Title

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Journal

Kidney International, 96(2)

ISSN

0085-2538

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Publication Date

2019-08-01

DOI

10.1016/j.kint.2019.03.024

Peer reviewed

HHS Public Access

Author manuscript

Kidney Int. Author manuscript; available in PMC 2020 August 01.

Published in final edited form as:

Kidney Int. 2019 August ; 96(2): 470–479. doi:10.1016/j.kint.2019.03.024.

The SPRINT trial suggests that markers of tubule cell function in the urine associate with risk of subsequent acute kidney injury while injury markers elevate after the injury

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DISCLOSURE

All authors declared no competing interests.

Supplementary information is available at Kidney International's website.

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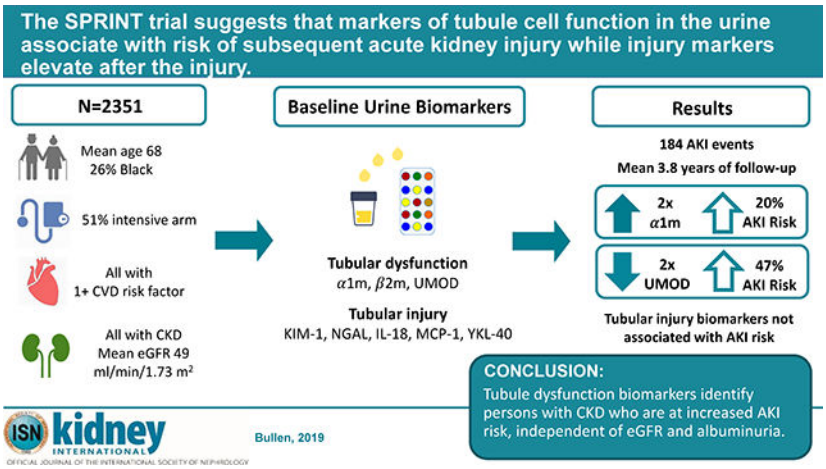
Abstract

Urine markers can quantify tubular function including reabsorption (α–1 microglobulin (α–1 m)) and β–2 microglobulin (β–2 m)) and protein synthesis (uromodulin). Individuals with tubular dysfunction may be less able to compensate to insults than those without, despite similar estimated glomerular filtration rate (eGFR) and albuminuria. Among participants in SPRINT with an eGFR under 60 ml/min/1.73m^2, we measured urine markers of tubular function and injury (NGAL, KIM-1, IL-18, MCP-1, and YKL-40) at baseline. Cox models evaluated associations with subsequent acute kidney injury risk, adjusting for clinical risk factors, baseline eGFR and albuminuria, and the function and injury markers. In a random subset, we remeasured biomarkers after four years, and compared changes in biomarkers in those with and without intervening acute kidney injury. Among 2351 participants, 184 experienced acute kidney injury during 3.8 years mean follow-up. Lower uromodulin (hazard ratio per two-fold higher (0.68, 95% confidence interval [0.56, 0.83]) and higher α–1m (1.20; [1.01, 1.44]) were associated with subsequent acute kidney injury, independent of eGFR and albuminuria. None of the five injury markers were associated with eventual acute kidney injury. Among 59 patients with intervening acute kidney injury in a random subset of 947 patients with repeated measurements, longitudinal increases were evident in urine NGAL, IL-18, and YKL-40 in those with acute kidney injury versus only one marker of tubule function (α–1 m). Thus, joint evaluation of tubule function and injury provided novel insights to factors predisposing to acute kidney injury, and responses to kidney injury.

GRAPHICAL ABSTRACT



Keywords

acute kidney injury; alpha-1 microglobulin (α1m); beta-2 microglobulin (β2m); uromodulin (UMOD); neutrophil gelatinase-associated lipocalin (NGAL); kidney injury molecule-1 (KIM-1); interleukin-18 (IL-18); monocyte chemoattract protein-1 (MCP-1); chitinase-3-like protein (YKL-40)

INTRODUCTION

Acute kidney injury (AKI) is a common clinical syndrome, and is associated with more rapid progression of chronic kidney disease (CKD),[1] cardiovascular disease (CVD),[2] and death [3–5]. The Systolic Blood Pressure Intervention Trial (SPRINT) randomized hypertensive, non-diabetic individuals to intensive (< 120 mm Hg) versus standard (< 140 mm Hg) systolic blood pressure targets and demonstrated lower risks of CVD and death in the intensive arm, but higher risk of AKI.[6] AKI events may be particularly problematic in patients with CKD, as they have less renal reserve, and may therefore be at higher risk of developing end-stage renal disease and electrolyte abnormalities from AKI. Thus, among patients with CKD, identifying factors that predispose to AKI may allow identification of subsets of patients who are at particularly high risk of adverse events where closer monitoring and preventive strategies may be beneficial.

Urinary concentrations of proteins that reflect kidney tubule injury increase rapidly in the setting of established AKI.[7–9] Prior studies have shown that these tubule injury markers are also detectable in the urine of community-living persons without AKI, and are associated with more rapid CKD progression, independent of the baseline level of estimated glomerular filtration rate (eGFR) or albuminuria (uACR). [10] As eGFR and uACR primarily mark glomerular function and injury, respectively, these findings suggest that subtle evidence of tubule damage may identify persons with kidney disease above and beyond established clinical glomerular markers. Thus, individuals with tubule cell injury or dysfunction may be less resilient to toxic, inflammatory or hemodynamic challenges than those without tubule cell injury or dysfunction, despite similar eGFR and uACR; this may manifest clinically by higher risk of AKI episodes.

Commonly evaluated markers of tubule cell injury in the setting of AKI have included neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), and interleukin-18 (IL-18), among others.[7–9] Several novel markers of tubule cell *function*, rather than injury, also exist, but their relationship with AKI risk is largely unknown. Urine alpha-1 microglobulin (uα1m)[11] and beta-2 microglobulin (uβ2m)[12] are low-molecular-weight proteins freely filtered by the glomerulus and reabsorbed by the proximal tubule in healthy individuals. In the presence of proximal tubule cell dysfunction, both uα1m and uβ2m concentrations are elevated in the urine.[13–15] We have previously demonstrated that higher ambulatory uα1m concentrations are associated with CKD incidence and progression in individuals with HIV infection and in stable kidney transplant recipients; these associations remained robust after adjustment for eGFR and uACR.[13,16] Urine uromodulin (uUMOD), the most abundant urine protein in healthy adults, is synthesized exclusively in the thick ascending limb of Henle's loop and the distal convoluted tubule of the kidney.[17,18] Higher uUMOD levels may serve as a surrogate for kidney tubule functional capacity.[19] We have previously demonstrated that lower uUMOD concentrations are associated with CKD progression, independent of eGFR and uACR.[20]

In this study, we evaluated the associations of markers of tubule cell function with subsequent risk of AKI in participants in SPRINT who had CKD at the time of the baseline study visit. *A priori*, we hypothesized that higher uα1m and uβ2m and lower uUMOD would each be associated with subsequent AKI risk. We also measured 5 markers of tubule cell injury at baseline (uKIM-1, uNGAL, uIL-18, uMCP-1, and uYKL-40), and evaluated associations of these markers with subsequent AKI risk, to provide a basis for comparisons. As intensive blood pressure lowering was associated with higher risk of AKI in SPRINT, [21] we also investigated whether baseline levels of tubule markers modify the relationship of intensive blood pressure lowering with AKI risk, and examined associations stratified by randomized treatment arm. Finally, among a random subset of 947 participants, we re-measured the 8 biomarkers of tubule function and injury in urines obtained at the year 4 SPRINT visit, and compared relative changes in each biomarker from baseline to year 4 among those with and without an AKI event in the intervening period.

RESULTS

Baseline Characteristics

Among 2351 SPRINT participants with CKD at baseline, the mean age was 73 ± 9 years, 40% were women, and 26% were black. The mean eGFR at baseline was 49 ± 11 mL/min/1.73m^2, and median ACR was 15 (interquartile range [IQR] 7 – 48) mg/g. Approximately half (1149 participants) were randomized to the standard arm, and 1202 participants were randomized to the intensive arm. The median and IQR for uUMOD, uα1m, and uβ2m concentrations were 6,595 ng/g (4,395 – 10,026 ng/g), 14 mg/g (7 – 25 mg/g), and 104 ng/g (39 – 334 ng/g), respectively. The median and IQR for the tubule injury markers were, uKIM-1 pg/mL 855 (391–1596), uNGAL ng/mL 28 (15 – 60), uIL-18 pg/mL 31 (17–57), uMCP-1 pg/mL 181 (91–326), and uYKL-40 pg/mL 555 (220–1279).

During 3.8 years of follow-up, 184 (7.8%) individuals experienced AKI hospitalizations or emergency room visits. Table 1 displays participant demographics and clinical

characteristics, stratified by AKI status. Compared to those who did not experience AKI events, those with AKI were more frequently male and black, were more frequently randomized to the intensive arm of SPRINT, had higher prevalence of CVD, and had lower eGFR and higher urine ACR at the baseline study visit.

Relationship of Tubule Function Markers with Risk of AKI

When modeled continuously, each two-fold higher uUMOD concentration was associated with a 39% lower risk of AKI in a model adjusted for age, gender, race, randomization arm, and urine creatinine (Model 1), as shown in Table 2. Although modestly attenuated, uUMOD remained significantly associated with lower AKI risk, after additional adjustment for eGFR and uACR and AKI risk factors (Model 2). This association remained robust after further adjustment for all the other markers of tubule function and injury, such that each two-fold higher uUMOD was associated with a 32% lower risk of AKI. Results were similar evaluating quartiles of uUMOD. Compared to the lowest quartile, the highest uUMOD quartile was associated with a 51% to 66% lower risk of AKI across the series of models.

Baseline uα1m was also associated with risk of AKI (Table 2). This association was in the opposite direction from uUMOD, whereby higher uα1m concentrations were associated with risk of AKI. The associations of uα1m were of smaller magnitude compared to those with uUMOD, but followed a similar pattern (Figure 1). In models adjusted for age, gender, race, randomization arm, and urine creatinine (Model 1), a two-fold higher uα1m concentration was associated with a 39% higher risk of AKI. This association no longer reached statistical significance after adjustment for eGFR and uACR and AKI risk factors (Model 2). However, it was strengthened by additional adjustment for the other tubule function and injury markers (Model 3), such that a two-fold higher uα1m was associated with a statistically significant 20% higher risk of AKI.

In contrast to uUMOD and uα1m, we found no association between uβ2m and risk of AKI in either unadjusted or adjusted models.

In sensitivity analyses we indexed each tubule function marker to urine creatinine, rather than adjusting for urine creatinine as a separate covariate, yielding similar results (Supplemental Table 1). In evaluations of whether the tubule function markers had similar associations with AKI risk across randomized treatment arms, higher uUMOD appeared more strongly associated with subsequent AKI in the intensive arm of SPRINT (Table 3); however, the p-value for interaction did not reach statistical significance (p for interaction=0.11). We found no evidence that either uα1m or uβ2m had differential strengths of association for AKI risk in either treatment arm (p for interactions=0.42 and 0.50, respectively).

Relationships of Tubule Injury Markers with Risk of AKI

A two-fold higher uKIM-1 concentration was associated with a 21% higher risk of AKI in a model adjusted for age, gender, race, randomization arm, and urine creatinine (Model 1). Additional adjustment for eGFR, uACR and AKI risk factors rendered the association no longer statistically significant (p=0.071). Results were similar when further adjusted for the other markers of tubule cell function and injury (Model 3; p=0.068).

Higher levels of uNGAL and uYKL-40 were modestly associated with 9–12% higher risk of AKI in minimally adjusted models (Model 1) and in those further adjusted for eGFR, uACR, and AKI risk factors (Model 2). These associations, however, were markedly attenuated with adjustment for the other markers of tubule function and injury. The other injury markers did not have significant associations with AKI risk after adjustment for eGFR, uACR, and AKI risk factors.

We found no evidence that the tubular injury markers had differential strengths of association with AKI risk by randomized treatment arm (p for interactions all > 0.35 Table 3). In sensitivity analyses which indexed each tubule injury marker to urine creatinine, the association of uKIM-1 with AKI risk was stronger than in our primary analyses. In the fully adjusted model that included all injury and function biomarkers, each two-fold higher uKIM-1 level was significantly associated with a 22% higher risk of AKI. No other marker of tubule injury was significantly associated with AKI risk in the fully adjusted model (Supplemental Table 1).

Relationship of Changes in Serum Creatinine from Baseline with Risk of AKI

We evaluated the change in serum creatinine from baseline to month 3 and its association with subsequent risk of AKI. When comparing the highest to the lowest quartiles, both uUMOD and uα1m at baseline were more strongly associated with subsequent risk of AKI (HR: 2.04 and 1.57, respectively) than the 3-month serum creatinine change (HR: 1.27) (Supplemental Table 2).

Changes in Biomarkers over 4 Years Among Persons with and without CKD

Among the random sub-sample of 947 participants with CKD who had repeated tubule injury and function measurements at the SPRINT year 4 visit, 59 (6.2%) experienced AKI events between the study baseline and year 4 visit (Supplemental Table 3). Four-year changes in uα1m were significantly greater (33%) in those with versus without AKI, whereas changes in uβ2M and uUMOD did not differ (Figure 2a). In contrast, 4-year changes in three of the five markers of tubule cell injury (uNGAL, uIL-18, and uYKL-40) were significantly greater in the AKI group compared to those who did not experience AKI (Figure 2b).

DISCUSSION

Abnormal tubule cell function may render the kidneys less capable to withstand insults and therefore more vulnerable to AKI.[22–25] The conventional clinical markers of kidney health, eGFR and albuminuria, primarily reflect glomerular function and injury. While a few prior studies have evaluated biomarkers of tubule cell injury with AKI risk,[26–29] none to our knowledge has previously assessed tubule cell function at baseline with future risk of AKI. Utilizing a panel of three urine markers of tubule cell function (α1M, β2M, and UMOD), and with comparisons to five urine markers of tubule cell injury, we evaluated associations of kidney tubule health with subsequent risk of AKI among individuals with CKD who participated in SPRINT. We found that two of the three markers of kidney tubular dysfunction (lower uUMOD concentrations and higher uα1m) were associated with greater

risk of subsequent AKI, independent of eGFR, uACR, other risk factors, as well as baseline markers of tubule cell injury. These findings have important implications for non-invasive assessment of kidney tubular health, and potentially for monitoring CKD patients at risk of AKI when intensive blood pressure therapy is considered.

The concurrent assessment of tubule injury, as reflected by uKIM-1, uIL-18, uNGAL, uMCP-1, and uYKL-40, provided useful comparisons relative to the three markers of tubule cell function. In contrast to the strong association of uUMOD and uα1m with AKI risk, markers of kidney tubule cell injury were generally not associated with subsequent AKI risk. Urine KIM-1 was the one potential exception. While it was not associated with future AKI risk in our primary models, the association became statistically significant in sensitivity analyses which indexed rather than adjusted for urine creatinine. Therefore, at most, 1 of 5 markers of tubule cell injury was associated with subsequent AKI risk. Moreover, the magnitude of the associations of uUMOD and uα1m with subsequent AKI was stronger than that of uKIM-1. In total, these findings affirm our initial hypothesis that tubule cell dysfunction may identify clinically stable ambulatory individuals who have abnormal kidney health (attenuated residual functional reserve) not captured by eGFR or albuminuria, and who are at higher risk for subsequent AKI.

In contrast to the markers of tubule cell dysfunction, the markers of kidney tubule cell injury provided an important signature after AKI. Among a random subset of 947 individuals who had repeat markers of both tubule cell injury and dysfunction after 4 years, those who experienced AKI in the intervening time had substantially greater elevations in three of five markers of tubule injury, and only one of three markers of tubule cell dysfunction, relative to those who did not experience AKI. The markers of tubule cell injury are known to be sensitive markers of acute tubule necrosis in hospitalized patients as they are experiencing AKI events. Thus, the finding of substantial increases in tubule cell injury markers over 4 years suggests that these markers may reflect residual/perpetual cycle of injury or repair mechanisms triggered by AKI or recurrent AKI, or worse kidney health in general that persist long after the AKI event, a finding that was also observed for uα1M but not the other two function measures.

While uUMOD and uα1M were strongly associated with future AKI risk, we did not observe similar associations when evaluating uβ2m, another marker of tubule cell function. The biology represented by higher uα1m and uβ2m concentrations are believed to be similar, as higher urine concentrations of both markers reflect decreased proximal tubule reabsorptive capacity. The reasons for the lack of association with uβ2m are uncertain, though urinary pH may have contributed. Several prior studies have noted that the measurement of uβ2m is Ph-dependent and unstable at a pH <5.5, postulated to be secondary to enzymatic degradation. [30,31]. The urine specimens from SPRINT were not treated with acid or alkali at time of collection; thus, the urine pH may have influenced uβ2m concentrations and biased associations with AKI towards the null. However, we lacked data on baseline urine pH and future studies with concurrent measurement of uβ2m, uα1m, and urine pH, with or without treatment with acid or alkali, will be required to test this hypothesis.

Several other findings in our study are worth special consideration. We observed that lower baseline uUMOD concentrations were strongly associated with subsequent AKI particularly in individuals randomized to the intensive arm. An assessment for effect modification approached, but did not reach statistical significance (p=0.11). Acutely, lowering of SBP may hemodynamically stress the kidney, which in turn, may predispose to AKI, particularly if there is minimal functional renal reserve to react to this insult. If baseline uUMOD concentrations can identify vulnerable individuals at higher risk of such hemodynamic-induced insults, then uUMOD could potentially risk-stratify individuals who might benefit from more gradual blood pressure lowering and closer surveillance.

As stated previously, uKIM-1's association with AKI differed by whether we indexed to or adjusted for urine creatinine. Beyond marking urine tonicity, we have previously found that urine creatinine concentrations indicate differences in muscle mass,[32] and 1/urine creatinine itself is associated with adverse clinical outcomes in ambulatory individuals.[33] Because of these findings, we have preferred to adjust for urine creatinine as a separate covariate in models. Adjustment allows us to evaluate the association of the biomarker in the numerator (uKIM-1 in this instance) independent of these determinants on urine creatinine, while still accounting for urine tonicity at the time of specimen collection. However, other investigators commonly index biomarkers to urine creatinine, and analyses presented in our sensitivity analyses may therefore be useful for comparisons of strengths of associations observed here to other studies.

Strengths of this study include the use of a well-characterized multicenter clinical trial with a large sample of persons with CKD. Availability of a panel of eight markers capturing both tubule cell function and injury enabled us to compare these two different biological processes with risk of future AKI. We repeated measures of these markers after 4 years in a subset of individuals with CKD, which also allowed comparison of biomarkers changes in those with and without intervening AKI episodes. Each marker was measured twice and averaged to improve precision.

The study also has limitations for consideration. This study evaluated associations of tubule health biomarkers with AKI risk in individuals with CKD at baseline. Future studies are required to determine whether results may generalize to other settings. AKI episodes were captured by the SPRINT safety monitoring committee, and represent hospital admissions and emergency room visits where the diagnosis of AKI was clinically evident and treated. While these AKI episodes were clinically recognized and important, subclinical AKI events may have been missed.[34,35]

In conclusion, among persons with non-proteinuric CKD who participated in SPRINT, lower baseline concentrations of uUMOD and higher baseline concentrations of uα1M – two proteins that reflect kidney tubule cell dysfunction – were each associated with future risk of AKI. These associations were stronger than markers of kidney tubule cell injury and independent of eGFR, uACR, and other CKD and AKI risk factors. In contrast, individuals who experienced AKI episodes predominantly had increases of markers of tubule cell injury after the AKI event, rather than changes in markers of tubule cell dysfunction. Our findings suggest that tubular cell function markers may reflect a vulnerable kidney with diminished

capacity to counter acute insults and thus identify CKD individuals at heightened risk of future AKI. While larger studies are needed to confirm findings, AKI appears to impart long-term residual tubular injury.

METHODS

Study Design and Participants

SPRINT was an open-label clinical trial that randomized persons with elevated risk of CVD events to a systolic blood pressure (SBP) target of <120 mm Hg ("intensive") vs. <140 mm Hg ("standard"). The primary results of SPRINT have been previously published.[21] Participants were recruited from 102 centers in the United States and Puerto Rico and were required to meet the following inclusion criteria: age ≥50 years, SBP between 130 and 180 mm Hg, and increased risk for CVD events (defined by prior clinical or subclinical CVD other than stroke, 10-year risk of CVD of ≥15% on the Framingham risk score, CKD defined as eGFR 20–59 ml/min/1.73m^2, or age ≥ 75 years). Major exclusion criteria included diabetes mellitus, proteinuria >1 gram/day, polycystic kidney disease, prior stroke, symptomatic heart failure, or a left ventricular ejection fraction <35%. A total of 9,361 participants were enrolled between November 2010 and March 2013. Participants were randomly assigned in a 1:1 ratio to the two treatment arms. The antihypertensive regimens were adjusted to maintain SBPs according to the randomized treatment target. Participants attended visits monthly for the first 3 months and then every 3 months thereafter.[36] Venous blood and urine specimens were processed immediately, shipped overnight on ice, and stored at -80 degrees Celsius at the central laboratory. All participants provided written informed consent and Institutional Review Boards of all participating institutions approved the study.

This ancillary study included all SPRINT participants who had CKD and available urine specimens at the baseline visit. We measured serum cystatin C concentrations in all SPRINT participants at the baseline examination, and defined the subset with CKD based on an eGFR <60 ml/min/1.73m^2 by the combined CKD-EPI equation for creatinine and cystatin C.[37] There were 2,514 individuals meeting inclusion criteria, which differs slightly from 2,646 with eGFR<60 ml/min/1.73m^2 by the four variable Modification of Diet in Renal Disease equation [38]used in the SPRINT primary results manuscript. One hundred and sixty-three participants were excluded due to unavailable urine specimens at baseline, resulting in a final sample of 2,351 for this analysis.

In addition, among our CKD subset, we used a random number generator to identify a random subset of 1000 individuals. Among these individuals, we obtained urine specimens from the year 4 SPRINT visit and measured markers of tubule cell function and injury. Among these 1000 individuals, 53 had died, did not complete the year 4 visit, or did not provide urine specimens, resulting in 947 participants who had available urine biomarker data at both baseline and Year 4.

Urine Tubule Function Biomarker Measurements

All urine biomarkers were measured at the Laboratory for Clinical Biochemistry Research at the University of Vermont. Urine specimens were stored at −80°C until biomarker measurement, without prior thaw. To minimize analytic drift in repeated biomarker measurements, urine samples from baseline and year 4 visits were measured at the same time. Laboratory personnel measuring the biomarker assays were blinded to clinical information. For each urine sample, all biomarkers were measured in duplicate, and results were averaged to increase precision. Urine β2m and uUMOD measurements were performed using a multiplex assay on a MESO Scale Diagnostics (MSD) platform (Rockville, Maryland, USA). The analytic ranges were 1.2–5020 ng/ml and 0.6–2510 ng/ml, respectively, and the inter-assay coefficients of variation (CVs) were 15–16% and 13–16%, respectively. Urine α1m was measured using a Siemens nephelometric assay with a detectable range from 5–480 mg/L and inter-assay CV of 3.5–8.8%.

Urine Tubule Injury Biomarker Measurements

Urine KIM-1, IL-18, MCP-1, and YKL-40 were measured together on multiplex assays using the MSD platform. The analytic ranges were 4–200,000 pg/ml, 2–10,000 ng/ml, 3–10,000 pg/ml, and 10–500,000 ng/ml, respectively. The inter-assay CVs were 6.1–13.0%, 4.9–13.7%, 7.1–12.0%, and 6.5–11.1%, respectively. Urine NGAL was measured with a multiplex assay along with β2m and umod, with an analytic range of 6–251,000 ng/mL and an inter-assay CV of 11–19%.

Urine creatinine and albumin were measured by an enzymatic procedure (Roche, Indianapolis, IN) and by a nephelometric method (Siemens, Tarrytown, NY), respectively. [39]

Acute Kidney Injury

Data on occurrence of AKI episodes were collected in the course of safety monitoring for serious adverse events in SPRINT. Participants were considered to have AKI if, during a hospitalization, an AKI diagnosis was listed in the hospital discharge summary, and it was determined by the central SPRINT safety committee to be one of the top 3 reasons for admission or continued hospitalization. Some cases of AKI were noted in emergency department visits without subsequent hospitalization, these were also included in our analysis.

Statistical Analysis

We stratified participants into those who experienced AKI during follow-up versus the remainder, and examined the distribution of demographics and risk factors by AKI status. We then evaluated the association of the tubular function and injury urine biomarkers at baseline with time to incident AKI using Cox proportional hazards models. Given skewed distributions, we log-base-2 transformed each biomarker to facilitate interpretation of parameter estimates as “per two-fold higher” level of each biomarker. To assess the functional form of associations, we also evaluated each biomarker by quartiles, setting the lowest as the reference category. When associations were observed to change monotonically across quartiles, we focused our interpretation on the results of the continuous models to

maximize precision. Because urine creatinine is susceptible to bias by muscle mass and health status,[32] tubular marker concentrations were analyzed without standardization to urine creatinine. Instead, we adjusted for urine creatinine in the multivariable models to correct for urine tonicity. However, we also conducted sensitivity analyses wherein tubular markers were indexed to urine creatinine to assure that main results were consistent. Covariates for multivariable models were selected *a priori* based on biological plausibility. Model 1 adjusted for age, sex, race, randomization arm, and urine creatinine. Model 2 added baseline eGFR, urine albumin, systolic and diastolic blood pressures, prevalent CVD, ACE-inhibitor or angiotensin receptor blocker (ARB) use, and diuretic use. Model 3 additionally included the other markers of tubular function and injury to determine the degree to which the relationship of each biomarker with AKI was independent of the other tubular markers. We tested for interactions of each tubular function and injury marker by randomized treatment arm on risk of AKI, and we explored analyses stratified by randomized treatment arm.

We also evaluated the change in serum creatinine from baseline to month 3 and its association with subsequent risk of AKI to provide a framework of comparison for strengths of association of the urine biomarkers relative to clinically recognizable changes in kidney function measures. We had aimed to compare the beta coefficients per doubling of the 3-month changes in serum creatinine to those for the tubular function and injury biomarkers at baseline. However, since change in serum creatinine included negative numbers, we were unable to evaluate the association of serum creatinine change (modeled as two-fold higher) with AKI. Therefore, we were limited to quartile comparisons.

Finally, we evaluated a random sample of 947 individuals who had repeated tubular biomarker measurements at year 4. We computed percent change in each biomarker relative to the baseline level. We utilized Student t-tests to compare differences in percent changes among the subset who experienced AKI in the intervening period versus the remainder.

All analyses were conducted using Stata/MP Version 15.1 (StataCorp LCC, College Station, TX). P values <0.05 were considered statistically significant for all analyses including interaction terms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

Dr. Alexander L. Bullen was supported by a Ruth L. Kirschstein training grant from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK; T32DK104717). Dr. Joachim H. Ix was supported by a mid-career mentoring award from the NIDDK (K24DK110427). The ancillary study measurements and data analysis were supported by an R01 award from the NIDDK to Drs. Ix and Shlipak (R01DK098234) and an AHA award to Dr. Ix (14EIA18560026). Dr. Michelle Estrella is supported by an R01 from the NIDDK (R01DK103574).

The Systolic Blood Pressure Intervention Trial is funded with Federal funds from the National Institutes of Health (NIH), including the National Heart, Lung, and Blood Institute (NHLBI), the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), the National Institute on Aging (NIA), and the National Institute of Neurological Disorders and Stroke (NINDS), under Contract Numbers HHSN268200900040C, HHSN268200900046C, HHSN268200900047C, HHSN268200900048C, HHSN268200900049C, and Inter-

Agency Agreement Number A-HL-13-002-001. It was also supported in part with resources and use of facilities through the Department of Veterans Affairs. The SPRINT investigators acknowledge the contribution of study medications (azilsartan and azilsartan combined with chlorthalidone) from Takeda Pharmaceuticals International, Inc. All components of the SPRINT study protocol were designed and implemented by the investigators. The investigative team collected, analyzed, and interpreted the data. All aspects of manuscript writing and revision were carried out by the coauthors. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH, the U.S. Department of Veterans Affairs, or the United States Government. For a full list of contributors to SPRINT, please see the supplementary acknowledgement list: https://www.sprinttrial.org/public/dspScience.cfm

We also acknowledge the support from the following CTSAs funded by NCATS: CWRU: UL1TR000439, OSU: UL1RR025755, U Penn: UL1RR024134& UL1TR000003, Boston: UL1RR025771, Stanford: UL1TR000093, Tufts: UL1RR025752, UL1TR000073 & UL1TR001064, University of Illinois: UL1TR000050, University of Pittsburgh: UL1TR000005, UT Southwestern: 9U54TR000017-06, University of Utah: UL1TR000105-05, Vanderbilt University: UL1 TR000445, George Washington University: UL1TR000075, University of CA, Davis: UL1 TR000002, University of Florida: UL1 TR000064, University of Michigan: UL1TR000433, Tulane University: P30GM103337 COBRE Award NIGMS, Wake Forest University: UL1TR001420.

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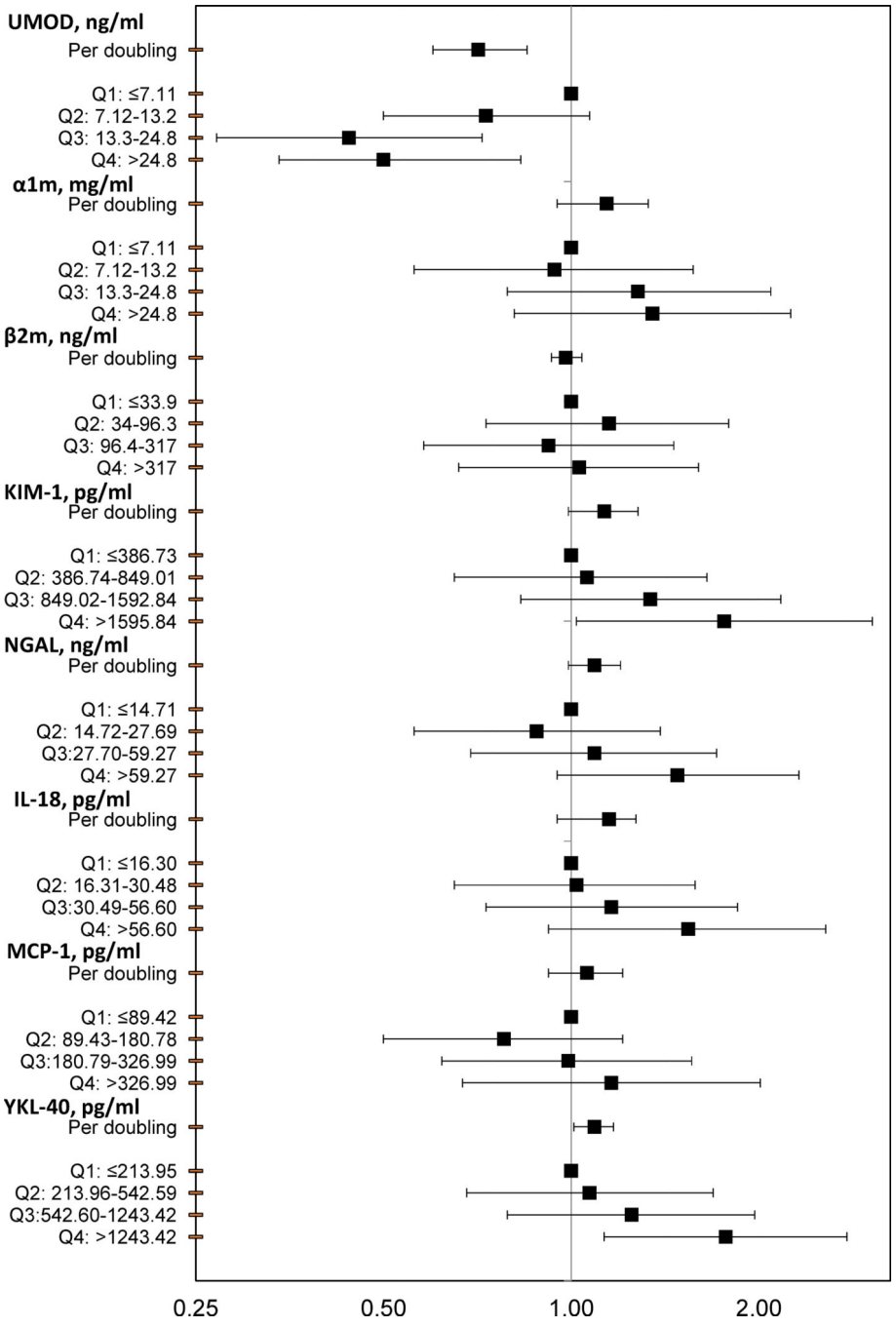

FIGURE 1. Baseline levels urine biomarkers of kidney tubular function, injury, inflammation and repair and relative hazard of AKI among SPRINT participants with baseline CKD (n=2531).

Squares denote the adjusted hazard ratio of AKI per doubling or per quartile higher of baseline urine biomarker concentration while the brackets denote the 95% confidence intervals.

Model adjusted for age, gender, race, randomization arm, urine creatinine, baseline eGFR and urine albumin, baseline systolic and diastolic blood pressures, prevalent cardiovascular

disease, and baseline use of ACE-inhibitors, angiotensin receptor blockers or diuretics. ("Model 2")

Abbreviations: Q1, quartile 1; Q2, quartile 2; Q3, quartile 3; Q4, quartile 4; α1m, alpha-1-microglobulin; β2m, beta-2-microglobulin; UMOD, uromodulin; KIM-1, kidney injury molecule-1; NGAL, neutrophil gelatinase-associated lipocalin; IL-18, interleukin-18; MCP-1, monocyte chemoattractant protein-1; YKL-40, chitinase-3-like protein

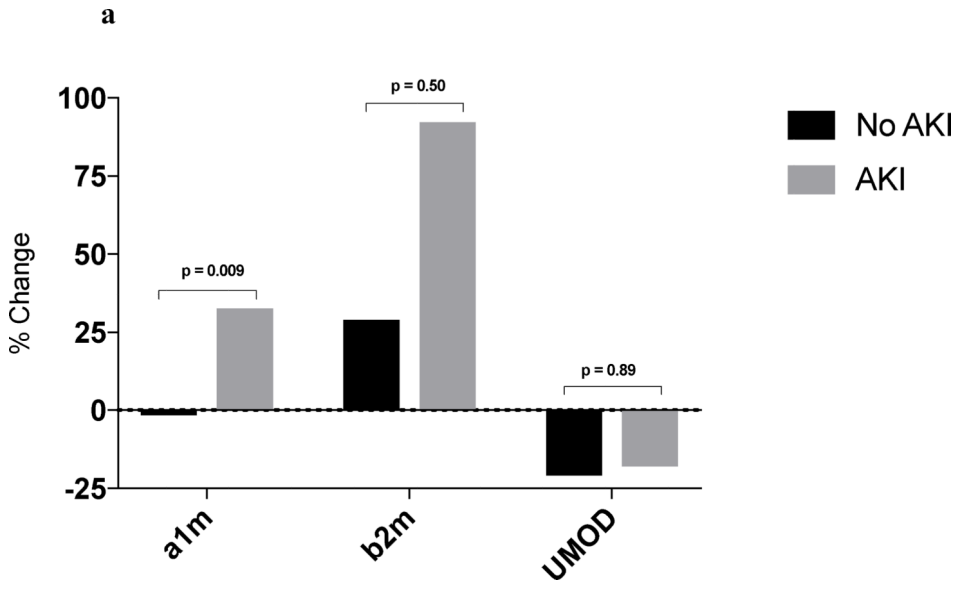

FIGURE 2a.

Change in tubule function markers from baseline to year four among a random sample of 947 individuals with CKD, stratified by those with intervening AKI (N=59) vs. all others (N=888)

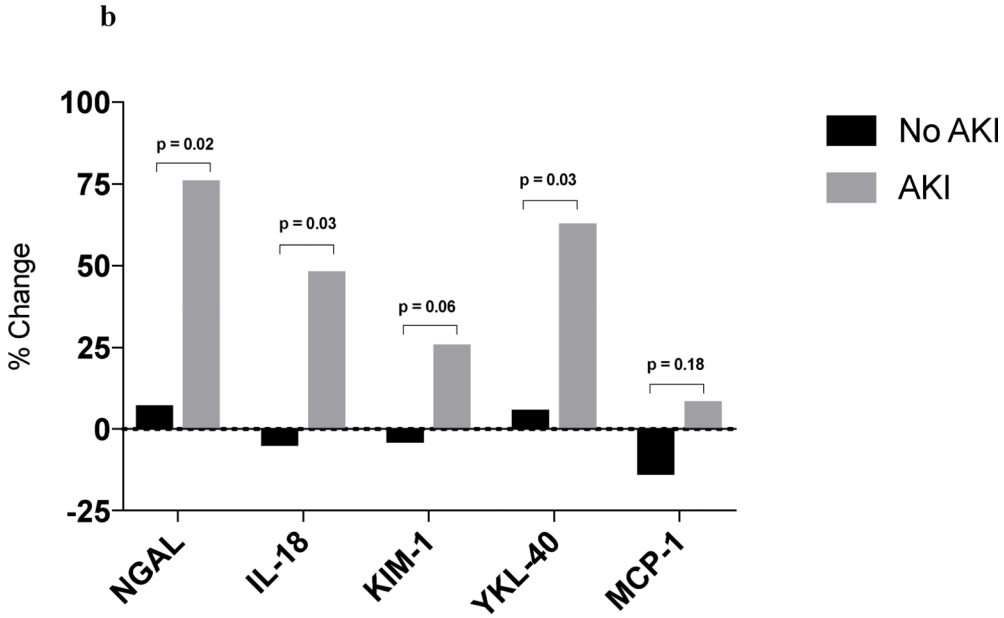

FIGURE 2b.

Change in tubule injury markers from baseline to year four among a random sample of 947 individuals with CKD, stratified by those with intervening AKI (N=59) vs. all others (N=888)

Table 1.

Baseline characteristics of SPRINT participants with CKD by AKI status

Characteristics	Without AKI (n=2167)	With AKI (n=184)
Age, years (SD)	73 (9)	74 (10)
Female, n (%)	883 (41)	59 (32)
Race, n (%)		
Non-Hispanic White	1597 (74)	118 (64)
Non-Hispanic Black	539 (25)	62 (34)
Hispanic and other	31 (1)	4 (2)
Intensive randomization arm, n (%)	1091 (50)	111 (60)
Cardiovascular disease or heart failure, n (%)	591 (27)	73 (40)
Mean systolic BP, mm Hg (SD)	139 (16)	141 (17)
Mean diastolic BP, mm Hg (SD)	75 (12)	73 (13)
Use of ACEi or ARBs, n (%)	1349 (62)	113 (61)
Use of diuretics, n (%)	1168 (54)	102 (55)
Median eGFR, (IQR)	50 (42, 57)	45 (35, 52)
Median urine ACR, mg/g (IQR)	14 (7,44)	27 (12, 156)
Median urine α1m, mg/g (IQR)	13 (7, 25)	18 (10, 32)
Median urine β2m, ng/ml (IQR)	103 (38, 322)	134 (47, 478)
Median urine UMOD, ng/ml (IQR)	7 (5, 10)	5 (3, 8)
Median urine KIM-1, pg/ml (IQR)	845 (387, 1582)	980 (458, 1862)
Median urine NGAL, ng/ml (IQR)	28 (15, 58)	36 (16, 76)
Median urine IL-18, pg/ml (IQR)	30 (16, 57)	31 (17, 61)
Median urine MCP-1, pg/ml (IQR)	180 (91, 325)	188 (88, 340)
Median urine YKL-40, pg/ml (IQR)	550 (219, 1251)	634 (234, 1732)

Abbreviations: ACEi, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers; eGFR, estimated glomerular filtration rate; ACR, albumin-creatinine ratio; α1m, alpha-1 microglobulin; β2m, beta-2 microglobulin; UMOD, uromodulin; KIM-1, kidney injury molecule-1; NGAL, neutrophil-gelatinase associated lipocalin; IL-18, interleukin 18; MCP-1, monocyte chemoattract protein-1; YKL-40, chitinase-3-like protein.

Table 2.

Association of Baseline Biomarkers of Tubular Health with AKI Events in SPRINT Participants with CKD

	Q1 HR (95% CI)	Q2 HR (95% CI)	Q3 HR (95% CI)	Q4 HR (95% CI)	Per 2-fold Higher HR (95% CI)	P Value
Uromodulin (uUMOD)						
Range	≤ 4.29 ng/ml	4.30 – 6.54 ng/ml	6.55 – 9.95 ng/ml	> 9.95 ng/ml		
# AKI/# at Risk (Rate)	72/566 (3.6%/yr)	53/597 (2.5%/yr)	29/595 (1.3%/yr)	30/593 (1.3%/yr)	184/2351 (2.1%/yr)	
Model 1 *	1.00 (Reference)	0.62 (0.43, 0.89)	0.32 (0.20, 0.51)	0.34 (0.21, 0.54)	0.61 (0.52, 1.71)	<0.001
Model 2 **	1.00 (Reference)	0.73 (0.50, 1.07)	0.44 (0.27, 0.72)	0.50 (0.31, 0.83)	0.71 (0.60, 0.85)	<0.001
Model 3 †	1.00 (Reference)	0.69 (0.47, 1.03)	0.44 (0.27, 0.72)	0.49 (0.30, 0.81)	0.68 (0.56, 0.83)	<0.001
Alpha-1 microglobulin (uα1m)						
Range	≤ 7.11 mg/ml	7.12 – 13.2 mg/ml	13.3 – 24.8 mg/ml	> 24.8 mg/ml		
# AKI/# at Risk (Rate)	29/564 (1.4%/yr)	33/589 (1.5%/yr)	53/593 (2.5%/yr)	69/605 (3.2%/yr)	184/2351 (2.1%/yr)	
Model 1 *	1.00 (Reference)	1.14 (0.68, 1.87)	1.81 (1.13, 2.89)	2.57 (1.61, 4.11)	1.39 (1.22, 1.58)	<0.001
Model 2 **	1.00 (Reference)	0.94 (0.56, 1.57)	1.28 (0.79, 2.09)	1.35 (0.81, 2.25)	1.14 (0.98, 1.33)	0.078
Model 3 †	1.00 (Reference)	0.98 (0.58, 1.67)	1.46 (0.86, 2.49)	1.57 (0.86, 2.88)	1.20 (1.01, 1.44)	0.042
Beta-2 microglobulin (uβ2m)						
Range	≤ 33.9 ng/ml	34.0 – 96.3 ng/ml	96.4 – 317.0 ng/ml	> 317.0 ng/ml		
# AKI/# at Risk (Rate)	36/530 (1.8%/yr)	45/598 (2.0%/yr)	39/608 (1.7%/yr)	64/615 (3.0%/yr)	184/2351 (2.1%/yr)	
Model 1 *	1.00 (Reference)	1.08 (0.69, 1.67)	0.92 (0.59, 1.46)	1.45 (0.96, 2.19)	1.04 (0.98, 1.10)	0.213
Model 2 **	1.00 (Reference)	1.15 (0.73, 1.79)	0.92 (0.58, 1.46)	1.03 (0.66, 1.60)	0.98 (0.93, 1.04)	0.534
Model 3 †	1.00 (Reference)	1.17 (0.74, 1.84)	0.84 (0.52, 1.36)	0.80 (0.48, 1.32)	0.94 (0.88, 1.00)	0.062
Kidney injury molecule-1 (uKIM-1)						
Range	≤ 386.73 pg/ml	386.74 – 849.01 pg/ml	849.02 – 1592.84 pg/ml	> 1595.84 pg/ml		
# AKI/# at Risk (Rate)	38/580 (1.8%/yr)	43/589 (2.0%/yr)	49/595 (2.2%/yr)	54/587 (2.5%/yr)	184/2351 (2.1%/yr)	
Model 1 *	1.00 (Reference)	1.32 (0.84, 2.08)	1.73 (1.08, 2.78)	2.52 (1.48, 4.31)	1.21 (1.08, 1.37)	0.002
Model 2 **	1.00 (Reference)	1.03 (0.65, 1.65)	1.34 (0.83, 2.17)	1.76 (1.02, 3.04)	1.13 (0.99, 1.28)	0.071
Model 3 †	1.00 (Reference)	1.13 (0.67, 1.90)	1.47 (0.84, 2.58)	1.83 (0.96, 3.48)	1.16 (0.99, 1.37)	0.068
Neutrophil-gelatinase associated lipocalin (uNGAL)						

	Q1	Q2	Q3	Q4	Per 2-fold Higher	P Value
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)	
Range	≤ 14.71 ng/ml	14.72 – 27.69 ng/ml	27.70 – 59.27 ng/ml	> 59.27 ng/ml		
# AKI/# at Risk (Rate)	42/575 (2.0%/yr)	38/592 (1.7%/yr)	45/593 (2.0%/yr)	59/591 (2.8%/yr)	184/2351 (2.1%/yr)	
Model 1 *	1.00 (Reference)	0.94 (0.60, 1.47)	1.21 (0.78, 1.88)	1.85 (1.20, 2.85)	1.12 (1.02, 1.23)	0.009
Model 2 **	1.00 (Reference)	0.88 (0.56, 1.39)	1.09 (0.69, 1.71)	1.48 (0.95, 2.32)	1.09 (0.99, 1.20)	0.067
Model 3 †	1.00 (Reference)	0.86 (0.54, 1.38)	1.03 (0.64, 1.66)	1.22 (0.73, 2.04)	1.03 (0.92, 1.17)	0.593
Interleukin-18 (uIL-18)						
Range	≤ 16.30 pg/ml	16.31 – 30.48 pg/ml	30.49 – 56.60 pg/ml	> 56.60 pg/ml		
# AKI/# at Risk (Rate)	43/577 (2.0%/yr)	46/597 (2.1%/yr)	44/583 (2.1%/yr)	51/594 (2.4%/yr)	184/2351 (2.1%/yr)	
Model 1 *	1.00 (Reference)	1.12 (0.73, 1.72)	1.24 (0.79, 1.95)	1.69 (1.03, 2.76)	1.13 (1.00, 1.29)	0.052
Model 2 **	1.00 (Reference)	1.02 (0.65, 1.58)	1.16 (0.73, 1.85)	1.54 (0.92, 2.56)	1.15 (0.98, 1.27)	0.108
Model 3 †	1.00 (Reference)	0.86 (0.54, 1.37)	0.88 (0.53, 1.45)	0.95 (0.53, 1.69)	0.96 (0.82, 1.13)	0.648
Monocyte chemoattractant protein-1 (uMCP-1)						
Range	≤ 89.42 pg/ml	89.43 – 180.78 pg/ml	180.79 – 326.99 pg/ml	> 326.99 pg/ml		
# AKI/# at Risk (Rate)	48/579 (2.3%/yr)	40/598 (1.8%/yr)	49/594 (2.3%/yr)	47/583 (2.2%/yr)	184/2351 (2.1%/yr)	
Model 1 *	1.00 (Reference)	0.85 (0.55, 1.31)	1.13 (0.71, 1.79)	1.21 (0.71, 2.08)	1.10 (0.97, 1.25)	0.124
Model 2 **	1.00 (Reference)	0.78 (0.50, 1.21)	0.99 (0.62, 1.56)	1.16 (0.67, 2.01)	1.06 (0.92, 1.21)	0.415
Model 3 †	1.00 (Reference)	0.67 (0.41, 1.09)	0.77 (0.45, 1.33)	0.79 (0.42, 1.50)	0.97 (0.81, 1.16)	0.739
Chitinase-3-like protein (uYKL-40)						
Range	≤ 213.95 pg/ml	213.96 – 542.59 pg/ml	542.60 – 1243.42 pg/ml	> 1243.42 pg/ml		
# AKI/# at Risk (Rate)	42/569 (2.0%/yr)	40/587 (1.8%/yr)	42/590 (1.9%/yr)	60/605 (2.8%/yr)	184/2351 (2.1%/yr)	
Model 1 *	1.00 (Reference)	1.02 (0.66, 1.58)	1.14 (0.73, 1.78)	1.93 (1.24, 3.00)	1.11 (1.03, 1.19)	0.004
Model 2 **	1.00 (Reference)	1.07 (0.68, 1.69)	1.25 (0.79, 1.97)	1.77 (1.13, 2.77)	1.09 (1.01, 1.17)	0.018
Model 3 †	1.00 (Reference)	1.03 (0.65, 1.64)	1.18 (0.74, 1.91)	1.52 (0.90, 2.56)	1.06 (0.97, 1.16)	0.185

* Model 1 adjusted for age, sex, race, randomization arm, and urine Cr

** Model 2 adjusted for Model 1 variables and baseline eGFR, urine albumin, systolic and diastolic blood pressures, prevalent CVD, ACE-inhibitor use, ARB use, and diuretic use

† Model 3 adjusted for Model 2 variables and the other urine biomarkers of tubular function and injury.

Table 3.

Associations of Kidney Tubule Function Markers with Risk of AKI Stratified by Randomization Arm in SPRINT*

Tubule Function Marker	Randomization Arm	HR per 2-fold higher (95% CI)	P-value	Interaction p-value
Uromodulin (uUMOD)				
	Standard	0.88 (0.67, 1.17)	0.389	0.111
	Intensive	0.62 (0.49, 0.78)	<0.001	
Alpha-1 microglobulin (uα1m)				
	Standard	1.18 (0.93, 1.49)	0.184	0.419
	Intensive	1.14 (0.94, 1.38)	0.192	
Beta-2 microglobulin (uβ2m)				
	Standard	0.97 (0.89, 1.07)	0.582	0.501
	Intensive	1.00 (0.93, 1.07)	0.896	
Kidney injury molecule-1 (uKIM-1)				
	Standard	1.00 (0.99, 1.50)	0.056	0.985
	Intensive	1.08 (0.92, 1.27)	0.847	
Neutrophil-gelatinase associated lipocalin (uNGAL)				
	Standard	1.08 (0.92, 1.26)	0.343	0.579
	Intensive	1.10 (0.98, 1.25)	0.106	
Interleukin-18 (uIL-18)				
	Standard	1.09 (0.87, 1.35)	0.458	0.445
	Intensive	1.14 (0.97, 1.35)	0.123	
Monocyte chemoattractant protein-1 (uMCP-1)				
	Standard	1.02 (0.93, 1.25)	0.855	0.354
	Intensive	1.10 (0.92, 1.31)	0.297	
Chitinase-3-like protein (uYKL-40)				
	Standard	1.12 (0.99, 1.25)	0.052	0.946
	Intensive	1.08 (0.99, 1.18)	0.081	

*Models adjusted for age; sex; race; and baseline urine creatinine, eGFR, urine albumin, baseline systolic and diastolic blood pressures, prevalent CVD, ACE-inhibitor use, ARB use, and baseline diuretic use. (“Model 2”)