A predictive model for sepsis-associated acute kidney injury in mice

James D Odum, MD[1]; Matthew N Alder, MD, PhD[1,2]; Steve Standage, MD[1,2] ; Basilia Zingarelli, MD, PhD[1,2] ; Prasad Devarajan, MD[2,3]; Hector R Wong, MD[1,2]

[1]Division of Critical Care Medicine, Cincinnati Children's Hospital Medical Center [2]Department of Pediatrics, University of Cincinnati College of Medicine [3]Division of Nephrology and Hypertension, Cincinnati Children's Hospital Medical Center

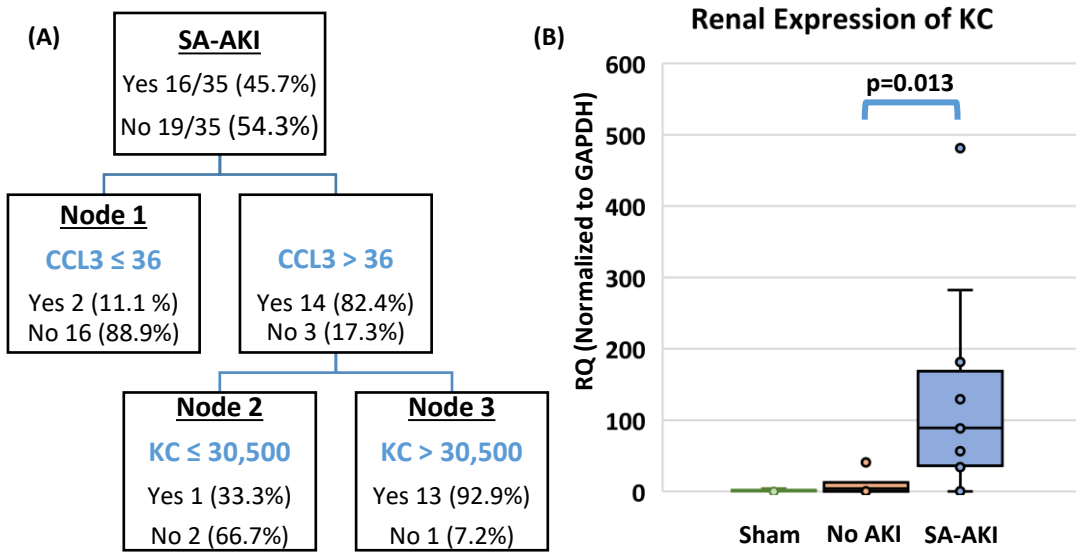
Introduction

- Sepsis results in significant morbidity and mortality, and acute kidney injury is a frequent complication that portends worse outcomes
- Sepsis-associated acute kidney injury (SA-AKI) is caused by a complex interaction of perfusion abnormalities, oxidant stress, and inflammation
  - Significant heterogeneity exists and seemingly similar patients often develop different degrees of SA-AKI
- It remains unclear how the principal components of the sepsis inflammatory cascade contribute to the development of SA-AKI
  - We do not know if SA-AKI is triggered by the systemic production of inflammatory mediators that are filtered by the glomerulus or if SA-AKI is instigated by local tissue-level initiation of the inflammatory cascade in the kidney
- To address this knowledge gap, we interrogated the biological relevance of biomarkers currently used to predict outcomes in sepsis
  - The Pediatric Sepsis Biomarker Risk Model (PERSEVERE) is clinically validated, provides insight into a patient's sepsis-related inflammatory response, and predicts mortality from sepsis (*Wong, Sci Transl Med, 2019*)
  - PERSEVERE biomarkers measured within 24 hours of septic shock have also recently been shown to reliably predict severe, persistent SA-AKI at day 3 of hospitalization in children (*Stanski, Am J Respir Crit Care Med, 2020*)
- We developed a murine model of SA-AKI and analyzed the murine homologs to the PERSEVERE biomarkers (mPERSEVERE)

Methods and Materials

- Eight-week-old C57BL/6 male mice underwent cecal ligation and puncture to induce a polymicrobial, intraperitoneal form of sepsis
- mPERSEVERE biomarkers were collected via cheek bleed at 8-hours post-CLP and antibiotics were administered
  - Keratinocyte-derived chemokine (KC)
  - Macrophage inflammatory protein-1alpha (MIP-1α)
  - IL-6
  - Granzyme B (GZMB)
  - IL-1α
  - Macrophage inflammatory protein-1beta (MIP-1β)
  - Matrix metallopeptidase 8 (MMP8)
- At 24-hours, kidneys were harvested and serum creatinine was measured
- Classification and regression tree analysis (CART) was used to develop a predictive model based on 8-hour mPERSEVERE biomarkers using the binary outcome of 24-hour SA-AKI development (creatinine > 0.4 mg/dL) vs. No AKI
- Renal expression of biomarkers was determined using qPCR (normalized to GAPDH)
- Immunofluorescence was used to spatially localize KC expression
- Single cell RNA sequencing (scRNAseq) was performed on kidneys at 24-hours

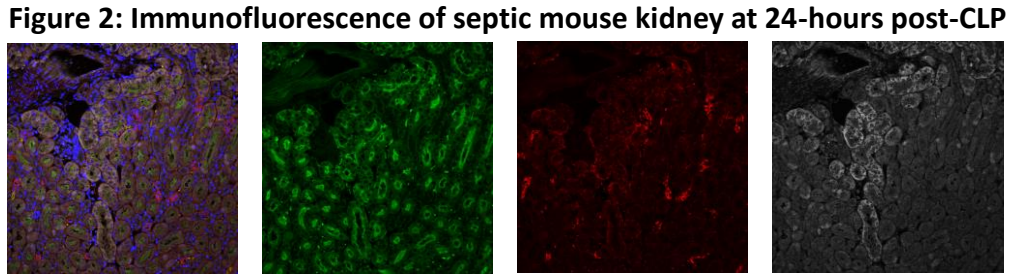
Figure 1: CART derived SA-AKI prediction model and KC renal expression levels



Results

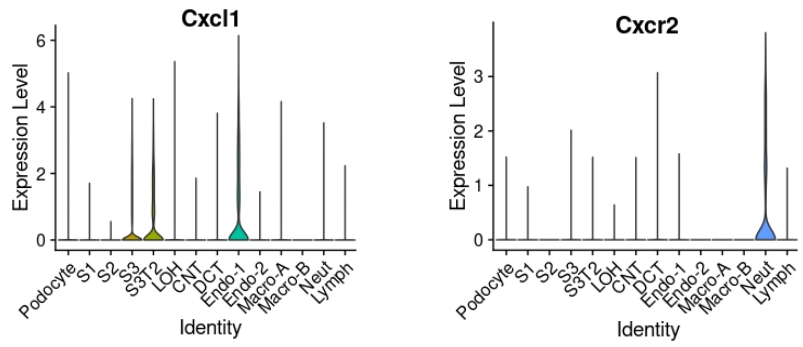
- KC and CCL3 at 8-hours were most predictive of SA-AKI development at 24-hours (**Fig 1A**)
  - Sensitivity 81%, Specificity of 95%, and AUC of 0.90
  - Nodes 1 and 2 predict No AKI
  - Node 3 predicts SA-AKI
- Septic mice had significantly higher renal expression of KC (p=0.004) and CCL3 (p=0.002) compared to sham mice
- Mice with SA-AKI at 24-hours had increased in renal expression of KC compared to mice with No AKI (p=0.013) (**Fig 1B**)
- Immunofluorescence localized KC to proximal tubular epithelial cells via co-localization to LTL (**Fig 2**)
- scRNAseq demonstrated KC expression in the S3 and S3T2 proximal tubular segments as well as endothelial cells, whereas expression of its receptor, CXCR2, was only seen in neutrophils (**Fig 3**)

Figure 2: Immunofluorescence of septic mouse kidney at 24-hours post-CLP



Aggregate Immunofluorescence | Proximal Tubular Cells (LTL) | Distal Tubular Cells (DBA) | KC

Figure 3: scRNAseq localizes KC expression to S3/S3T2 proximal tubules



Discussion

This is the first predictive murine model that ties a host's sepsis-related inflammatory signature to later development of SA-AKI

- KC and CCL3 are chemokines associated with chemotaxis of innate immune cells, particularly neutrophils, but it is unclear if they are mechanistically tied to the underlying pathophysiology of SA-AKI
- KC is expressed and translated within kidney epithelial cells - the S3/S3T2 proximal tubular segments - as opposed to solely being secreted systemically
- Further investigation is needed to determine what initiates transcription of KC upstream and what role activating this chemotactic pathway plays in the development of renal injury.
- Strength: this study directly correlates to a clinical SA-AKI predictive model using PERSEVERE-II biomarkers in children (*Stanski, Am J Respir Crit Care Med 2020)*
- Limitations: polymicrobial nature of CLP and the singular time point of 24-hours by which kidney tissue was analyzed.

Conclusions

- CCL3 + KC reliably estimates SA-AKI development in mice at 24-hours following CLP
- Both chemokines are expressed in whole kidney tissue
- KC demonstrated increased expression in mice that develop SA-AKI and localized to the proximal tubule cells by immunofluorescence and scRNAseq.