



Uric Acid Lowering and Biomarkers of Kidney Damage in CKD Stage 3: A Post Hoc Analysis of a Randomized Clinical Trial

Loni Perrenoud, Nicholas T. Kruse, Emily Andrews, Zhiying You, Michel Chonchol, Chaorong Wu, Patrick Ten Eyck, Diana Zepeda-Orozco, and Diana Jalal

Rationale & Objective: Hyperuricemia is associated with chronic kidney disease (CKD) progression. We evaluated whether lowering serum uric acid levels improves levels of biomarkers of kidney damage.

Study Design: Post hoc analysis of clinical trial participants.

Setting & Participants: A double-blind randomized placebo-controlled study designed to lower serum uric acid levels. 80 patients with stage 3 CKD and asymptomatic hyperuricemia were randomly assigned to allopurinol treatment or placebo (300 mg/d) for 12 weeks.

Exposure/Predictor: Allopurinol treatment versus placebo.

Outcomes & Measures: We evaluated the change from baseline for the following urinary biomarkers of kidney damage: albumin-creatinine ratio (ACR), neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), and transforming growth factor β 1 (TGF- β 1). Additionally, we evaluated CKD Epidemiology Collaboration (CKD-EPI)-estimated glomerular filtration rate (eGFR) and cystatin C eGFR.

Analytical Approach: Generalized linear mixed modeling was used.

Results: After 12 weeks, allopurinol (compared to placebo) significantly lowered serum uric acid levels with an estimate of -3.3 mg/dL (95% CI, -4.1 to -2.5 mg/dL; $P < 0.001$). Estimates for the change for allopurinol versus placebo over time were 1.09 (95% CI, 0.77-1.54) for ACR, 0.77 (95% CI, 0.36-1.63) for NGAL, and 2.36 (95% CI, 0.97-5.70) for TGF- β 1. The model did not converge for KIM-1, but Wilcoxon signed rank test showed no significant difference in change from baseline between study groups. There was no significant change observed in CKD-EPI eGFR or cystatin C eGFR.

Limitations: Post hoc analysis and short duration of the study.

Conclusions: Uric acid-lowering with allopurinol is not associated with improvement in levels of biomarkers of kidney damage in patients with asymptomatic hyperuricemia and stage 3 CKD.

Funding: The study was funded by the National Institutes of Health through a career development award, K23DK088833, and the Clinical and Translational Science Award UL1TR002537.

Trial Registration: NCT01228903.

Complete author and article information provided before references.

Correspondence to D. Jalal (diana-jalal@uiowa.edu)

Kidney Med. 2(2):155-161. Published online February 12, 2020.

doi: 10.1016/j.xkme.2019.11.007

© 2020 The Authors. Published by Elsevier Inc. on behalf of the National Kidney Foundation, Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Chronic kidney disease (CKD) is increasingly recognized as a global public health epidemic reported to afflict $\sim 11.5\%$ of the US population.¹ Despite the many advances made in the care of patients with CKD, kidney disease progression and end-stage kidney disease remain an important clinical problem. The most recent report by the US Renal Data System indicates a crude prevalence of 2,161 cases per million in the United States.² Therefore, it is important to explore treatment strategies that may mitigate CKD progression and reduce levels of biomarkers of kidney disease damage.

One characteristic of CKD is elevated serum uric acid levels (ie, hyperuricemia), which has been implicated as an independent risk factor for progression of kidney disease in many epidemiologic studies, as summarized elsewhere.³ Experimental evidence suggests that hyperuricemia may lead to kidney disease by a variety of mechanisms, including induction of oxidative stress and interstitial inflammation.⁴⁻⁷ Lowering uric acid levels by xanthine oxidase inhibition has furthermore been shown to reduce kidney damage in several animal models of

kidney disease.^{6,8-11} Specifically, lowering uric acid levels has been shown to reduce glomerular renal tubular oxidative stress⁶ and tubulointerstitial inflammation and fibrosis.^{10,11} Although a few clinical studies have suggested that lowering uric acid levels may slow kidney disease progression in humans,¹²⁻¹⁴ these studies have mostly evaluated change in glomerular filtration rate (GFR). The potential impact of lowering uric acid levels on biomarkers of kidney damage remains unknown.

Several biomarkers of kidney tubular injury and fibrosis have recently been explored as biomarkers of kidney disease progression. Neutrophil gelatinase-associated lipocalin (NGAL), known to increase with acute kidney injury (AKI), has recently been found to correlate with interstitial fibrosis and tubular atrophy in biopsies of patients with CKD.¹⁵ Several studies have reported that NGAL level is an independent predictor of CKD progression.¹⁶⁻¹⁸ Kidney injury molecule 1 (KIM-1) is a transmembrane protein that is upregulated in the proximal tubule after ischemic injury. When induced long term, KIM-1 leads to kidney fibrosis in animal models.¹⁹ Urinary KIM-1 has

further been shown to associate with CKD progression in humans.^{17,18} Transforming growth factor β (TGF- β) has long been recognized as a potent mediator of kidney fibrosis in several models of CKD.²⁰ Urinary TGF- β 1 excretion has shown to correlate with kidney fibrosis and associate with significantly higher risk for kidney disease progression.²¹

The purpose of the present study was to evaluate whether uric acid-lowering therapy would improve levels of biomarkers of kidney damage (including NGAL, KIM-1, and TGF- β 1) in patients with CKD. We evaluated this in a post hoc analysis of a 12-week randomized clinical trial aimed at lowering serum uric acid levels with allopurinol in patients with stage 3 CKD.²²

METHODS

Participants

This is a post hoc analysis from a previous “parent” study, for which the results have been published elsewhere.²² Briefly, the parent study was a double-blind randomized placebo-controlled study to determine whether 12 weeks of allopurinol treatment lowered serum uric acid levels and improved vascular endothelial function in 80 patients with stage 3 CKD and asymptomatic hyperuricemia. Participants were randomly allocated to receive allopurinol or a placebo over a 12-week period; this involved consuming 100 mg/d during the first week, 200 mg/d during the second week, and 300 mg/d for weeks 3 to 12.

To qualify for the study, patients had to be aged 18 to 74 years and have documentation of the following: (1) serum albumin level > 3.0 mg/dL, (2) stage 3 CKD identified as estimated GFR (eGFR) between 30 and 59 mL/min/1.73 m², and (3) serum uric acid levels ≥ 7.0 mg/dL for men and ≥ 6.0 mg/dL for women. Exclusion criteria consisted of life expectancy less than 1 year; uncontrolled hypertension; history of severe liver disease or congestive heart failure; active infection or using antibiotics; pregnant, breastfeeding, or unwilling to use adequate birth control; history of hospitalization within the last 3 months; expected to undergo living related kidney transplantation in the next 6 months; history of immunosuppressive therapy in the last 6 months; history of warfarin use; or body mass index ≥ 40 kg/m². Last, individuals who were taking allopurinol or other uric acid-lowering agents were excluded.

The nature, risks, and benefits of all study procedures were explained to participants, and their written informed consent was obtained before participation. All procedures were reviewed and approved by the Colorado Multiple Institutional Review Board (protocol number 10-0625) and all procedures were conducted at The Clinical and Translational Research Center at the University of Colorado Anschutz Medical Campus. This study is registered at [ClinicalTrials.gov](https://clinicaltrials.gov) (NCT01228903).

Measurement of Biomarkers of Kidney Damage and Function

Midstream urine samples were obtained and a sample was provided for the clinical laboratory at the University of Colorado Hospitals for measurement of urinary albumin-creatinine ratio (ACR). The remaining sample was transported immediately to the wet laboratory on ice, where samples were centrifuged at 1,000g for 10 minutes. The supernatant was then stored at -80°C in 1.5-mL aliquots until the time of analysis.²³ NGAL was measured using Quantikine Immunoassay Solid Phase Sandwich enzyme-linked immunosorbent assay (ELISA; R&D Systems).²⁴ Intra- and interassay variability for the assay are 3.6% and 7.1%, respectively.²⁵ Analysis of KIM-1 was performed using ELISA Colorimetric detection (Enzo Life Sciences) with reported intra- and interassay variability (according to the manufacturer) of 1.8% and 6.2%, respectively. TGF- β 1 measurements were performed using Quantikine Immunoassay Solid Phase Sandwich ELISA (R&D Systems).²⁶ The assay intra-assay coefficient is 7.5% and interassay coefficient is 12.2%.²⁶ All biomarker measurements were performed in duplicate. Serum creatinine and serum cystatin C were measured by the clinical laboratory. Creatinine and cystatin C eGFRs were calculated using the CKD Epidemiology Collaboration (CKD-EPI) formulas.¹

Other Variables

Race/ethnicity was evaluated by questionnaire. Similarly, smoking was evaluated by questionnaire as history of smoking (current or former) or no history of smoking. The parent study was stratified by history of diabetes mellitus (DM) defined as a diagnosis of DM and receiving antiglycemic agents. Blood pressure was measured using an automated cuff after 10 minutes of rest at the beginning of each visit and reported in mm Hg. Medication data were collected for all study participants at baseline and change in medication prescriptions was documented during the 6- and 12-week study visits.

Statistical Analysis

Baseline characteristics are reported as mean \pm standard deviation for continuous variables and percentages for categorical variables. Spearman correlation coefficients were generated to evaluate the correlation between baseline serum uric acid levels and baseline CKD-EPI eGFR and urinary ACR, NGAL, KIM-1, and TGF- β 1 levels. To evaluate for a potential treatment effect, the analysis used generalized linear mixed modeling to evaluate the interaction between time (defined as week 0 and week 12) and treatment group (defined as placebo and allopurinol). Generalized linear mixed models were fit using an identity link for variables with normal distribution, including CKD-EPI eGFR, cystatin C eGFR, and urinary ACR. A random intercept for patient was included for all outcomes. The estimates for these normally distributed variables represent the mean change difference between study arms. Urinary

NGAL, KIM-1, and TGF- β 1 levels were right skewed so the generalized linear mixed models were fit with a log link function. The estimates reported for these non-normally distributed variables represent the ratio for the change difference between the study arms. Because the parameter estimates for these outcomes did not converge, the random effects were removed. In addition to this unadjusted analysis, the potential interaction with DM status was explored. SAS software (SAS Institute Inc; version 9.4) was used to conduct all analyses. For all outcome variables, alpha was set at 0.05.

RESULTS

Parent Study Results

Results of the parent study have been published elsewhere.²² Briefly, of the 80 patients who were randomly assigned at baseline, 70 completed the study procedures. Allopurinol effectively lowered serum uric acid levels after 12 weeks, whereas no significant change was observed in the placebo group (P value comparing the change for both study groups was <0.001). However, allopurinol did not improve vascular endothelial function (measured by brachial artery flow-mediated dilation) or affect systemic or endothelial markers of inflammation compared with placebo. Of the patients who had participated in the parent study and completed the study visits, 69 patients had adequate urinary samples and were included in this analysis.

Baseline Characteristics and Descriptive Statistics

Participant baseline characteristics are illustrated in Table 1. There were no significant differences between study groups

in age, sex, or race. Baseline history of DM, hemoglobin A_{1c} level, systolic and diastolic blood pressures, CKD-EPI eGFR, and cystatin C eGFR were similar in both groups. The proportion of patients receiving angiotensin-converting enzyme (ACE) inhibitors (ACE) or angiotensin 2 receptor blockers (ARBs) was high but did not differ significantly between the placebo and allopurinol groups. Baseline urinary markers of kidney damage are shown in Figure 1. No significant difference was observed in baseline measurements of biomarkers of kidney damage between the placebo and allopurinol groups.

Correlation of Baseline Serum Uric Acid Levels and Biomarkers of Kidney Function and Damage

These data are shown in Table 2. Baseline serum uric acid levels were significantly and inversely correlated with baseline eGFRs assessed using both CKD-EPI eGFR and cystatin C eGFR. We found that the correlation between baseline serum uric acid levels and baseline levels of urinary biomarkers of kidney damage, including urinary ACR, NGAL, KIM-1, and TGF- β 1, was not significant.

Effect of Allopurinol Versus Placebo on Markers of Kidney Damage and Function

Figure 1 illustrates baseline and end-of-study values for CKD-EPI eGFR and the urinary biomarkers of kidney damage, including urinary ACR, NGAL, KIM-1, and TGF- β 1. CKD-EPI eGFR tended to increase after 12 weeks in the allopurinol group (1.8; 95% confidence interval [CI], -0.48 to 4.1 mL/min/ 1.73 m²) and to decrease in the placebo group (-0.82 ; 95% CI, -3.0 to 1.4 mL/min/ 1.73 m²). When comparing the treatment and placebo groups, allopurinol treatment (vs placebo) successfully lowered serum uric acid levels. The estimate for the generalized linear mixed model was -3.3 mg/dL (95% CI, -4.1 to -2.5 ; $P < 0.0001$) for allopurinol compared to placebo, indicating a change difference of 3.3 mg/dL between groups. Of note, there was no significant difference between study groups in the change in eGFR over the 12-week study period whether estimated using CKD-EPI or cystatin C eGFR.

Table 3 illustrates results of generalized linear mixed modeling comparing treatment group with placebo over the duration of the study. As shown, there was no significant difference between allopurinol and placebo in change in urinary ACR, NGAL, or TGF- β 1 levels. The model did not converge for urinary KIM-1, so Wilcoxon signed rank test was applied to evaluate whether the change in urinary KIM-1 levels from baseline to week 12 differed between allopurinol and placebo. Consequently, no significant differences were identified between study groups ($P = 0.7$). Of note, we obtained similar results when analyses were repeated in the subset of participants with elevated NGAL, KIM-1, and TGF- β 1 levels above the median for each biomarker. We found no significant interaction for allopurinol versus placebo with DM in any of our analysis outputs.

Table 1. Baseline Characteristics According to Study Group

	Placebo (n = 36)	Allopurinol (n = 33)
Age, y	58 \pm 9	59 \pm 12
Male sex	29 (80%)	27 (82%)
Race		
White	31 (86%)	20 (60%)
African American	3 (8%)	9 (27%)
Other	2 (6%)	4 (12%)
Hispanic	9 (25%)	5 (15%)
History of DM	22 (61%)	22 (67%)
Systolic blood pressure, mm Hg	129 \pm 16	127 \pm 15
Diastolic blood pressure, mm Hg	77 \pm 9	77 \pm 11
Hemoglobin A _{1c} , %	6.3 \pm 1.4	6.5 \pm 1.5
Serum creatinine, mg/dL	1.77 \pm 0.40	1.82 \pm 0.37
CKD-EPI eGFR, mL/min/ 1.73 m ²	41.7 \pm 9.3	41.4 \pm 9.3
Cystatin C, mg/L	1.78 \pm 0.40	1.72 \pm 0.41
Cystatin C eGFR, mL/min	39.0 \pm 14.5	41.4 \pm 9.3
Medication status (ACEi/ARB)	28 (77%)	26 \pm (78%)

Note: Values are expressed as mean \pm standard deviation or number (percent) unless otherwise noted. Conversion factors for units: creatinine in mg/dL to μ mol/L, $\times 88.4$.

Abbreviations: ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin 2 receptor blocker; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate.

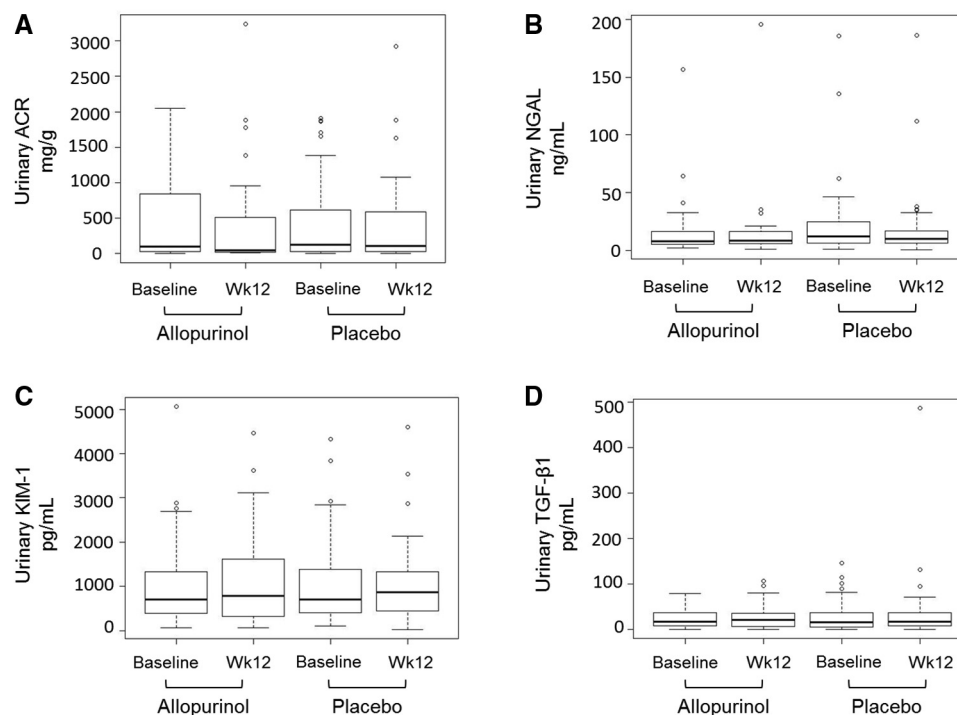


Figure 1. Baseline and end-of-study values for urinary (A) albumin-creatinine ratio (ACR), (B) neutrophil gelatinase-associated lipocalin (NGAL), (C) kidney injury molecule 1 (KIM-1), and (D) transforming growth factor β 1 (TGF- β 1) according to treatment group. There was no difference in baseline values for any of the biomarkers of kidney damage between the allopurinol and placebo groups.

Table 2. Correlation Between Baseline Serum Uric Acid Levels and Biomarkers of Kidney Function and Damage

	Spearman Correlation Coefficients (95% CI)	P
CKD-EPI eGFR	-0.51 (-0.65 to -0.31)	<0.001
Cystatin C eGFR	0.60 (0.43 to 0.73)	<0.001
Urine ACR (mg/g)	0.16 (-0.09 to 0.39)	0.2
Urine NGAL (ng/mL)	0.07 (-0.16 to 0.29)	0.6
Urine KIM-1 (pg/mL)	0.04 (-0.19 to 0.27)	0.7
Urine TGF- β 1 (pg/mL)	-0.07 (-0.30 to 0.16)	0.5

Abbreviations: ACR, albumin-creatinine ratio; CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; KIM-1, kidney injury molecule 1; NGAL, neutrophil gelatinase-associated lipocalin; TGF- β 1, transforming growth factor β 1.

DISCUSSION

In this analysis we evaluated whether lowering uric acid levels with allopurinol treatment is associated with improvement in kidney function or levels of biomarkers of kidney damage within a randomized double-blind study of patients with stage 3 CKD. We found no significant correlation between baseline serum uric acid levels and levels of urinary biomarkers of kidney damage, including urinary ACR, NGAL, KIM-1, or TGF- β 1. As expected, baseline serum uric acid levels correlated significantly and inversely with eGFR, reflecting reduced uric acid filtration in CKD. Our data indicate that 12 weeks of allopurinol treatment significantly lowered serum uric acid levels in patients

Table 3. Effect of Treatment Group Compared to Placebo Over the 12-Week Study Period

	Estimate for Treatment Group \times Time	95% CI	P
CKD-EPI eGFR (mL/min/1.73 m ²) ^a	2.62	-0.55 to 5.78	0.1
Cystatin C eGFR (mL/min) ^a	0.55	-2.27 to 3.37	0.7
Urinary ACR (mg/g) ^b	1.09	0.77 to 1.54	0.6
Urinary NGAL (ng/mL) ^b	0.77	0.36 to 1.63	0.5
Urinary TGF- β 1 (pg/mL) ^b	2.36	0.97 to 5.70	0.06

Abbreviations: ACR, albumin-creatinine ratio; CI, confidence interval; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; NGAL, neutrophil gelatinase-associated lipocalin; TGF- β 1, transforming growth factor β 1.

^aVariables were normally distributed and the estimates for the generalized linear mixed model represent the mean difference for the change over time between study groups.

^bVariables were not normally distributed and estimates for the log scale were exponentiated and re-expressed on the relative scale.

with stage 3 CKD. However, contrary to our hypothesis, we found no significant change in urinary ACR, NGAL, KIM-1, or TGF- β 1 levels. Additionally, we found no significant difference between study groups regarding the change in eGFR, whether by CKD-EPI or cystatin C eGFR. To our knowledge, this is the first study to examine the potential effects of uric acid lowering on biomarkers of kidney damage.

The prevalence of hyperuricemia is known to be increased in patients with CKD. Several factors likely contribute to this, including reduced clearance of uric acid due to low eGFR.³ Although it is possible that hyperuricemia in CKD is merely a marker of reduced GFR, there is evidence that hyperuricemia may also be a complicating factor in CKD. Many observational studies, reviewed elsewhere, have associated elevated serum uric acid levels with incident CKD and kidney disease progression.³ Additionally, several experimental studies have provided several potential underlying mechanisms by which uric acid may be a mediator of kidney disease, such as glomerular hypertension, activation of oxidative stress pathways, and tubulointerstitial inflammation and fibrosis.^{6,9-11} A few pilot studies in patients with CKD have furthermore suggested that uric acid-lowering therapies may slow the decline in GFR indicative of CKD progression.¹²⁻¹⁴

Urinary NGAL and KIM-1 levels are both well-established biomarkers for AKI, known to increase rapidly after AKI and to signify cellular injury within the kidney tubular structures.²⁷ However, more recent data have indicated both NGAL and KIM-1 levels to be increased in patients with CKD compared with healthy adults,²⁸⁻³⁰ and there is evidence to suggest that tubular injury, detected by elevated urinary NGAL and KIM-1 levels, confers a higher risk for CKD progression in the absence of AKI.¹⁸ Furthermore, urinary NGAL and KIM-1 levels are both increased in association with hyperuricemia even in the absence of significant kidney disease.³¹ As such, both these biomarkers represent appealing surrogate markers of kidney disease progression in a study such as ours.

It is interesting that we found no significant correlation between serum uric acid level and either biomarker level and no effect of uric acid lowering on urinary NGAL or KIM-1 excretion in our patients with CKD. One possible explanation for our negative findings is that the patients with CKD included in the study had limited evidence of tubular injury to begin with.^{31,32} This was unexpected considering that hyperuricemia was an inclusion criterion and that preceding data indicate that both NGAL and KIM-1 associate with hyperuricemia in CKD. Of note, when the analyses were repeated in the subgroup of patients with higher NGAL and KIM-1 levels (greater than median for each biomarker), we observed similar negative findings. Collectively, our data suggest that lowering uric acid levels with allopurinol may not modulate tubular injury in patients with CKD.

TGF- β plays an important role in kidney disease progression and is an important target of therapy to slow CKD progression.³³ Additionally, studies suggest that urinary TGF- β 1 is associated with kidney disease progression.^{21,34} Uric acid lowering has been shown to reduce TGF- β 1 expression in experimental diabetic kidney disease.¹¹ A previous report had shown increased urinary TGF- β 1 levels when allopurinol treatment was withdrawn from patients with stages 3 and 4 CKD.³⁵ Based on these data, we anticipated a significant correlation between baseline

serum uric acid and urinary TGF- β 1 levels, as well as a significant decline in urinary TGF- β 1 levels in the group treated with allopurinol. However, in contrast to these published reports, we found no effect of allopurinol therapy on urinary TGF- β 1 levels in our study.

One possible reason behind our findings is the large number of study participants receiving ACE inhibitor/ARB therapy. In the study by Talaat and el-Sheikh³⁵ in which allopurinol therapy was withdrawn in patients with CKD, the increase in urinary TGF- β 1 excretion was not noted in patients receiving blockers of the renin-angiotensin system.³⁵ Collectively, these data suggest that uric acid lowering with allopurinol may be of no additional benefit in patients receiving ACE inhibitor/ARB therapy for their CKD.

Our findings are at odds with previously published studies suggesting that uric acid-lowering with allopurinol slows kidney disease progression.¹²⁻¹⁴ For example, Siu et al¹² randomly assigned a total of 51 patients with CKD and hyperuricemia to allopurinol versus the standard of care and showed significantly improved creatinine levels in the treatment group starting at 3 months post follow-up and extending through the end of the study at 12 months. Several characteristics of this study may explain the positive findings. Of note, the patients included in this study were at high risk for CKD progression considering their baseline proteinuria (protein excretion > 2 g/d) and that many had above-goal blood pressure at baseline. Additionally, the study reported a significant reduction in systolic blood pressure in the treatment arm over the duration of follow-up, which likely influenced the results.

Subsequently, Goicoechea et al¹³ reported that 100 mg per day of allopurinol (compared to standard of care) slowed kidney disease progression in 113 patients with CKD over a period of 7 years. The positive findings here may be explained by the longer duration of follow-up in addition to older age of the participants (>70 years). Of interest, it is unclear what percent of participants in either study had asymptomatic hyperuricemia because neither study excluded individuals with gout. Although results of such studies may have seemed promising, their limitations were elegantly highlighted in a recent meta-analysis by Su et al,¹⁴ who noted the low quality of the published studies including the lack of a placebo arm in the majority of the published literature and the clinical heterogeneity of the randomly assigned study participants.

Although our data are in conflict with some of the preceding literature, our findings are in agreement with the findings of FEATHER (Febuxostat Versus Placebo Randomized Controlled Trial Regarding Reduced Renal Function in Patients With Hyperuricemia Complicated by CKD Stage 3) that showed no effect of uric acid lowering with febuxostat on kidney disease progression.³⁶ FEATHER was a multicenter randomized placebo-controlled study conducted in Japan. A total of 443 patients were randomly assigned to treatment with febuxostat or placebo and monitored for approximately 2 years (through eGFR),

rendering this the largest and longest study of uric acid lowering and CKD progression published to date.³⁶ The study participants included in FEATHER shared many similarities with the participants included in our study, such as CKD stage, asymptomatic hyperuricemia, predominantly male, a large number of participants with DM, majority prescribed ACE inhibitor/ARB therapy, and with well-controlled hypertension and DM at baseline. It is possible that the findings of our study and those of FEATHER are due to the low risk for CKD progression in the included participants. Additionally, participants in both studies may have been at low risk for uric acid–related disease because we included only patients with asymptomatic hyperuricemia. Collectively, our data and the findings of FEATHER suggest no added benefit (beyond the standard of care) of uric acid lowering in patients with stage 3 CKD and asymptomatic hyperuricemia.

Our study is not without limitations. First, this is a post hoc analysis, the outcomes of which were not predetermined. Second, our study was short, inclusion/exclusion criteria were not designed to identify potential participants at high risk for CKD progression, and the study was not powered to evaluate hard outcomes that pertain to CKD progression. Third, although we evaluated well-established biomarkers of kidney damage, we evaluated only a select few.

Notwithstanding these limitations, our study has several strengths, including the original study design being double blind and placebo controlled, the evaluation of surrogate markers of kidney damage that are predictive of CKD progression, and that a large number of our participants were prescribed ACE inhibitor/ARB therapy and had well-controlled hypertension and DM at baseline, allowing us to evaluate whether uric acid lowering would be of added benefit to the current standard of care.

We report that allopurinol effectively lowers serum uric acid levels in patients with stage 3 CKD. In this short study, we found that allopurinol was not associated with improvement in levels of biomarkers of kidney function or damage in patients with asymptomatic hyperuricemia and well-controlled hypertension and DM. Future studies to evaluate the potential effects of uric acid–lowering on CKD progression should include a larger number of patients at high risk for CKD progression and longer duration of follow up. Additionally, future studies should consider the inclusion of patients at high risk for uric acid–related disease, such as those with an established history of gout.

ARTICLE INFORMATION

Authors' Full Names and Academic Degrees: Loni Perrenoud, BS, Nicholas T. Kruse, PhD, Emily Andrews, MA, Zhiying You, PhD, Michel Chonchol, MD, Chaorong Wu, PhD, Patrick Ten Eyck, PhD, Diana Zepeda-Orozco, MD, and Diana Jalal, MD.

Authors' Affiliations: Division of Renal Diseases and Hypertension, Department of Medicine, University of Colorado Anschutz Medical Campus, Aurora, CO (LP, EA, ZY, MC); Division of Nephrology,

Department of Internal Medicine, Carver College of Medicine (NTK, DJ); and Institute for Clinical and Translational Science (CW, PTE) and Division of Pediatric Nephrology, Dialysis and Transplantation, Stead Family Department of Pediatrics, University of Iowa, Iowa City, IA (DZ-O).

Address for Correspondence: Diana Jalal, MD, Carver College of Medicine, Division of Nephrology, 200 Hawkins Dr, E300C GH, Iowa City, IA 52242. E-mail: diana-jalal@uiowa.edu

Authors' Contributions: Research idea and study design: DJ, MC; data acquisition: LP, EA, ZY, DJ; data analysis and interpretation: LP, NTK, ZY, MC, DZ-O, DJ; statistical analysis: ZY, CW, PTE. 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.

Support: The study was supported by a career development award, K23DK088833, from the National Institutes of Health (NIH) by the National Institute of Diabetes and Digestive and Kidney Diseases and the Clinical and Translational Science Award grant funded from the NIH (grant number UL1TR002537). Additionally, this material is the result of work supported with resources and the use of facilities at the Veterans Affairs (VA) Eastern Colorado Health Care System, Denver, CO.

Financial Disclosure: The authors declare that they have no relevant financial interests.

Acknowledgements: We thank Dr Alkesh Jani, who has overseen the regulatory issues for the study at the VA Eastern Colorado Health Care System, Denver, CO, the after the PI, Dr Jalal, moved from Denver.

Peer Review: Received June 20, 2019. Evaluated by 2 external peer reviewers, with direct editorial input from the Statistical Editor and the Editor-in-Chief. Accepted in revised form November 14, 2019.

REFERENCES

1. Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009;150(9):604-612.
2. Saran R, Robinson B, Abbott KC, et al. US Renal Data System 2017 Annual Data Report: epidemiology of kidney disease in the United States. *Am J Kidney Dis.* 2018;71(3)(suppl 1):A7.
3. Jalal DI, Chonchol M, Chen W, Targher G. Uric acid as a target of therapy in CKD. *Am J Kidney Dis.* 2013;61(1):134-146.
4. Kang DH, Nakagawa T, Feng L, et al. A role for uric acid in the progression of renal disease. *J Am Soc Nephrol.* 2002;13(12):2888-2897.
5. Mazzali M, Hughes J, Kim YG, et al. Elevated uric acid increases blood pressure in the rat by a novel crystal-independent mechanism. *Hypertension.* 2001;38(5):1101-1106.
6. Sanchez-Lozada LG, Soto V, Tapia E, et al. Role of oxidative stress in the renal abnormalities induced by experimental hyperuricemia. *Am J Physiol Renal Physiol.* 2008;295(4):F1134-F1141.
7. Roncal CA, Mu W, Croker B, et al. Effect of elevated serum uric acid on cisplatin-induced acute renal failure. *Am J Physiol Renal Physiol.* 2007;292(1):F116-F122.
8. Mazali FC, Johnson RJ, Mazzali M. Use of uric acid-lowering agents limits experimental cyclosporine nephropathy. *Nephron Exp Nephrol.* 2012;120(1):e12-e19.
9. Sanchez-Lozada LG, Tapia E, Soto V, et al. Treatment with the xanthine oxidase inhibitor febuxostat lowers uric acid and

- alleviates systemic and glomerular hypertension in experimental hyperuricaemia. *Nephrol Dial Transplant*. 2008;23(4):1179-1185.
10. Sanchez-Lozada LG, Tapia E, Soto V, et al. Effect of febuxostat on the progression of renal disease in 5/6 nephrectomy rats with and without hyperuricemia. *Nephron Physiol*. 2008;108(4):69-78.
 11. Kosugi T, Nakayama T, Heinig M, et al. Effect of lowering uric acid on renal disease in the type 2 diabetic db/db mice. *Am J Physiol Renal Physiol*. 2009;297(2):F481-F488.
 12. Siu YP, Leung KT, Tong MK, Kwan TH. Use of allopurinol in slowing the progression of renal disease through its ability to lower serum uric acid level. *Am J Kidney Dis*. 2006;47(1):51-59.
 13. Goicoechea M, Garcia de Vinuesa S, Verdalles U, et al. Allopurinol and progression of CKD and cardiovascular events: long-term follow-up of a randomized clinical trial. *Am J Kidney Dis*. 2015;65(4):543-549.
 14. Su X, Xu B, Yan B, Qiao X, Wang L. Effects of uric acid-lowering therapy in patients with chronic kidney disease: a meta-analysis. *PLoS One*. 2017;12(11):e0187550.
 15. Nickolas TL, Forster CS, Sise ME, et al. NGAL (Lcn2) monomer is associated with tubulointerstitial damage in chronic kidney disease. *Kidney Int*. 2012;82(6):718-722.
 16. Bolignano D, Lacquaniti A, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. *Clin J Am Soc Nephrol*. 2009;4(2):337-344.
 17. Lobato GR, Lobato MR, Thome FS, Veronese FV. Performance of urinary kidney injury molecule-1, neutrophil gelatinase-associated lipocalin, and N-acetyl-beta-D-glucosaminidase to predict chronic kidney disease progression and adverse outcomes. *Braz J Med Biol Res*. 2017;50(5):e6106.
 18. Dubin RF, Judd S, Scherzer R, et al. Urinary tubular injury biomarkers are associated with ESRD and death in the REGARDS Study. *Kidney Int Rep*. 2018;3(5):1183-1192.
 19. Humphreys BD, Xu F, Sabbisetti V, et al. Chronic epithelial kidney injury molecule-1 expression causes murine kidney fibrosis. *J Clin Invest*. 2013;123(9):4023-4035.
 20. Meng XM, Nikolic-Paterson DJ, Lan HY. TGF-beta: the master regulator of fibrosis. *Nat Rev Nephrol*. 2016;12(6):325-338.
 21. Mansour SG, Puthumana J, Coca SG, Gentry M, Parikh CR. Biomarkers for the detection of renal fibrosis and prediction of renal outcomes: a systematic review. *BMC Nephrol*. 2017;18(1):72.
 22. Jalal DI, Decker E, Perrenoud L, et al. Vascular function and uric acid-lowering in stage 3 CKD. *J Am Soc Nephrol*. 2017;28(3):943-952.
 23. Fliser D, Novak J, Thongboonkerd V, et al. Advances in urinary proteome analysis and biomarker discovery. *J Am Soc Nephrol*. 2007;18(4):1057-1071.
 24. Krzeminska E, Wyczalkowska-Tomasik A, Korytowska N, Paczek L. Comparison of two methods for determination of NGAL levels in urine: ELISA and CMA. *J Clin Lab Anal*. 2016;30(6):956-960.
 25. Kift RL, Messenger MP, Wind TC, et al. A comparison of the analytical performance of five commercially available assays for neutrophil gelatinase-associated lipocalin using urine. *Ann Clin Biochem*. 2013;50(pt 3):236-244.
 26. Tsakas S, Goumenos DS. Accurate measurement and clinical significance of urinary transforming growth factor-beta1. *Am J Nephrol*. 2006;26(2):186-193.
 27. Schrezenmeier EV, Barasch J, Budde K, Westhoff T, Schmidt-Ott KM. Biomarkers in acute kidney injury - pathophysiological basis and clinical performance. *Acta Physiol (Oxf)*. 2017;219(3):554-572.
 28. Bolignano D, Coppolino G, Campo S, et al. Neutrophil gelatinase-associated lipocalin in patients with autosomal-dominant polycystic kidney disease. *Am J Nephrol*. 2007;27(4):373-378.
 29. Bolignano D, Coppolino G, Campo S, et al. Urinary neutrophil gelatinase-associated lipocalin (NGAL) is associated with severity of renal disease in proteinuric patients. *Nephrol Dial Transplant*. 2008;23(1):414-416.
 30. Waanders F, Vaidya VS, van Goor H, et al. Effect of renin-angiotensin-aldosterone system inhibition, dietary sodium restriction, and/or diuretics on urinary kidney injury molecule 1 excretion in nondiabetic proteinuric kidney disease: a post hoc analysis of a randomized controlled trial. *Am J Kidney Dis*. 2009;53(1):16-25.
 31. Tomczak J, Wasilewska A, Milewski R. Urine NGAL and KIM-1 in children and adolescents with hyperuricemia. *Pediatr Nephrol*. 2013;28(9):1863-1869.
 32. Haase M, Bellomo R, Devarajan P, Schlattmann P, Haase-Fielitz A; NGAL Meta-analysis Investigator Group. Accuracy of neutrophil gelatinase-associated lipocalin (NGAL) in diagnosis and prognosis in acute kidney injury: a systematic review and meta-analysis. *Am J Kidney Dis*. 2009;54(6):1012-1024.
 33. Isaka Y. Targeting TGF-beta signaling in kidney fibrosis. *Int J Mol Sci*. 2018;19(9):2532.
 34. Grenda R, Wuhl E, Litwin M, et al. Urinary excretion of endothelin-1 (ET-1), transforming growth factor-beta1 (TGF-beta1) and vascular endothelial growth factor (VEGF165) in paediatric chronic kidney diseases: results of the ESCAPE trial. *Nephrol Dial Transplant*. 2007;22(12):3487-3494.
 35. Talaat KM, el-Sheikh AR. The effect of mild hyperuricemia on urinary transforming growth factor beta and the progression of chronic kidney disease. *Am J Nephrol*. 2007;27(5):435-440.
 36. Kimura K, Hosoya T, Uchida S, et al. Febuxostat therapy for patients with stage 3 CKD and asymptomatic hyperuricemia: a randomized trial. *Am J Kidney Dis*. 2018;72(6):798-810.