD-2 scores from the two calendar days were averaged. After matching, baseline characteristics, complement activation, and patient outcomes were reported by AKI and dialysis status. Median and interquartile range were used to summarize continuous variables that have a skewed distribution, while counts and percentages were used to summarize categorical variables. Associations with AKI (and dialysis status for those with AKI) were assessed with the Kruskal-Wallis test for continuous variables, and Fisher’s Exact test for categorical variables. We used logistic regression to examine the association between the binary outcomes of interest (MAKE30 and severe AKI rates) and complement variables, and both unadjusted model and model with adjustment for age were included. A two-sided significance level of 0.05 was used in making conclusions. Analyses were performed using SAS 9.4 (SAS Institute; Cary, NC).
Results:

Patient Characteristics:

14 patients with stage 3 AKI (9 without RRT requirement; 5 with RRT requirement) were matched to 14 patients with no AKI. There were no differences in patients’ age, gender, ethnicity, PRISM-III score, or PELOD-2 score (without sCr) between the three groups (Table 1). There was no difference in PICU admission category or chronic diagnoses, but patients with AKI were more likely to have sepsis as their primary diagnosis category (p =0.031).

Timing of Biospecimen collection:

There was no significant difference in time from study enrollment to urine collection for patients without AKI (35 hours; IQR 24-54) compared to patients with AKI (28 hours; IQR 17-41), p 0.296. Similar findings were seen for plasma collection time. The median time between urine and plasma collection was 3.9 hours (IQR 0.3-20.5) for patients without AKI and 4.1 hours (IQR 1.2 – 17.7) for patients with AKI, p 0.670. Of patients diagnosed with stage 3 AKI there was a variety of timepoints between urine acquisition and AKI diagnosis. Six patients had urine samples obtained while meeting stage 3 AKI criteria: 3 on initial day of stage 3 AKI diagnosis, 2 on second day of stage 3 AKI diagnosis, and 1 on fourth day of stage 3 AKI diagnosis. Three patients had urine samples obtained after stage 3 AKI resolved (2 had stage 2 AKI and 1 had stage 1 AKI) and 5 patients had urine obtained prior to stage 3 AKI diagnosis (three patients had urine obtained on day prior to diagnosis, two patients had urine obtained 4-5 days prior to diagnosis). Of the 5 subjects with RRT requirement, 4 had urine and plasma obtained prior to RRT initiation, and 1 had urine and plasma obtained on day of RRT initiation.

Urine factor Ba increases in patients with severe AKI:

Urine factor Ba, C4a, C3a, and sC5b-9 were measured in all patients. Urine C3a levels and urine C4a levels were at the lower limits of detection in a substantial number of patients (n
=24 and n=11, respectively) so were not analyzed further. There was no significant difference in urine sC5b-9 levels. Figure 1 and table 2 shows the stepwise increase in urine factor Ba levels as severity of AKI increased: children without AKI (median 174; IQR 39-542); children with stage 3 AKI without RRT requirement (median 697; IQR 215-2157); children with stage 3 AKI with RRT requirement (median 3454; IQR 422-5013); p = 0.027. There was no significant difference in a pairwise comparison between stage 3 AKI patients who did not require RRT and stage 3 AKI patients who required RRT.

Plasma complement factors increase as AKI severity increases: Figure 1 shows the stepwise increase in plasma complement levels between patients without AKI, stage 3 AKI, and stage 3 AKI requiring RRT. Table 2 shows the significant increase in plasma C4a levels: no AKI (474; 235-802); stage 3 AKI without RRT (1384; 1012-1685); and stage 3 AKI with RRT (2406; 127-2652), p <0.001) and a significant increase in plasma C3a levels: no AKI (78; 50-143); stage 3 AKI without RRT (143; 110-321); and stage 3 AKI with RRT (266; 212-439), p = 0.037. Although not reaching significance, similar trends were seen in both plasma factor Bb (p = 0.075) and sC5b-9 levels (p = 0.069). There was no significant difference in stage 3 AKI patients who did and did not require RRT amongst all complement fragment measurements.

MAKE 30 outcomes: MAKE30 as the outcome of interest was then assessed. Plasma C4a levels were significantly higher in patients who developed MAKE30 outcomes compared to patients who did not develop MAKE30 outcomes (OR 3.2; IQR 1.1-8.9; p 0.028). This association continued after adjusting for patient age (OR 3.0; IQR 1.1-8.6; p 0.038).

Severe AKI outcomes: Severe AKI (stage 2 or 3 KDIGO) on day 3 of ICU admission was then assessed via multi-regression analysis. Table 3 shows that urine Ba, plasma Bb, plasma C4a,
and plasma C3a were significantly elevated in patients who developed severe AKI. After adjusting for age in the regression analysis similar results were found.

**Patient Outcomes:**

Relevant outcomes for critically ill children were compared between the three groups of patients. Patients with AKI with RRT requirement had significantly longer hospital length of stay (61.3; 55.5-76.6) compared to patients without AKI (16.2; 14.0-30.2) and patients with AKI without RRT need (23.6; 17.1, 53.4), p =0.023, Table 4. Similar results were seen with PICU LOS (p = 0.06). There was no significant differences in ventilator free days or mortality rates amongst the three groups of patients (Table 3).
Discussion:

This is the first study examining complement activation fragments in urine and plasma in a heterogeneous cohort of critically ill children with and without AKI. Urine Ba and plasma C4a levels showed the greatest difference between patients with and without AKI. The magnitude of the increase in urine Ba, plasma C4a, and plasma C3a correlated with severity of AKI. Plasma C4a levels were independently associated with increased risk of MAKE30 outcomes after adjusting for age. Complement activation fragments in urine and plasma were also independently associated with increased risk of severe AKI on day 3 of PICU admission after adjusting for age. Elevation of complement activation fragments is not simply a marker of severity of illness as patients with and without AKI had similar degree of organ dysfunction as measured by PELOD-2 scores.

The findings of this study add to the abundance of basic science evidence that implicates the alternative complement pathway in AKI pathogenesis, although this observational study cannot prove mechanistic involvement of the complement system in AKI. In tissue biopsies from adult patients with tubular injury, immunostaining showed evidence of alternative pathway complement activation in the damaged tubulointerstitium[23]. Tissue specimens also showed sC5b-9 deposition in tubular epithelial cells which correlated with plasma sC5b-9 concentration[24]. Alternative pathway fragments (Ba) were also seen in urine of patients with AKI after cardiac surgery, and elevation of urine factor Ba preceded the rise of serum creatinine (sCr), showing an ability to use urine factor B as a mechanistic biomarker predicting AKI development[13].

Activation of complement can lead to chemoattraction and leukocyte activation releasing proinflammatory mediators, increased vascular permeability and vascular leak, and both sub-lytic effects and lysis of renal cells[4]. Normally, complement proteins are continuously activated and then inactivated by cell surface regulatory proteins, but when these regulatory proteins are disrupted by cellular injury complement becomes dysregulated. In models of sepsis and bilateral
IRI, mice with targeted genetic deletion of factor B ($fB^+/-$ mice) demonstrated improved survival during sepsis, and had attenuated kidney tissue damage, lower AKI biomarkers levels, and reduced generation of reactive oxygen species and cytokine response[6, 16]. Similar findings were seen in wildtype mice treated with a direct factor B inhibitor[5, 17]. Together with this study’s findings, these animal models add biologic plausibility that complement factor B plays a mechanistic role in the development of AKI in critically ill pediatric patients.

Factor B is particularly important as it plays a role in feedback amplification that causes even higher levels of uncontrolled activation and resulting injury, as well as increased levels of C3a [4] which was observed in our study. Potentially, the increased levels of plasma C3a and C4a are due to the low GFR in patients with AKI as the molecular weights of C3a, C4a, and factor Ba are small compared with sC5b-9. Because of this small molecular size, plasma levels of these components may be affected by low glomerular filtration rate (GFR) in patients with severe AKI. However, there was an increase in urine Ba levels which would not be seen if a low GFR was the sole reason for higher plasma levels. Further, although non-significant, a stepwise increase in levels of plasma Bb and sC5b-9 was observed between AKI severity levels, which are less affected by low GFR. Similar to the study of adult post-cardiac surgery patients[13], we did not see a significant increase in urine sC5b-9, which may be due to shorter half-lives of this fragments or decreased stability in the urine. Overall, it remains unknown whether these increased complement plasma levels are primarily due to AKI-induced GFR decrease, or systemic inflammatory state induced complement activation that then causes AKI and decreased GFR. Future studies evaluating the trajectory of complement levels over the course of critical illness are indicated.

Changes in complement activation fragment levels have numerous potential clinical implications. First, complement levels may be used as a prognostic enricher for studies evaluating CRRT. For example, patients with a severely elevated urine factor Ba or plasma C4a level may be stratified into an early v. late CRRT initiation group. Second, these same
stratification tools may be used to trial complement inhibitors or other interventions to treat or prevent clinical AKI.

Our study has some limitations. This was a pilot study performed post hoc that included a small sample size. There was variable timing in specimen acquisition in this study, and some patients had sCr-diagnosed AKI prior to urine and plasma collection. Biospecimens were obtained at a single timepoint and so the longitudinal changes in complement fragments were not assessed. Given the limited amount of urine available for specimen analysis, we were unable to test for proteinuria and thus unable to account for this potential confounder. The optimal time to assess complement fragments in patients at risk of AKI remains unknown. Finally, this was an observational study and causality cannot be determined.

Our study also has multiple strengths. This is the first to examine the association between complement activation fragments and AKI development in children. Our findings were compatible with prior reports in animal models as well as post-cardiac surgery adults with and without AKI. The complement factors were measured in a CLIA-certified laboratory with robust experience quantifying complement levels. This was also a heterogeneous population of critically ill children and potentially more generalizable.

In conclusion we have found that urine Ba, plasma C3a, and plasma C4a levels increase as magnitude of AKI severity increases. Very high levels of urine Ba or plasma C4a may identify patients at risk for severe AKI, hemodialysis, and MAKE30 outcomes. Urine Ba and plasma C4a may be useful as a functional biomarker of complement activation and may identify those patients to study complement inhibition to treat or prevent AKI in critically ill children. Interestingly, factor B specific inhibitors are under development in clinical trials. Our study shows the need for future investigation of the role of complement activation in critically ill pediatric patients at risk of AKI, as well as longitudinal assessment of complement levels.
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References


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<th>AKI with RRT (n = 5)</th>
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<td>4.0 [1.5, 6.9]</td>
<td>2.4 [1.3, 11.4]</td>
<td>4 [1.7, 9.9]</td>
<td>0.907*</td>
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<td>PELOD-2 score without sCr</td>
<td>9.5 [8.0, 11.0]</td>
<td>9.0 [8.5, 11.0]</td>
<td>10.0 [8.0, 13.0]</td>
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<td>PRISM-III score</td>
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<td>17 [14, 21]</td>
<td>9 [9, 12]</td>
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<td>3 (33)</td>
<td>3 (60)</td>
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<td>4 (44)</td>
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*Kruskal-Wallis Test
**Fisher's Exact Test

All variables are frequencies (%) except continuous variables which are expressed as median [Q1, Q3].
PELOD-2=Pediatric Logistic Organ Dysfunction-2, PRISM=Pediatric Risk of Mortality Score, PICU=Pediatric Intensive Care Unit, GI/GU/Metabolic=Gastrointestinal/Genitourinary/Metabolic, HEME/Onc/BMT=Hematologic/Oncological/Bone Marrow Transplant, RRT = renal replacement therapy
Table 2: Urine and plasma complement factor levels in patients with and without severe AKI.

<table>
<thead>
<tr>
<th></th>
<th>No AKI (n = 14)</th>
<th>AKI without RRT (n = 9)</th>
<th>AKI with RRT (n = 5)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urinary levels</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor Ba (ng/mL)</td>
<td>174 [39, 542]</td>
<td>697 [215, 2157]</td>
<td>3454 [422, 5013]</td>
<td>0.027¹</td>
</tr>
<tr>
<td>sC5b-9 (ng/mL)</td>
<td>11.8 [10.1, 15.2]</td>
<td>12.9 [10.7, 15.2]</td>
<td>12.9 [12.4, 25.3]</td>
<td>0.3¹</td>
</tr>
<tr>
<td><strong>Plasma levels</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor Bb (mcg/mL)</td>
<td>1.3 [0.6, 1.5]</td>
<td>1.8 [1.3, 3.7]</td>
<td>2.0 [1.9, 3.0]</td>
<td>0.075¹</td>
</tr>
<tr>
<td>sC5b-9 (ng/mL)</td>
<td>197 [125, 262]</td>
<td>250 [151, 325]</td>
<td>708 [286, 710]</td>
<td>0.069¹</td>
</tr>
<tr>
<td>C4a (ng/mL)</td>
<td>474 [235, 802]</td>
<td>1384 [1012, 1685]</td>
<td>2406 [1274, 2652]</td>
<td>0.001¹</td>
</tr>
<tr>
<td>C3a (ng/mL)</td>
<td>78 [50, 143]</td>
<td>143 [110, 321]</td>
<td>266 [212, 439]</td>
<td>0.037¹</td>
</tr>
</tbody>
</table>

¹ Kruskal-Wallis Test. All variables display median [Q1,Q3]

RRT = renal replacement therapy
Table 3: MAKE30 Outcomes and Severe AKI outcomes on PICU day 3.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MAKE 30</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urine Ba</td>
<td>1.2 (0.9-1.6)</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>Plasma Bb</td>
<td>1.13 (0.61-2.10)</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>Plasma C4a</td>
<td>3.2 (1.1-8.9)</td>
<td>0.028</td>
</tr>
<tr>
<td></td>
<td>Plasma C3a</td>
<td>1.47 (0.76-2.83)</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>Urine sC5b-9</td>
<td>1.64 (0.68-4.0)</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Plasma sC5b-9</td>
<td>1.07 (0.64-1.79)</td>
<td>0.80</td>
</tr>
<tr>
<td><strong>Stage 2-3 AKI on PICU day 3</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urine Ba</td>
<td>1.9 (1.1-3.1)</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>Plasma Bb</td>
<td>2.7 (1.1-6.8)</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>Plasma C4a</td>
<td>13.0 (1.6-106.6)</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>Plasma C3a</td>
<td>3.3 (1.3-8.4)</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>Urine sC5b-9</td>
<td>2.11 (0.57-7.9)</td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td>Plasma sC5b-9</td>
<td>1.84 (0.91-3.7)</td>
<td>0.088</td>
</tr>
</tbody>
</table>

MAKE30 = major adverse kidney events at 30 days
Table 4: Patient outcomes

<table>
<thead>
<tr>
<th>Patient Outcome</th>
<th>Overall (n = 28)</th>
<th>No AKI (n = 14)</th>
<th>AKI without RRT (n = 9)</th>
<th>AKI with RRT (n = 5)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days (out of 28 days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICU LOS (days)</td>
<td>10.6 [7.3, 15.9]</td>
<td>10.2 [7.1, 12.0]</td>
<td>8.8 [7.0, 14.7]</td>
<td>54.9 [14.2, 63.5]</td>
<td>0.06*</td>
</tr>
<tr>
<td>Hospital LOS (days)</td>
<td>23.7 [15.8, 54.5]</td>
<td>16.2 [14.0, 30.2]</td>
<td>23.6 [17.1, 53.4]</td>
<td>61.3 [55.5, 76.6]</td>
<td>0.023*</td>
</tr>
<tr>
<td>Mortality</td>
<td>5 (18)</td>
<td>2 (14)</td>
<td>1 (11)</td>
<td>2 (40)</td>
<td>0.442*</td>
</tr>
</tbody>
</table>

*Kruskal-Wallis Test  
Fisher's Exact Test

All variables are frequencies (%) except continuous variables which are expressed as median [Q1, Q3].

Figure Legend:

**Figure 1: Stepwise increase in complement activation fragments as AKI severity increases.**
Complement fragment levels based on severity of acute kidney injury. Urine samples were collected within the first 4 days of PICU admission. For patients requiring RRT samples were obtained prior to RRT initiation. As AKI severity increased from no AKI to stage 3 AKI without RRT to stage 3 AKI with RRT need, complement fragments urine Ba (A); plasma C4a (C); and plasma C3a (F) increased accordingly. There was no significant change in complement factors urine sC5b-9 (B), plasma Bb (D), or plasma sC5b-9 (E).
Figure 1: Stepwise increase in complement activation fragments as AKI severity increases.

RRT = renal replacement therapy

Fig. 1. Complement fragment levels based on severity of acute kidney injury. Urine samples were collected within the first 4 days of PICU admission. For patients requiring RRT samples were obtained prior to RRT initiation. As AKI severity increased from no AKI to stage 3 AKI without RRT to stage 3 AKI with RRT need, complement fragments urine Ba (A); plasma C4a (C); and plasma C3a (F) increased accordingly. There was no significant change in complement factors urine sC5b-9 (B), plasma Bb (D), or plasma sC5b-9 (E).