**Title**: Markers of Kidney Tubule Secretion and Future Risk of Sepsis-Associated Acute Kidney Injury among REGARDS participants

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Kidney tubular secretion eliminates many endogenous and exogenous substances that are not eliminated by glomerular filtration, including numerous drugs, such as furosemide, rivaroxaban, and cisplatin.1 Historically, tubular secretion has been assessed with time-intensive clearance studies using exogenous tracers, such as para-amino hippuric acid and mercaptoacetyltriglycine. These measures required infusions and frequent timed collections, making them of limited value in the clinical setting. Recently, endogenous secreted solutes (hippurate, indoxyl sulfate, p-cresol sulfate, and cinnamoylglycine, among others) have been identified,2 which have facilitated the assessment of tubular secretion using simple paired blood and urine samples.1

Secretion occurs primarily in the proximal tubules, which is the tubule segment most susceptible to damage during acute kidney injury (AKI).3 Among critically ill patients, decreased tubular secretion is associated with a higher risk of major adverse kidney events (MAKE; defined as estimated glomerular filtration rate decline, need for replacement therapy, and death) (ref?). We were interested in understanding future risk of AKI in stable ambulatory individuals. Specifically, when stressed by sepsis, we wish to understand why some individuals develop AKI whereas others do not, despite seemingly similar baseline glomerular kidney function, severity of infections and systemic responses. We hypothesized that lower tubular secretion among healthy outpatients might identify subtle abnormalities in kidney health not detected by estimated glomerular filtration rate (eGFR) or albuminuria and might signal individuals at higher AKI risk when subjected to a kidney stressor.

We developed a nested case-control study within the Reasons for Geographic and Racial Differences in Stroke (REGARDS) study. We identified 352 participants who had available urine samples from a REGARDS study visit and subsequently had a hospitalization with sepsis and AKI (≥ Kidney Diseases Improving Global Outcomes [KDIGO] stage 1). These cases were matched 1:1 by age, sex, race, and time from baseline study visit to REGARDS participants who had hospitalization with sepsis but did not develop AKI (total N=352). Paired blood and urine specimens from the baseline study visit were stored at -80°C until solute measurement without prior thaw. Plasma and urine secretion markers were measured by liquid chromatography-tandem mass spectrometry at the University of Minnesota, Minneapolis, MN, as previously described.4 Consistent with prior studies, a summary secretion score was created by averaging across the standardized spot urine-to-plasma ratios of eleven secretory solutes to provide a single, overall assessment of tubular secretion.5,6 Conditional logistic regression was used to evaluate the association between the summary secretion score and future risk of sepsis-associated AKI. We evaluated the summary secretion score as a continuous, linear predictor (per 1-SD lower). We also modeled the summary secretion per quartiles to assess the functional form using the highest secretion score quartile as the reference group. We adjusted for baseline eGFR and albuminuria. All analyses were conducted using the SAS system, version 9.4 (SAS Institute, Inc., Cary, NC). P values <0.05 were considered statistically significant.

Among the 352 participants in our study, the mean age was 70 years, 42% were female, and 35% were Black. Median baseline eGFR among cases and controls was 74 and 83 mL/min/1.73m2, and median albuminuria was 16 and 10 mg/g, respectively. The median time between the baseline study visit and the hospitalization was 4.3 (2.5-6) years. The mean and distribution of the summary secretion score was 55 ± 14. Most AKI events were KDIGO stage 1 (91.2%). The median summary secretion score was 56 among cases and 58 among controls (p-value for non-zero difference: ?). In a multivariable model adjusted for eGFR and albuminuria, the baseline secretion score was not associated with risk of sepsis-associated AKI (OR: 1.00, 95% confidence interval 0.99 to 1.02). In analyses using quartiles of the secretion score, Compared to the highest secretion score quartile, the lower three quartiles had numerically greater risk of sepsis-associated AKI, but the associations did not reach statistical significance.

Serum creatinine and albuminuria, the most widely used metrics to evaluate kidney function, primarily reflect glomerular health and do not fully capture essential tubular functions, such as proximal tubule reabsorptive capacity, defense from infection, tubule protein synthesis, secretion, and acid-base homeostasis. Lower tubular secretion is associated with greater severity of biopsy-proven interstitial fibrosis and tubular atrophy, which is the final common pathway in the progression of chronic kidney disease.5 In this cohort of individuals who were hospitalized due to sepsis, we did not find an association between lower secretory solute clearance at baseline with future risk of sepsis-associated AKI.

Among persons with pre-renal etiologies for AKI, we previously showed that lower tubular secretion at time of health?? is associated with increased risk of future AKI, serious electrolyte abnormalities, and other adverse outcomes, independent of eGFR, albuminuria, and other risk factors.6 Among critically ill patients, others have shown that lower tubular secretion at the time of the AKI event is associated with higher risk of MAKE independent of the severity of illness, serum creatinine, and tubular injury markers.7 Our findings here stand in contrast with our original hypothesis that worse tubular secretory function at baseline is associated with higher risk of future sepsis-associated AKI. The reasons underlying these null findings are unclear, but several possibilities deserve consideration. First, the number of events was a relatively small and we may have been underpowered. Second, although we matched elapsed time between baseline and the hospitalization, this time was variable, and longer time between secretion measurement and the AKI event may have led to weaker signals. Although, we have found associations between tubular dysfunction and AKI after similar times, it is plausible that due to the different pathophysiology of septic AKI this study was null. Third, over 90% of the participants had stage 1 AKI, thus more severe AKI episodes may have led to different results. Finally, we did not have access to other important information about severity of sepsis such as Sequential Organ Failure Assessment scores.

Strengths of this study include the use of a well-characterized, sizeable cohort with a significant prevalence of diabetes and hypertension, placing participants at high risk for AKI. The size and available follow-up of REGARDS provided over 2000 hospital admissions with sepsis and access to urine specimens collected before these events occurred. We also evaluated multiple markers of tubular secretion as a composite score and accounted for baseline eGFR and albuminuria.

In summary, estimated tubular secretion in the ambulatory setting did not provide a strong signal for risk of future sepsis-associated AKI. Future studies in larger cohorts are needed to evaluate the role of this key tubular function measure among patients at high risk of kidney outcomes and their downstream consequences.

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| **Table 1. Baseline characteristics of REGARDS participants stratified by acute kidney injury status** | | | |
| **Baseline Characteristic** | | **Controls** | **Cases** |
| N |  | 176 | 176 |
| Age, yr (SD) |  | 70 (8) | 70 (9) |
| Female, n (%) |  | 74 (42) | 74 (42) |
| Race, n (%) |  |  |  |
|  | White | 114 (65) | 114 (65) |
|  | Black | 62 (35) | 62 (35) |
| Diabetes mellitus, n (%) | | 50 (29) | 74 (42) |
| Mean systolic BP, mm Hg (SD) | | 131 (18) | 132 (18) |
| Mean diastolic BP, mm Hg (SD) | | 75 (10) | 75 (11) |
| Use of ACEi, n (%) | | 56 (32) | 66 (38) |
| Use of ARBs, n (%) | | 31 (18) | 39 (22) |
| Use of mineralocorticoids, n (%) | | 7 (4) | 9 (5) |
| Use of NSAID, n (%) | | 38 (22) | 46 (26) |
| Admission destination, n (%) | |  |  |
|  | ICU | 16 (10) | 39 (22) |
|  | General ward | 131 (82) | 130 (74) |
|  | Other/Unknown | 12 (8) | 7 (4) |
| hsCRP >3.0 at baseline, mg/dl n (%) | | 84 (49) | 92 (53) |
| Median cystatin C, mg/dl (IQR) | | 1.06 (0.93, 1.25) | 1.21 (1.02, 1.56) |
| Median eGFR, ml/min/1.73m2 (IQR) | | 83 (65, 92) | 74 (55, 110) |
| Urine ACR, mg/g (IQR) | | 10 (5, 21) | 16 (7, 70) |
| Median summary secretion score, IQR | | 58 (48, 65) | 56 (47, 63) |
| Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; NSAID, non-steroidal anti-inflammatory drug, ICU, intensive care unit; hsCRP, high sensitivity C-reactive protein; ACR, urine albumin-to-creatinine ratio | | | |

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| **Table 2. Association of the summary secretion score with risk of future sepsis associated acute kidney injury** | |
| **Summary Secretion Score** | **Model\*** |
| Per 1-SD lower secretion score | 1.00 (0.99, 1.02) |
| Quartile 1 | 1.58 (0.79, 3.15) |
| Quartile 2 | 1.36 (0.70, 2.61) |
| Quartile 3 | 1.32 (0.61, 2.89) |
| Quartile 4 | Reference |
| \*Cases and controls were matched for age, sex, race, and time from baseline study visit. Models were adjusted for baseline estimated glomerular filtration rate and urine albumin-to-creatinine ratio | |