Title
Serum Uromodulin: A Biomarker of Long-Term Kidney Allograft Failure

Journal
American Journal of Nephrology, 47(4)

ISSN
0250-8095

Authors
Bostom, Andrew
Steubl, Dominik
Garimella, Pranav S
et al.

Publication Date
2018

DOI
10.1159/000489095

Peer reviewed

HHS Public Access

Author manuscript

Am J Nephrol. Author manuscript; available in PMC 2019 September 21.

Published in final edited form as:

Am J Nephrol. 2018 ; 47(4): 275–282. doi:10.1159/000489095.

Serum Uromodulin: A Biomarker of Long-Term Kidney Allograft Failure

Andrew Bostom[a], **Dominik Steubl**[b], **Pranav S. Garimella**[c], **Nora Franceschini**[d], **Mary B. Roberts**[a], **Andreas Pasch**[e], **Joachim H. Ix**[c], **Katherine R. Tuttle**[f], **Anastasia Ivanova**[d], **Theresa Shireman**[g], **S. Joseph Kim**[h], **Reginald Gohh**[i], **Daniel E. Weiner**[j], **Andrew S. Levey**[j], **Chi-yuan Hsu**[k], **John W. Kusek**[l], **Charles B. Eaton**[m,n]

[a]Center For Primary Care and Prevention, Memorial Hospital of Rhode Island, Pawtucket, RI, USA; [b]Klinikum rechts der Isar, Technische Universität, München, Germany; [c]Division of Nephrology-Hypertension, Department of Medicine, University of California, San Diego, CA, USA; [d]Department of Epidemiology, Gillings School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [e]University of Bern, Bern, Switzerland; [f]Providence Medical Research Center, University of Washington, Spokane, WA, USA; [g]Center for Gerontology and Healthcare Research, Brown University, Providence, RI, USA; [h]Division of Nephrology and the Kidney Transplant Program, Toronto General Hospital, Toronto, ON, Canada; [i]Division of Hypertension and Kidney Diseases, Department of Medicine, Rhode Island Hospital, Providence, RI, USA; [j]Division of Nephrology, Department of Medicine, Tufts Medical Center, Boston, MA, USA; [k]Division of Nephrology, University of California-San Francisco, San Francisco, CA, USA; [l]National Institute of Diabetes, Digestive, and Kidney Diseases, National Institutes of Health, Bethesda, MD, USA; [m]Department of Family Medicine, Warren Alpert Medical School, Brown University, Providence, RI, USA; [n]Department of Epidemiology, School of Public Health, Brown University, Providence, RI, USA

Abstract

Background: Uromodulin is a kidney-derived glycoprotein and putative tubular function index. Lower serum uromodulin was recently associated with increased risk for kidney allograft failure in a preliminary, longitudinal single-center European study involving 91 kidney transplant recipients (KTRs).

Methods: The Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) trial is a completed, large, multiethnic controlled clinical trial cohort, which studied chronic, stable KTRs. We conducted a case cohort analysis using a randomly selected subset of patients (random

Andrew Bostom, MD, Center For Primary Care and Prevention Memorial Hospital of Rhode Island, 111 Brewster Street, Pawtucket, RI 02860 (USA), abostom@cox.net.

Author Contributions

Research idea and study design: A.B., D.S., P.G., A.I., J.H.I., K.R.T., A.P., M.B.R., and C.B.E.; data acquisition: A.B., A.P., T.S., and M.B.R.; data analysis/interpretation: A.B., D.S., P.G., N.F., M.B.R., A.P., J.H.I., K.R.T., A.I., T.S., S.J.K., R.G., D.E.W., A.S.L., C.H., J.W.K., and C.B.E.; statistical analysis: M.B.R. and A.I. Each author contributed important intellectual content during manuscript drafting or revision, and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Disclosure Statement

The authors declare that they have no relevant financial or other potential conflicts of interest to disclose.

subcohort, $n = 433$), and all individuals who developed kidney allograft failure (cases, $n = 226$) during follow-up. Serum uromodulin was determined in this total of $n = 613$ FAVORIT trial participants at randomization. Death-censored kidney allograft failure was the study outcome.

Results: The 226 kidney allograft failures occurred during a median surveillance of 3.2 years. Unadjusted, weighted Cox proportional hazards modeling revealed that lower serum uromodulin, tertile 1 vs. tertile 3, was associated with a threefold greater risk for kidney allograft failure (hazards ratio [HR], 95% CI 3.20 [2.05–5.01]). This association was attenuated but persisted at two-fold greater risk for allograft failure, after adjustment for age, sex, smoking, allograft type and vintage, prevalent diabetes mellitus and cardiovascular disease (CVD), total/high-density lipoprotein cholesterol ratio, systolic blood pressure, estimated glomerular filtration rate, and natural log urinary albumin/creatinine: HR 2.00, 95% CI (1.06–3.77).

Conclusions: Lower serum uromodulin, a possible indicator of less well-preserved renal tubular function, remained associated with greater risk for kidney allograft failure, after adjustment for major, established clinical kidney allograft failure and CVD risk factors, in a large, multiethnic cohort of long-term, stable KTRs.

Keywords

Serum uromodulin; Kidney transplantation; Kidney allograft failure

Uromodulin is an 85 kDa glycoprotein synthesized exclusively in the kidney – especially within the nephron's thick ascending limb but also in the distal convoluted tubule. Urinary and plasma or serum concentrations of uromodulin have emerged as unique correlates of renal tubular function or integrity [1–8]. Preliminary longitudinal studies have further suggested that reduced levels of plasma or serum uromodulin are associated with renal function decline in established or presumed coronary heart disease patients [9] and progression to end-stage renal disease in patients with chronic kidney disease (CKD) [10]. Reduced serum or urine uromodulin concentrations may also be more sensitive indicators of kidney allograft dysfunction not detected by serum glomerular filtration markers or proteinuria/albuminuria [11, 12]. In a pilot study, lower baseline serum uromodulin concentrations were associated with kidney transplant recipient (KTR) allograft loss during follow-up [13]. This preliminary investigation had a limited number of events ($n = 13$). Accordingly, we examined the prospective association between serum uromodulin concentrations and the development of kidney allograft failure [13] in a sizable, multicenter, multiethnic cohort of chronic, stable KTRs, that is, the Folic Acid for Vascular Outcome Reduction in Transplantation (FAVORIT) trial cohort [14].

Methods

Study Population

A large, multicenter, multiethnic controlled clinical trial [14, 15], the completed FAVORIT study (NCT00064753; Rhode Island Hospital blanket ancillary study exemption for de-identified secondary data analyses granted, July 23, 2015) presented conclusive evidence that high-dose, compared to low-dose, B-vitamin-based lowering of plasma homocysteine – an amino acid by-product of methionine metabolism, and ostensible atherothrombotic risk

factor [15] – failed to reduce hard, centrally adjudicated cardiovascular disease (CVD) events, kidney allograft failure, or all-cause mortality in chronic stable KTRs [15]. KTRs were eligible for the parent study if they provided informed consent, were aged 35–75 years, had clinically stable kidney function and elevated total homocysteine levels. Stable kidney function was ascertained by medical chart review to establish that the patient's current allograft had been functioning for at least 6 months post-transplantation and there was no documented clinical indication of kidney function deterioration. All enrolled participants had a screening Cockcroft-Gault estimated creatinine clearance (Ccr) of 30 mL/min or greater and elevated total homocysteine (≥12.0 μmol/L for men or ≥11.0 μmol/L for women) based on central laboratory analysis of screening specimens. The Ccr eligibility criteria were reduced to 25 mL/min or greater for women recruited after July 2005, acknowledging the lower Ccr distribution routinely observed in women relative to the distribution in men. Individuals who had a chronic illness limiting life expectancy to less than 2 years, or whose CVD risk was modified because of recent CVD-related events or procedures were excluded [14, 16]. We combined the 2 randomized treatment arms in all the analyses for this report because the high-dose vitamin intervention did not result in a significant reduction in the event rates for any of these outcomes compared with the low-dose B-vitamin treatment [15].

To evaluate the potential association between serum uromodulin and kidney allograft failure, we took advantage of an efficient, preexisting case-cohort selection scheme employed for prior FAVORIT ancillary studies [17–19], in particular the report of Ix et al. [19]. A 530-member subcohort with complete baseline data for key laboratory variables, including serum creatinine and lipid studies, as well as urine albumin to creatinine ratio, was selected at random from the entire cohort, regardless of whether or not the participants had experienced kidney allograft failure during follow-up [17–20]. We also selected all participants in the full cohort who had a kidney allograft failure event, irrespective of whether they were sampled in the random subcohort. Through a National Institutes of Diabetes and Digestive and Kidney Diseases (NIDDK) Central Repository approved specimen request (1 X01 DK113962), and after excluding participants who lacked banked sera, and were missing key parent study laboratory covariables at baseline, we obtained sera to determine serum uromodulin concentrations in a subcohort of $n = 433$ individuals, and 226 kidney allograft failure cases. Given that 46 of the 226 allograft failure cases were also sampled in the random subcohort, the total case-cohort study population included 613 individuals.

Measurement of Serum Uromodulin

Serum uromodulin assays were performed at the University of Minnesota (UMN) Advanced Research and Diagnostic Laboratory using a commercially available uromodulin enzyme-linked immunosorbent assay (ELISA; Euroimmun, Medzinische Labor-diagnostika, Luebeck Germany [21]). With this method, immunoglobulin G1 of 2 affinity-purified monoclonal antibodies directed against uromodulin isolated from urine are used either for coating microtiter plates or as detection antibody, and processed according to the manufacturer's manual [21]. The absorbance of each well was detected using a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA, USA), and concentration was determined from a standard curve prepared with the assay. Inter-assay coefficients of variation for uromodulin concentrations determined by this ELISA [21] are 7.8, 6.6, and

6.6% at 35.4, 96.3, and 228.1 ng/mL respectively. Specimens for the serum uromodulin assays in our study were required to undergo 2 freeze-thaw cycles to preserve this precious resource. Before receiving the specimens from the NIDDK Repository, we established the validity of our specimen-handling scheme, using independent duplicate serum samples from KTRs not part of the FAVORIT cohort, banked at minus 80 ° C, since 2011 (at Rhode Island Hospital, Providence, RI, USA), and shipped overnight on dry ice to the UMN-ADRL. Consistent with the published findings of Scherberich et al. [8], and the unpublished ELISA kit package insert data [21], these analyses indicated that serum uromodulin was stable through at least 3 freeze-thaw cycles, the comparison concentration results remaining within the range of overall coefficients of variations of the described method [21], and independent published reports utilizing the same ELISA [6, 13] (Ms. Valerie Arends, UMN-ADRL, personal communication, April 7, 2017). Additional blind replicate pair analyses ($n = 11$) from the FAVORIT study samples yielded the mean between pair percent differences of <5% for serum uromodulin.

Kidney Allograft Failure

Initiation of dialysis therapy, as determined by local study staff, defined kidney allograft failure. Event time was considered from randomization to the date of kidney allograft failure, last follow-up visit, or culmination of the study period. Follow-up contacts occurred every 6 months from January 31, 2010, through June 24, 2009 to obtain study-related outcomes. We censored for death; hence death with a functioning kidney allograft was not designated the status of a kidney allograft failure in these analyses.

Other Measurements

Data collected at study enrollment [14–16], germane to these analyses, included: demographics (age, gender, self-designated ethnicity/race); smoking status (current, former, or never); past medical history (baseline CVD, diabetes mellitus); transplant characteristics (living donor kidney, and time since transplant, “allograft vintage”); physical examination findings (body mass index [BMI], systolic and diastolic blood pressure [BP]); and laboratory variables (creatinine, total cholesterol, high density lipoprotein, high-density lipoprotein (HDL)-cholesterol, and spot urinary [22] albumin and creatinine). Baseline BP was the average of 2 measurements and hypertension [23] was defined by a systolic BP ≥140 mm Hg, a diastolic ≥90 mm Hg, or antihypertensive medication use at study enrollment. Diabetes mellitus was defined by the use of insulin or oral hypoglycemic medications or patient history. Baseline CVD was characterized as prior myocardial infarction, coronary artery revascularization, stroke, carotid arterial revascularization, abdominal or thoracic aortic aneurysm repair, and/or lower extremity arterial revascularization [14–16]. Distinct from the use of the Cockroft-Gault estimate of renal function during the screening phase of FAVORIT, glomerular filtration rate (GFR) for subsequent baseline assessment of patients at their enrollment visit was estimated by the CKD-EPI [24] equation. Race categories were white, black, or other [14–16], with individuals who identified as “other” assigned as non-African Americans for estimated GFR (eGFR) calculation. BMI was calculated using the formula: weight (kg)/height $(m)^2$ [14–16].

Statistical Methods

Jonckheere-Terpstra tests for ordered alternatives (for continuous variables) and a Wilcoxon-type test for trend (for categorical variables) were used to compare baseline characteristics across tertiles of serum uromodulin concentrations. Weighted Cox proportional hazards regression models were employed to evaluate the association of baseline serum uromodulin concentrations and time to death-censored kidney allograft failure, in accordance with the case-cohort design [25, 26]. The distribution of serum uromodulin concentration values was right skewed, so these data were log base-2 scale transformed, such that coefficients can be interpreted as "per halving," or "per twofold lower" serum uromodulin. After assessing the unadjusted associations between serum uromodulin (per unit log base 2 lower; lower across tertiles, with tertile 3 as the "referent"), and kidney allograft failure, these initial models were adjusted, a priori, for serum uromodulin, plus systolic BP, age, sex, race, preexisting CVD or diabetes mellitus, eGFR, and natural log urinary albumin/creatinine ratio. On the basis of additional data available from the FAVORIT trial, extended ("fully-adjusted") models were further adjusted, a priori, for smoking, BMI, total cholesterol/HDL cholesterol ratio, kidney allograft vintage and type (deceased vs. living donor), calcineurin inhibitor use, and lipid-lowering drug use. Accordingly, we settled upon 3 main models: unadjusted, that is, serum uromodulin, only, as a continuous variable (model 1); serum uromodulin plus all the variables described above, except the 2 kidney measures, eGFR and natural log urinary albumin/creatinine (model 2); and the fully adjusted model that included the kidney measures (model 3). Proportional hazards assumptions were assessed by the examination of transformed martingale residuals plotted over survival time, and the supremum test. Interaction terms were evaluated between serum uromodulin and preexisting diabetes mellitus or CVD, age, and eGFR <45 (vs. ≥45) mL/min/1.73 m^2, as well as urinary albumin-creatinine ratio (UACR) ≥30 (vs. <30) μg/mg, to detect for potential effect modification within these higher-risk sub-groups, in particular. Finally, a sensitivity analysis was performed restricting the sample to those participants with an eGFR between 20 and 74 mL/min/1.73 m^2. Analyses were performed using SPSS software (version 23.0), and SAS software (version 9.4). Two sided p values <0.05 were considered statistically significant for all analyses.

Results

Study Population

As depicted in Table 1, key baseline characteristics of the random subcohort (n = 433) were concordant with those of the full FAVORIT cohort. The mean age for these 433 participants was 51.2 ± (SD) 9.0 years, 39% were women, 24% were non-white, 19% had a history of CVD, and 36% had a history of diabetes mellitus. Their baseline mean eGFR was 45.5 ± 18.1 mL/min/1.73 m^2 and the median time since transplantation ("allograft vintage") was 3.9 years, while 43% received a living donor kidney allograft. Serum uromodulin concentration (ng/mL) was right skewed, but normalized with log base-2 transformation, and is presented in tertiles, with tertile 3 as the referent: tertile 3, >74.05–309.59 ng/mL; tertile 2, >46.81–74.05 ng/mL; tertile 1, 5.62–46.81 ng/mL. Table 1 demonstrates that compared with those in the referent third uromodulin tertile, subcohort participants with lower uromodulin concentrations were more likely to smoke (p = 0.11), have a greater BMI (p =

0.01) and total cholesterol/HDL cholesterol ($p = 0.01$), lesser eGFR ($p < 0.01$), and higher UACR ($p < 0.01$).

Outcome Analyses

The Methods section described how we identified 226 kidney allograft failure events, 46 of these sampled cases overlapping with the randomly selected 433-member subcohort. These 226 events occurred during a median follow-up period of 3.2 years. Subsequently, utilizing the entire 613 case-cohort sample, we evaluated the association between serum uromodulin and kidney allograft failure. Proportional hazards assumptions were met for the kidney allograft failure outcome analyses. A robust, graded association between serum uromodulin concentrations and kidney allograft failure was observed in unadjusted analyses (model 1; Table 2). Each halving of uromodulin concentrations (i.e., per unit $\log_2$-transformed uromodulin, lower) was associated with a 51% greater risk for kidney allograft failure (hazards ratio [HR] per halving = 1.51; 95% CI [1.23–1.85]), while a 3.20-fold increased risk (HR 3.20; 95% CI [2.05–5.01]) was evident comparing the concentrations of uromodulin in tertile 1, relative to tertile 3. Multivariable adjustment (Table 2; model 2) for treatment assignment, systolic BP, age, sex, race, preexisting CVD or diabetes mellitus, smoking, BMI, total cholesterol/HDL cholesterol, kidney allograft vintage and type (deceased vs. living donor), calcineurin inhibitor use, and lipid lowering drug use, had no effect on the association between uromodulin and kidney allograft failure per each halving lower (HR 1.51; 95% CI [1.20–1.90]), while slightly diminishing the association comparing tertile 1 to tertile 3, HR 2.96; 95% CI (1.80–4.81). Full adjustment for all the variables in model 2, plus the kidney measures eGFR and UACR (Table 2; model 3), attenuated these associations, somewhat, but they persisted, significantly: per each halving lower of uromodulin, HR 1.31, 95% CI (1.01–1.69); tertile 1 to tertile 3 comparison, HR 2.00, 95% CI (1.06–3.77).

Interaction analyses revealed no evidence for effect modification by preexisting diabetes mellitus and CVD ($p = 0.486$), age ($p = 0.096$), eGFR <45 (vs. ≥45) mL/min/1.73 m^2 ($p = 0.309$), or UACR ≥30 (vs. <30) μg/mg ($p = 0.735$). Lastly, a sensitivity analysis restricting the sample to those $n = 549$ participants with an eGFR between 20 and 74 mL/min per 1.73 m^2 ($n = 199$ kidney allograft failure events experienced in this subgroup) yielded results concordant with those from the entire case-cohort sample: per unit log base 2 lower serum uromodulin, fully adjusted (model 3), HR 1.47, 95% CI (1.10–1.96); comparing tertile 1 relative to tertile 3, fully adjusted (model 3), HR 2.13, 95% CI (1.10–4.11).

Conclusion

A recent preliminary, longitudinal assessment of 91 kidney/kidney-pancreas transplant recipients by Steubl et al. [13] reported that lower serum uromodulin concentrations were associated with subsequent allograft loss, after adjustment for age, gender, BMI, and living versus cadaveric allograft. Of the 13 allograft losses recorded during a mean follow-up of 3.6 ± 2.2 years, 9 occurred among those with uromodulin concentrations in the lowest quartile [13]. Our prospective investigation, which evaluated the potential association between baseline serum uromodulin concentrations, and the development of more than 17-

fold (i.e., 226 vs. 13) the number of kidney allograft failure events, provides considerable external validation of this pioneering study [13]. We found that after adjusting not only for the same 4 variables modeled by Steubl et al. [13], but expanding our multivariable modeling to include allograft vintage, preexisting diabetes mellitus or CVD, smoking history, systolic BP, and total/HDL cholesterol ratio, each halving of serum uromodulin was associated with a 51% greater risk for kidney allograft failure, while relative to the third tertile, the risk for developing kidney allograft failure in the first uromodulin tertile increased threefold. Most important, although attenuated, these associations remained moderately robust, and significant, upon further adjustment for the quintessential clinical kidney measures, eGFR, and UACR, with a 31% increased risk for kidney allograft failure per uromodulin halving, and twofold greater risk comparing uromodulin tertile 1, to tertile 3 respectively.

Notwithstanding a decided emphasis on studies of urinary uromodulin – since the glycoprotein's discovery as Tamm-Horsfall protein in 1950 [27], through 1990 [28], which has continued till now [5, 7, 12, 29–35] – normative ranges for serum/plasma uromodulin concentrations were established over 3 decades ago by radioimmunoassay [2, 3]. Seminal reports published in 1981 [2] and 1985 [3] by St. Bartholomew's Hospital (London) investigators, hinging upon serum/plasma and urinary radioimmunoassay determination of uromodulin, revealed that the glycoprotein was a normal component of human serum, as well as urine, whose concentrations were closely related to the volume of functioning renal mass. These findings have been validated in current, larger studies employing serum uromodulin ELISAs [4, 6, 9, 10].

Perhaps due, in part, to methodologic limitations unique to urinary assays of uromodulin, compared to the serum assay [8, 36], studies of the potential associations between concentrations of the glycoprotein in urine and clinical outcomes in CKD and non-CKD populations have produced conflicting results [29–35]. Youhanna et al. [36] have shown that storage conditions, centrifugation, vortexing, pH, electrolytes, and osmolality may negatively affect the stability of uromodulin in urine samples, possibly altering its polymerization, and causing conformational changes that modulate antigenic binding sites and impair urinary assay reproducibility [36]. Two groups, in contrast, have reported finding only monomeric uromodulin, free of any aggregation processes in serum [8, 37]. Scherberich et al. [8] also described how serum uromodulin exhibited remarkable stability over weeks, even at increased temperatures. This potential methodologic advantage of determining serum (or plasma), vs. urine uromodulin, might limit 1 critical source of error in clinico-epidemiologic studies of uromodulin concentrations, and renal outcomes, yielding less discordant results. For example, our finding in KTRs of a prospective association between lower serum uromodulin and kidney allograft failure confirms the preliminary KTR study of Steubl et al. [13], and is also consistent with 2 small prospective investigations, one linking lower plasma uromodulin to ESRD development in CKD patients [10] and the other reporting that reduced serum uromodulin predicted the occurrence of stage 3 CKD in patients with known or suspected CHD, whose baseline eGFRs were >60 mL/min/1.73 m^2 [9].

Although hypothesized disadvantages [8, 36] of assessing urine vs. serum or plasma uromodulin merit consideration, the absence of simultaneous urine uromodulin determinations in our analyses is one of the study's drawbacks. Indeed, we hope to procure matching urines from this case-cohort sample ([38]; and Table 1, within 38) for the purpose of directly comparing the relative associations between serum vs. urine uromodulin concentrations, and kidney allograft failure. Additionally, lack of currently available resources constrained our ability to undertake further rigorous, exploratory analyses [39] of the potential clinical utility of uromodulin to predict kidney allograft failure beyond the clinical kidney measures eGFR and UACR [22, 24], or ostensible urinary renal tubular injury markers [19, 20]. Our hope is to complete such analyses in the near future patterned after those reported by Hsu et al. from the CRIC cohort [40], within the limitations imposed by the case-cohort design itself [39]. We also did not evaluate possible associations between serum uromodulin concentrations and CVD outcomes, or all-cause mortality in FAVORIT, as suggested by 2 independent reports from Western European cohorts with established or presumptive angiographic coronary artery disease [41, 42]. Examining these relationships is planned as well, pending additional resources. The design of FAVORIT as a study of a chronic, stable KTR cohort [14–16] also precludes its ability to explore any utility of serum uromodulin concentration in detecting early (i.e., within the first year), more subtle kidney allograft dysfunction, or even kidney allograft failure, which occurs during the initial 12-months post-transplantation. Finally, there are no genetic data available from FAVORIT, which precluded exploring the potential influence, for example, of either the relatively common rs129177707 polymorphism at the uromodulin gene locus, or rare pathogenic mutations [38, 42]. It should be noted, however, that irrespective of genetic phenotypes, in both CHD populations and cohorts with established Autosomal Dominant Tubulointerstitial Kidney Disease (ADTKD-UMOD), serum uromodulin concentrations were independently associated with hard outcomes in the former [42], or were lower in all affected patients with a novel missense mutation in the UMOD gene (457T>G; Cys153Gly), relative to all patients of the reference eGFR-matched CKD groups, and healthy, unaffected family members [43].

In conclusion, our study, conducted within a large, multiethnic cohort of long-term, stable KTRs [14–16], provides evidence that lower serum uromodulin concentrations, which may be a surrogate for less well-preserved renal tubular function [1–13], were associated with greater risk for kidney allograft failure, and this relationship persisted after adjustment for major, established kidney allograft failure and CVD risk factors [21–24]. Future studies that elucidate the potential clinical value of determining serum, and/or urine uromodulin concentrations to enhance kidney allograft failure prediction, and possibly other KTR outcomes, are warranted.

Acknowledgments

The authors acknowledge Ms. Valerie Arends of the UMN Advanced Research and Diagnostic Laboratory for her technical role in serum uromodulin assay performance and validation.

Support

The serum aliquots for the uromodulin assays were provided (to Dr. Andrew Bostom) from the NIDDK Central Repository via 1 X01 DK113962. The serum uromodulin determinations were made possible by generous private donations from Dr. Leslie Robinson-Bostom, and Ms. Rita Schacter. The FAVORIT Trial was supported by

cooperative agreement U01 DK61700 from the National Institute of Diabetes and Digestive and Kidney Diseases (to Dr. Andrew Bostom) and by the Office of Dietary Supplements at the National Institutes of Health. The funders of this study (Dr. John W. Kusek, retired September, 2017, only) contributed to the writing of this report but had no role in the study design, collection, or data analysis.

References

1. El-Achkar TM, McCracken R, Liu Y, Heitmeier MR, Bourgeois S, Ryerse J, Wu XR: Tamm-Horsfall protein translocates to the basolateral domain of thick ascending limbs, interstitium, and circulation during recovery from acute kidney injury. Am J Physiol Renal Physiol 2013;304:F1066–F1075. [PubMed: 23389456]
2. Dawnay AB, Cattell WR: Serum Tamm-Horsfall glycoprotein levels in health and in renal disease. Clin Nephrol 1981;15:5–8. [PubMed: 7011620]
3. Thornley C, Dawnay A, Cattell WR: Human Tamm-Horsfall glycoprotein: urinary and plasma levels in normal subjects and patients with renal disease determined by a fully validated radioimmunoassay. Clin Sci (Lond) 1985;68:529–535. [PubMed: 3979015]
4. Risch L, Lhotta K, Meier D, Medina-Escobar P, Nydegger UE, Risch M: The serum uromodulin level is associated with kidney function. Clin Chem Lab Med 2014;52:1755–1761. [PubMed: 24933630]
5. Pruijm M, Ponte B, Ackermann D, Paccaud F, Guessous I, Ehret G, Pechère-Bertschi A, Vogt B, Mohaupt MG, Martin PY, Youhanna SC, Nägele N, Vollenweider P, Waeber G, Burnier M, Devuyst O, Bochud M: Associations of urinary uromodulin with clinical characteristics and markers of tubular function in the general population. Clin J Am Soc Nephrol 2016;11: 70–80. [PubMed: 26683888]
6. Steubl D, Block M, Herbst V, Nockher WA, Schlumberger W, Satanovskij R, Angermann S, Hasenau AL, Stecher L, Heemann U, Renders L, Scherberich J: Plasma uromodulin correlates with kidney function and identifies early stages in chronic kidney disease patients. Medicine (Baltimore) 2016; 95:e3011. [PubMed: 26962815]
7. Garimella PS, Sarnak MJ: Uromodulin in kidney health and disease. Curr Opin Nephrol Hypertens 2017;26:136–142. [PubMed: 27898524]
8. Scherberich JE, Gruber R, Nockher WA, Christensen EI, Schmitt H, Herbst V, Block M, Kaden J, Schlumberger W: Serum uromodulin-a marker of kidney function and renal parenchymal integrity. Nephrol Dial Transplant 2017;33:284–295.
9. Leiherer A, Muendlein A, Saely CH, Brandtner EM, Geiger K, Fraunberger P, Drexel H: The value of uromodulin as a new serum marker to predict decline in renal function. J Hypertens 2018;36:110–118. [PubMed: 28858977]
10. Tan F, Zeng Y, Yan L, Zhang D: Low plasma uromodulin is a predictor of early stage chronic kidney disease progression. Int J Clin Exp Med 2017;10:8055–8059.
11. Avis PJ, Williams JD, Salaman JR, Asscher AW, Newcombe R: Serum Tamm-Horsfall protein (THP) concentration in cyclosporin treated renal transplant recipients. Lancet 1985;2:154.
12. McLaughlin PJ, Aikawa A, Davies HM, Ward RG, Bakran A, Sells RA, Johnson PM: Uromodulin levels are decreased in urine during acute tubular necrosis but not during immune rejection after renal transplantation. Clin Sci (Lond) 1993;84:243–246. [PubMed: 8382589]
13. Steubl D, Block M, Herbst V, Schlumberger W, Nockher A, Angermann S, Schmaderer C, Heemann U, Renders L, Scherberich J: Serum uromodulin predicts graft failure in renal transplant recipients. Biomarkers 2017;22: 171–177. [PubMed: 27790922]
14. Bostom AG, Carpenter MA, Hunsicker L, Jacques PF, Kusek JW, Levey AS, McKenney JL, Mercier RY, Pfeffer MA, Selhub J; FAVORIT Study Investigators: Baseline characteristics of participants in the folic acid for vascular outcome reduction in transplantation (FAVORIT) Trial. Am J Kidney Dis 2009; 53:121–128. [PubMed: 19022547]
15. Bostom AG, Carpenter MA, Kusek JW, Levey AS, Hunsicker L, Pfeffer MA, Selhub J, Jacques PF, Cole E, Gravens-Mueller L, House AA, Kew C, McKenney JL, Pacheco-Silva A, Pesavento T, Pirsch J, Smith S, Solomon S, Weir M: Homocysteine-lowering and cardiovascular disease outcomes in kidney transplant recipients: primary results from the folic acid for vascular outcome reduction in transplantation trial. Circulation. 2011; 123:1763–1770. [PubMed: 21482964]

16. FAVORIT Data Dictionary and Analysis Manual: Prepared by the FAVORIT Data Coordinating Center, Collaborative Studies Coordinating Center, Department of Biostatistics, Gillings School of Global Public Health University of North Carolina at Chapel Hill, 137 E. Franklin Street Suite #203, Chapel Hill, NC 27514–4145, Version 5.0, 2 1, 2013.
17. Jarolim P, Claggett BL, Conrad MJ, Carpenter MA, Ivanova A, Bostom AG, Kusek JW, Hunsicker LG, Jacques PF, Gravens-Mueller L, Finn P, Solomon SD, Weiner DE, Levey AS, Pfeffer MA: B-type natriuretic peptide and cardiac troponin I are associated with adverse outcomes in stable kidney transplant recipients. Transplantation 2017;101:182–190. [PubMed: 26910333]
18. Foster MC, Weiner DE, Bostom AG, Carpenter MA, Inker LA, Jarolim P, Joseph AA, Kusek JW, Pesavento T, Pfeffer MA, Rao M, Solomon SD, Levey AS: Filtration markers, cardiovascular disease, mortality, and kidney outcomes in stable kidney transplant recipients: the FAVORIT trial. Am J Transplant 2017;17:2390–2399. [PubMed: 28257169]
19. Ix JH, Katz R, Bansal N, Foster M, Weiner DE, Tracy R, Jotwani V, Hughes-Austin J, McKay D, Gabbai F, Hsu CY, Bostom A, Levey AS, Shlipak MG: Urine fibrosis markers and risk of allograft failure in kidney transplant recipients: a case-cohort ancillary study of the FAVORIT trial. Am J Kidney Dis 2017;69:410–419. [PubMed: 28024930]
20. Bansal N, Carpenter MA, Weiner DE, Levey AS, Pfeffer M, Kusek JW, Cai J, Hunsicker LG, Park M, Bennett M, Liu KD, Hsu CY: Urine injury biomarkers and risk of adverse outcomes in recipients of prevalent kidney transplants: the folic acid for vascular outcome reduction in transplantation trial. J Am Soc Nephrol 2016;27:2109–2121. [PubMed: 26538631]
21. Euroimmun Uromodulin ELISA, description https://goo.gl/iSCWoB.
22. John AA, Levey AS, Carpenter MA, Hunsicker LG, Kalil RS, Kusek JW, Pfeffer MA, Solomon SD, Weir MR, Weiner DE: Albuminuria and cardiovascular disease, kidney failure and death in stable kidney transplant recipients (abstract).J Am Soc Nephrol 2013;24: 72A.
23. Carpenter MA, John A, Weir MR, Smith SR, Hunsicker L, Kasiske BL, Kusek JW, Bostom A, Ivanova A, Levey AS, Solomon S, Pesavento T, Weiner DE: BP, cardiovascular disease, and death in the folic acid for vascular outcome reduction in transplantation trial. J Am Soc Nephrol 2014;25:1554–1562. [PubMed: 24627349]
24. Weiner DE, Carpenter MA, Levey AS, Ivanova A, Cole EH, Hunsicker L, Kasiske BL, Kim SJ, Kusek JW, Bostom AG: Kidney function and risk of cardiovascular disease and mortality in kidney transplant recipients: the FAVORIT trial. Am J Transplant 2012;12:2437–2445. [PubMed: 22594581]
25. Barlow WE: Robust variance estimation for the case-cohort design. Biometrics 1994;50: 1064–1072. [PubMed: 7786988]
26. Barlow WE, Ichikawa L, Rosner D, Izumi S: Analysis of case-cohort designs. J Clin Epidemiol 1999;52:1165–1172. [PubMed: 10580779]
27. Tamm I, Horsfall FL Jr: Characterization and separation of an inhibitor of viral hemagglutination present in urine. Proc Soc Exp Biol Med 1950;74:108–114.
28. Kumar S, Muchmore A: Tamm-Horsfall protein – uromodulin (1950–1990). Kidney Int 1990;37:1395–1401. [PubMed: 2194064]
29. Sejdiu I, Torffvit O: Decreased urinary concentration of Tamm-Horsfall protein is associated with development of renal failure and cardiovascular death within 20 years in type 1 but not in type 2 diabetic patients. Scand J Urol Nephrol 2008;42:168–174. [PubMed: 17907053]
30. Köttgen A, Hwang SJ, Larson MG, Van Eyk JE, Fu Q, Benjamin EJ, Dehghan A, Glazer NL, Kao WH, Harris TB, Gudnason V, Shlipak MG, Yang Q, Coresh J, Levy D, Fox CS: Uromodulin levels associate with a common UMOD variant and risk for incident CKD. J Am Soc Nephrol 2010;21:337–344. [PubMed: 19959715]
31. Shlipak MG, Li Y, Fox C, Coresh J, Grunfeld C, Whooley M: Uromodulin concentrations are not associated with incident CKD among persons with coronary artery disease. BMC Nephrol 2011;12:2. [PubMed: 21235779]
32. Reznichenko A, van Dijk MC, van der Heide JH, Bakker SJ, Seelen M, Navis G: Uromodulin in renal transplant recipients: elevated urinary levels and bimodal association with graft failure. Am J Nephrol 2011;34:445–451. [PubMed: 21968132]

33. Zhou J, Chen Y, Liu Y, Shi S, Wang S, Li X, Zhang H, Wang H: Urinary uromodulin excretion predicts progression of chronic kidney disease resulting from IgA nephropathy. PLoS One 2013;8:e71023. [PubMed: 23990922]
34. Garimella PS, Biggs ML, Katz R, Ix JH, Bennett MR, Devarajan P, Kestenbaum BR, Siscovick DS, Jensen MK, Shlipak MG, Chaves PH, Sarnak MJ: Urinary uromodulin, kidney function, and cardiovascular disease in elderly adults. Kidney Int 2015;88:1126–1134. [PubMed: 26154925]
35. Garimella PS, Katz R, Ix JH, Fried LF, Kritchevsky SB, Devarajan P, Bennett MR, Parikh CR, Shlipak MG, Harris TB, Gutiérrez OM, Sarnak MJ: Association of urinary uromodulin with kidney function decline and mortality: the health ABC study. Clin Nephrol 2017;87:278–286. [PubMed: 28332475]
36. Youhanna S, Weber J, Beaujean V, et al.: Determination of uromodulin in human urine: influence of storage and processing. Nephrol Dial Transplant 2014;29:136–145. [PubMed: 24097801]
37. Micanovic R, Khan S, Janosevic D, Lee ME, Hato T, Srour EF, Winfree S, Ghosh J, Tong Y, Rice SE, Dagher PC, Wu XR, El-Achkar TM: Tamm-Horsfall protein regulates mono-nuclear phagocytes in the kidney. J Am Soc Nephrol. 2018;29:841–856. [PubMed: 29180395]
38. Bostom AG, Steubl D, Friedman AN: Hypothesis: potential utility of serum and urine uromodulin measurement in kidney transplant recipients? Transplant Direct 2017; 3:e219. [PubMed: 29184908]
39. Sanderson J, Thompson SG, White IR, Aspelund T, Pennells L: Derivation and assessment of risk prediction models using case-cohort data. BMC Med Res Methodol 2013;13:113. [PubMed: 24034146]
40. Hsu CY, Xie D, Waikar SS, Bonventre JV, Zhang X, Sabbisetti V, Mifflin TE, Coresh J, Diamantidis CJ, He J, Lora CM, Miller ER, Nelson RG, Ojo AO, Rahman M, Schelling JR, Wilson FP, Kimmel PL, Feldman HI, Vasan RS, Liu KD; CRIC Study Investigators; CKD Biomarkers Consortium: Urine biomarkers of tubular injury do not improve on the clinical model predicting chronic kidney disease progression. Kidney Int 2017;91:196–203. [PubMed: 28029431]
41. Leiherer A, Muendlein A, Saely CH, Ebner J, Brandtner EM, Fraunberger P, Drexel H: Serum uromodulin is a predictive biomarker for cardiovascular events and overall mortality in coronary patients. Int J Cardiol 2017;231:6–12. [PubMed: 28089453]
42. Delgado GE, Kleber ME, Scharnagl H, Krämer BK, März W, Scherberich JE: Serum uromodulin and mortality risk in patients undergoing coronary angiography. J Am Soc Nephrol 2017;28:2201–2210. [PubMed: 28242751]
43. Satanovskij R, Bader A, Block M, Herbst V, Schlumberger W, Haack T, Nockher WA, Heemann U, Renders L, Schmaderer C, Angermann S, Wen M, Meitinger T, Scherberich J, Steubl D: A new missense mutation in UMOD gene leads to severely reduced serum uromodulin concentrations – a tool for the diagnosis of uromodulin-associated kidney disease. Clin Biochem 2017;50:155–158. [PubMed: 27729211]

Table 1.

Baseline full cohort characteristics, and SC characteristics by serum uromodulin concentration

Characteristic	Full cohort* (*n* = 3,530)	SC overall 5.62–309.59 ng/mL (*n* = 433)	SC tertile 3 >74.05–309.59 ng/mL (*n* = 144)	SC tertile 2 >46.81–74.05 ng/mL (*n* = 145)	SC tertile 1 5.62–46.81 ng/mL (*n* = 144)	*p* value
Age, years	51.8±9.4	51.2±9.0	52.0±9.1	50.6±8.6	51.1±9.2	0.30
Gender, male	2,220 (62.9)	266 (61.4)	83 (57.6)	96 (66.2)	87 (60.4)	0.63
White race	2,672 (75.7)	327 (75.5)	112 (77.8)	106 (73.1)	109 (75.7)	0.67
Transplant vintage, years	4.0 (1.7–7.3)	3.9 (1.8–7.1)	3.5 (1.6–6.1)	4.1 (1.9–8.0)	3.9 (1.8–7.5)	0.17
CVD history	702 (19.9)	84 (19.4)	30 (20.8)	24 (16.6)	30 (20.8)	1.00
Diabetes history	1,398 (39.6)	155 (35.8)	50 (34.7)	53 (36.6)	52 (36.1)	0.81
Current smoker	385 (10.9)	55 (12.7)	16 (11.1)	14 (9.7)	25 (17.4)	0.11
BMI, kg/m^2	29.2±6.2	29.0±5.8	27.8±5.5	29.6±5.6	29.5±6.2	0.01
Systolic BP, mm Hg	136.4±19.8	135.9±20.6	134.6±21.5	136.8±21.5	136.4±21.8	0.35
Living transplant donor type	1,500 (42.5)	187 (43.2)	61 (42.4)	71 (49.0)	55 (38.2)	0.48
Calcineurin inhibitor use[a]	3,113 (88.2)	383 (88.5)	124 (86.1)	129 (89)	130 (90.3)	0.27
Lipid-lowering drug use	1,945 (55.1)	229 (52.9)	77 (53.5)	81 (55.9)	71 (49.3)	0.48
Total/HDL cholesterol	4.3±2.0	4.3±1.3	4.0±1.2	4.4±1.3	4.5±1.4	0.01
eGFR, mL/min/1.73 m^2	49.0±17.6	45.5±18.1	53.5±17.7	45.0±17.9	38.0±15.2	<0.01
UACR, μg/mg[b]	33.1±5.5	35.5±6.0	24.1±5.5	38.3±6.2	48.6±5.9	<0.01
Uromodulin, ng/mL[c]	-	67.79±39.74 58.85 (39.13–83.08)	111.17±37.38 99.51 (83.03–125.28)	59.93±7.75 58.85 (52.96–67.02)	32.34±9.49 32.95 (25.74–39.14)	-

Values for categorical variables are given as *n* (%); values for continuous variables, as arithmetic or geometric mean ± SD, and/or median (interquartile range). *p* value comparisons across SC serum uromodulin categories are based on Wilcoxon-type tests for trend for categorical variables and Jonckheere-Terpstra tests for continuous variables.

*See Weiner et al. [24].

[a]Calcineurin inhibitors are cyclosporine or tacrolimus.

[b]Geometric mean ± SD (also as an antilog).

[c]Uromodulin data are presented as both mean ± SD, and median (interquartile range).

SC, subcohort; BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; UACR, urinary albumin-creatinine ratio.

Table 2.

Association of serum uromodulin with kidney allograft failure risk

Serum uromodulin	Number of events* (total *n* = 226)	Event rates per 100 person-years**	Model 1	Model 2	Model 3
Per $\log_2$ lower***			1.51 (1.23–1.85)	1.51 (1.20–1.90)	1.31 (1.01–1.69)
Tertile 3 >74.05–309.59 ng/mL	40	1.57	1.00 (reference)	1.00 (reference)	1.00 (reference)
Tertile 2 >46.81–74.05 ng/mL	77	2.45	1.97 (1.25–3.10)	1.70 (1.01–2.88)	1.25 (0.66–2.36)
Tertile 1 5.62–46.81 ng/mL	109	4.58	3.20 (2.05–5.01)	2.96 (1.80–4.87)	2.00 (1.06–3.77)
p value for trend comparing tertile medians			<0.001	<0.001	0.025

* From full case-cohort, *n* = 613;

** from random subcohort, *n* = 433;

*** per $\log_2$ lower = per "halving"; unless otherwise noted, values are provided as HRs (with 95% CIs).

Events indicate kidney allograft failures. Model 1: unadjusted, that is, serum uromodulin, only. Model 2: adjusted for treatment assignment, systolic BP, age, sex, race, preexisting cardiovascular disease or diabetes, smoking, BMI, total cholesterol/HDL cholesterol, kidney allograft vintage and type (deceased vs. living donor), calcineurin inhibitor use, and lipid lowering drug use. Model 3: model 2, with additional adjustment for eGFR (mL/min/1.73 m^2), and urinary albumin/creatinine ratio (μg/mg).

BP, blood pressure; BMI, body mass index; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration rate; HRs, hazards ratios.