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Relevance of uric acid and asymmetric dimethylarginine for modeling cardiovascular risk prediction in chronic kidney disease patients

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Abstract
Background Both elevated serum uric acid and serum asymmetric dimethylarginine (ADMA) are risk factors for cardiovascular disease. We hypothesized that combined elevation of uric acid and ADMA amplifies the risk of all-cause mortality and/or cardiovascular events (CVE) in patients with chronic kidney disease (CKD).
Methods A total of 259 patients with CKD stages 1–5 were followed up in a time-to-event analysis for all-cause mortality and fatal and non-fatal CVE (including death, stroke, and myocardial infarction). Baseline measurements included serum uric acid and ADMA and endothelial function [ultrasound determined flow-mediated dilatation (FMD)].
Results As a measure of endothelial function, log FMD value was positively associated with log eGFR, but negatively associated with log ADMA and log uric acid levels. During follow-up (median 38 months), 24 (9.3 %) deaths, 90 (34.7 %) CVE, and 95 (36.7 %) deaths and CVE (composite outcome) occurred. In the univariate Cox analysis, patients with both serum uric acid and ADMA levels above the median had an increased risk of all-cause mortality, CVE, and the composite outcome (HR 5.06, 95 % CI 2.01–12.76; HR 4.75, 95 % CI 2.98–7.59; and HR 4.13, 95 % CI 2.66–6.43, respectively). However, after adjustment for renal-specific risk factors (glomerular filtration rate, proteinuria, and hsCRP), this association was maintained only for CVE and the composite outcome. The addition of both biomarkers into a model with traditional and renal-specific risk factors did not increase the prediction abilities of the model for none of the three outcomes.
Conclusion Elevated serum uric acid and ADMA levels are associated with an increased cardiovascular risk, but their combination does not improve risk prediction. The effects are not additive, possibly because uric acid may lie in the causal pathway by which ADMA acts.

Keywords Uric acid · Asymmetric dimethylarginine · Cardiovascular disease · Chronic kidney disease

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Introduction

Uric acid is generated during the metabolism of nucleotides and adenosine triphosphate (ATP) and represents the end product of purine metabolism in humans [1]. While uric acid may act as an antioxidant in extracellular settings [2, 3], an elevated serum uric acid is an important risk factor for cardiovascular disease [4]. Elevated serum uric acid levels are associated with a variety of adverse pathological and cellular processes, including inflammation, endothelial

dysfunction, vasoconstriction, and hypertension, and, in turn, increased risk of renal failure and cardiovascular events (CVE): coronary artery disease, heart failure, or stroke [1, 5].

Uric acid plays an important role with respect to the nitric oxide system. Entry of uric acid into endothelial cells is associated with a reduction in nitric oxide (NO) bioavailability via blocking uptake of L-arginine [6], increased L-arginine degradation via arginase [7], and by scavenging of NO from uric acid-generated oxidants [8] or by uric acid itself [9]. Consequently, uric acid has been reported to inhibit the NO-dependent dilatation of isolated aortic rings in rats [10]. Reciprocally, lowering uric acid with xanthine oxidase inhibitors is strongly associated with improvement in endothelial function [11].

Asymmetric dimethylarginine (ADMA), an endogenous NO synthase inhibitor, plays a role in endothelial dysfunction and is likely involved in the pathogenesis of atherosclerosis [12, 13]. Elevated serum ADMA is associated with metabolic syndrome, endothelial dysfunction, and cardiovascular diseases such as hypertension and atherosclerosis [14, 15]. Elevated serum ADMA levels have also been reported in adolescents with hyperuricemia and in women with hyperuricemia and cardiovascular disease, suggesting a potential relationship between these molecules [13, 16]. Furthermore, one recent study showed that genetic polymorphisms associated with hyperuricemia synergize with ADMA levels as a risk factor for CKD [17].

Given the observation that uric acid and ADMA are both associated with decreased NO bioavailability and increased cardiovascular disease and mortality [18, 19], we hypothesized that elevation of both uric acid and ADMA would increase synergistically the risk prediction of all-cause mortality and/or development of CVE in a cohort of patients with stages 1–5 chronic kidney disease (CKD).

Materials and methods

Patients and study design

This prospective observational study included 259 patients attending the Nephrology Unit of the Gulhane School of Medicine Medical Center between January 2011 and December 2011. All subjects were previously diagnosed as having CKD according to National Kidney Foundation K/DOQI guidelines [20] and were followed up in our unit for at least 3 months in order to exclude acute kidney injury. Stages of CKD were determined using estimated glomerular filtration rates (eGFR) according to the Modification of Diet in Renal Disease (MDRD) equation [21]. None of the patients in stage 5 CKD were on dialysis. Some enrolled subjects were included in our previous study [22]. Patients with chronic infection or malignancy as well as those unwilling to enroll were not included in the study.

Arterial blood pressure was determined in the morning by a physician based on three consecutive measurements, each after a 15-min resting period, with the mean values calculated for systolic and diastolic pressure. Hypertension was defined as systolic blood pressure (SBP) $\geq$ 140 mmHg or diastolic blood pressure (DBP) $\geq$ 90 mmHg, or the current use of antihypertensive medications.

All included patients were followed up for time-to-event analysis, until occurrence of death and fatal or non-fatal CVE. Information on all-cause mortality and CVE including death, stroke, and myocardial infarction was obtained from the Gulhane School of Medicine Medical Center registries by investigators unaware of baseline parameters. If information could not be obtained, the patient was assumed to be lost to follow-up starting from the date of the last actual visit. The Gulhane School of Medicine Ethics Committee approved the study protocol, and all patients were included in the study after signing informed consent forms.

Biochemical analyses

All blood samples were obtained from patients in the morning, after 12 h of fasting, for measurement of serum creatinine, albumin, hsCRP, uric acid, calcium, phosphate, parathyroid hormone, fasting plasma glucose, total serum cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol. Serum ADMA was measured by high-performance liquid chromatography, as described by Yilmaz et al. [23].

Assessment of endothelial function

Endothelium-dependent vasodilatation (flow-mediated dilatation (FMD)) of the brachial artery was assessed non-invasively, using high-resolution ultrasound as described by Celermajer [24] using criteria established by the International Brachial Artery Reactivity Task Force [25]. Measurements were taken by a single observer using an ATL 5000 ultrasound system (Advanced Technology Laboratories Inc., Bothell, WA., USA) with a 12-Mhz probe. The maximum FMD diameter was calculated as the average of the three consecutive maximum diameter measurements. The FMD was calculated as the percentage change in diameter compared with baseline resting diameters. The intra-observer coefficient of variation for FMD was 5.9 %.

Statistical analysis

All calculations were made using SPSS for Windows, version 19.0.1, Chicago, IL, and R (version 3.2.0)—package

for statistical analysis (Foundation for Statistical Computing, Vienna, Austria).

Data are expressed as mean ± SD or as percentage frequency, as appropriate. Between-group comparisons were made for the categorical variables with the Chi-square test and by Mann–Whitney test or independent *T* test for the remaining variables, as appropriate. The normality of the distribution was assessed by the Shapiro–Wilk test, and logarithmic conversion was performed for non-normally distributed variables.

Pearson and point biserial correlation coefficient was used to determine correlations between FMD and all the other investigated variables. Backward stepwise multivariate regression analysis, including all univariate associates (with $p < 0.05$), was used to select the most informative model (based on Akaike information criterion (AIC)) to assess the determinants for FMD.

Time-to-event analysis of death was performed using Kaplan–Meier and Cox analyses. The Kaplan–Meier curves were compared using the log-rank test. Cox analysis was performed initially only with the two groups of patients (group 1: low ADMA–low uric acid, low ADMA–high uric acid, and high ADMA–low uric acid; group 2: high ADMA–high uric acid levels), subsequently adjusting for several groups of covariates. In model 1, we adjusted for conventional cardiovascular risk factors: age, gender, smoking status, diabetes, systolic blood pressure, HDL, and total cholesterol. In model 2, we adjusted for renal-specific cardiovascular risk factors: eGFR, proteinuria, and hsCRP. In model 3, we adjusted for all the variables used in the previous two models. To avoid the problem of overfitting due to the low number of incident outcomes, we performed bootstrapping validation, in order to determine the confidence intervals for estimating B in the Cox proportional hazard regression.

The diagnostic accuracy of the combination between ADMA and uric acid levels was tested by the C statistic difference, continuous net reclassification improvement (NRI), and integrated discrimination improvement (IDI) using methods accounting for censoring [26, 27] evaluated from the Cox proportional hazards models including traditional and renal-specific cardiovascular risk factors (age, gender, smoking status, diabetes, systolic blood pressure, HDL, total cholesterol, eGFR, proteinuria, and hsCRP) with and without continuous ADMA and uric acid levels. We used the Hosmer and Lemeshow test to evaluate the calibration of the models. Additionally, we calculated the Bayesian information criterion (BIC) and the AIC for each of the two Cox models; there is no statistical test to compare different BIC or AIC estimations, and a lower value indicates a better-fitted model.

Table 1 Demographic and clinical characteristics of the study population

	All $N = 259$	Group 1 $N = 159$	Group 2 $N = 100$	p[a]
Age (years)	51.5 ± 12.1	51.0 ± 11.9	52.2 ± 12.3	0.43
BMI (kg/m^2)	26.0 ± 2.7	26.3 ± 2.6	25.6 ± 2.9	0.12
Male [*N* (%)]	130 (50.2)	73 (45.9)	57 (57.0)	0.09
Current smoker [*N* (%)]	114 (44.0)	67 (42.1)	47 (47.0)	0.52
Diabetes [*N* (%)]	58 (22.4)	25 (15.7)	33 (33.0)	**0.001**
SBP (mmHg)	134.7 ± 10.9	133.9 ± 9.9	135.8 ± 12.2	0.32
DBP (mmHg)	84.1 ± 4.3	83.9 ± 4.2	84.6 ± 4.5	0.24
Hypertensive [*N* (%)]	44 (17.0)	20 (12.6)	24 (24.0)	**0.02**
Previous CVD [*N* (%)]	50 (19.3)	27 (17.0)	23 (23.0)	0.26

Data are expressed as mean ± SD or as number and percentage, as appropriate. Bold values are statistically significant

Group 1: patients with low ADMA–low uric acid, low ADMA–high uric acid, and high ADMA–low uric acid

Group 2: patients with high ADMA–high uric acid

BMI body mass index, *CVD* cardiovascular disease, *DBP* diastolic blood pressure, *SBP* systolic blood pressure

[a] Comparison between groups

Results

Baseline characteristics

Two hundred and fifty-nine patients (mean age 51.5 ± 12 years, 22.4 % diabetics) were included in the current study. Baseline demographic, clinical, biological, and vascular characteristics of the entire population are presented in Tables 1 and 2. The mean values of serum ADMA and uric acid were 3.1 μmol/L and 6.9 mg/dL, respectively. Serum uric acid levels were correlated closely with serum ADMA levels ($r = 0.73$, $p < 0.0001$). Patients were divided into two groups according to median values of ADMA and uric acid (group 1: low ADMA–low uric acid, low ADMA–high uric acid, and high ADMA–low uric acid; group 2: high ADMA–high uric acid) levels. Patients in group 2 had a higher prevalence of diabetes and hypertension (Table 1), worse inflammatory status (as assessed by hsCRP), and lower renal function (Table 2). There was also a significant difference between the two groups regarding calcium, phosphate, and PTH (as markers of CKD—mineral and bone disorder) and FMD (as a marker of vascular abnormalities) (Table 2). As shown in Table 3, log FMD value was positively associated with log eGFR, but negatively associated with log ADMA and log uric acid levels. When performing a stepwise multiple linear analysis

Table 2 Biological and vascular characteristics of the study population

	All $N = 259$	Group 1 $N = 159$	Group 2 $N = 100$	p[a]
eGFR (mL/min/1.73 m^2)	48.4 ± 32.9	65.1 ± 27.8	21.9 ± 20.6	**<0.001**
CKD stage [*N* (%)]				
Stage 1	44 (17.0)	43 (27.0)	1 (1.0)	**<0.001**
Stage 2	53 (20.5)	46 (28.9)	7 (7.0)	
Stage 3	57 (22.0)	44 (27.7)	13 (13.0)	
Stage 4	49 (18.9)	15 (9.4)	34 (34.0)	
Stage 5	56 (21.6)	11 (6.9)	45 (45.0)	
Proteinuria (g/day)	1.8 ± 0.9	1.9 ± 0.9	1.8 ± 0.9	0.18
hsCRP (mg/L)	17.8 ± 8.0	14.9 ± 6.5	22.4 ± 8.1	**<0.001**
iPTH (pg/mL)	146.5 ± 81.9	106.0 ± 65.4	210.8 ± 62.4	**<0.001**
Calcium (mg/dL)	8.4 ± 0.6	8.6 ± 0.6	8.1 ± 0.4	**<0.001**
Phosphorus (mg/dL)	5.2 ± 1.5	4.6 ± 1.1	6.3 ± 1.6	**<0.001**
Serum protein (g/dL)	6.8 ± 0.5	6.8 ± 0.4	6.7 ± 0.5	**0.02**
Serum albumin (g/dL)	3.9 ± 0.3	4.0 ± 0.3	3.9 ± 0.3	**0.04**
Glucose (mg/dL)	102.1 ± 37.3	94.9 ± 27.2	113.6 ± 47.1	**<0.001**
FMD (%)	6.7 ± 1.3	7.4 ± 0.9	5.7 ± 0.9	**<0.001**
Uric acid (mg/dL)	6.9 ± 2.1	5.7 ± 1.6	8.9 ± 1.3	**<0.001**
ADMA (μmol/L)	3.1 ± 1.8	2.1 ± 0.9	4.8 ± 1.4	**<0.001**
Total cholesterol (mg/dL)	203.1 ± 17.7	204.0 ± 18.4	201.6 ± 16.4	0.36
Triglycerides (mg/dL)	148.8 ± 14.9	148.4 ± 14.6	149.4 ± 15.6	0.54
LDL cholesterol (mg/dL)	128.9 ± 16.9	130.3 ± 16.5	126.8 ± 17.3	0.10
HDL cholesterol (mg/dL)	42.8 ± 5.8	43.3 ± 5.7	41.9 ± 5.8	0.05

Data are expressed as mean ± SD or as number and percentage, as appropriate. Bold values are statistically significant

Group 1: patients with low ADMA–low uric acid, low ADMA–high uric acid, and high ADMA–low uric acid

Group 2: patients with high ADMA–high uric acid

CKD chronic kidney disease, *eGFR* estimated glomerular filtration rate, *FMD* flow-mediated vasodilatation, *HDL* high-density lipoprotein, *hsCRP* high-sensitive C-reactive protein, *iPTH* intact parathyroid hormone, *LDL* low-density lipoprotein

[a] Comparison between groups

for assessing the independent determinants for FMD, all three variables remained in the final model (Table 3, R^2 of the model = 0.877). Interestingly, the final model included also the interaction term between ADMA and uric acid levels, showing a more important and negative association between uric acid and FMD values with increasing ADMA levels.

Survival and prognostic analysis

During the follow-up (mean 34.8, median 38 months), there were recorded 24 (9.3 %) all-cause deaths, 90 (34.7 %), CVE (fatal and non-fatal), and 95 (36.7 %) composite outcome (all-cause deaths and CVE). As shown in Table 4 and Fig. 1, patients from group 2 had a 5.06-, 4.75-, and 4.13-fold increase in the risk of all-cause mortality, CVE, and the composite outcome occurrence, respectively. In the Cox survival analysis, this association remained significant for CVE and the composite outcome after adjustment for traditional (model 1) and renal-specific risk factors (model 2), and even in the fully adjusted model (model 3). However, when analyzing the all-cause mortality outcome, the inclusion in the multivariable Cox survival analysis of the renal-specific risk factors (model 2 and model 3) made this association nonsignificant (Table 4).

Finally, we also determined whether using both ADMA and uric acid levels could improve the risk prediction of all-cause mortality, CVE, or the composite outcome, beyond clinical and biological parameters. Therefore, we tested the potential incremental prognostic value of adding ADMA and uric acid to the fully adjusted prediction model, based on both traditional (age, gender, smoking status, diabetes, systolic blood pressure, HDL, and total cholesterol) and renal-specific risk factors (eGFR, proteinuria, and hsCRP), using three measurements of performance: calibration, discrimination, and reclassification. All models showed good calibration ($p > 0.05$ for the Hosmer–Lemeshow—Table 5). The models that included ADMA and uric acid levels had the lowest AIC and BIC scores, showing better global goodness of fit than the baseline models for both outcomes (Table 5). Nevertheless, the addition of serum ADMA and uric acid did not increase the discrimination abilities for none of the outcomes (Table 5). Similarly, it did not improve the IDI or continuous NRI for all-cause mortality, although it did show some improved reclassification abilities over the baseline model for CVE or the composite outcome (NRI 17.1 and 16.3 %, respectively) (Table 5).

Discussion

We tested the hypothesis that the combination of elevated serum uric acid and ADMA levels carries a worse cardiovascular risk in subjects with CKD. Several findings were obtained. First, we showed that both uric acid and ADMA are correlated with endothelial dysfunction. Second, we demonstrated that higher uric acid and ADMA levels were

Table 3 Univariate and multivariate associates of log FMD in chronic kidney disease patients

Variables	Univariate ρ	p
Hypertension (yes-1)	−0.17	0.01
Systolic blood pressure (10 mmHg)	−0.15	**0.02**
Log eGFR (mL/min/1.73 m^2)	0.81	**<0.001**
Log hsCRP (mg/L)	−0.52	**<0.001**
Diabetes (yes-1)	−0.29	**<0.001**
Log glucose (mg/dL)	−0.28	**<0.001**
Log iPTH (pg/mL)	−0.72	**<0.001**
Log phosphate (mg/dL)	−0.58	**<0.001**
Log calcium (mg/dL)	0.21	**<0.01**
Log serum protein (g/dL)	0.23	**<0.001**
Log serum albumin (g/dL)	0.21	**<0.01**
Log ADMA (μmol/L)	−0.77	**<0.001**
Log uric acid (mg/dL)	−0.72	**<0.001**
Variables	**Multivariate B**	**p**
Systolic blood pressure (10 mmHg)	−0.004	0.14
Log eGFR (mL/min/1.73 m^2)	0.09	**<0.001**
Log hsCRP (mg/L)	0.02	0.21
Diabetes (yes-1)	−0.03	**<0.001**
Log serum protein (g/dL)	0.01	0.36
Log ADMA (μmol/L)	−0.05	**0.04**
Log uric acid (mg/dL)	−0.08	**0.01**
Log uric acid * log ADMA (mg/dL μmol/L)	−0.001	**0.01**

Bold values indicate $p < 0.001$

ADMA asymmetric dimethylarginine, *eGFR* estimated glomerular filtration rate, *FMD* flow-mediated dilatation, *hsCRP* high-sensitive C-reactive protein, *iPTH* intact parathyroid hormone

Table 4 Unadjusted and adjusted Cox survival analysis for all-cause mortality, fatal and non-fatal cardiovascular events, and composite outcome

	All-cause mortality		Cardiovascular events		Composite outcome	
	HR[a]	95 % CI	HR[a]	95 % CI	HR[a]	95 % CI
Unadjusted	5.06	2.01–12.76	4.75	2.98–7.59	4.13	2.66–6.43
Adjusted						
Model 1	4.30	1.60–11.56	3.83	2.36–6.23	3.37	2.13–5.33
Model 2	1.84	0.55–6.16	3.41	1.89–6.16	2.93	1.66–5.16
Model 3	1.18	0.33–4.26	2.46	1.33–4.54	2.13	1.17–3.85

Model 1 adjusted for age, gender, smoking status, diabetes, systolic blood pressure, HDL, and total cholesterol

Model 2 adjusted for eGFR, proteinuria, and hsCRP

Model 3 adjusted for variables included in model 1 and model 2

[a] Patients from group 1 were used as reference

associated with a higher risk of all-cause mortality and/or CVE, but this association remained independent of the renal-specific risk factors only for the CVE and composite outcome. Third, the addition of log ADMA and log uric acid into two baseline models for predicting cardiovascular death or CVE did not increase the risk prediction abilities of the models for any of the three outcomes.

Previous studies have shown that both uric acid [1, 18] and ADMA [12, 13] are independent risk factors for increased CVE in CKD and the general population. Both ADMA and uric acid are thought to be involved in important pathophysiologic processes such as inflammation, oxidative stress, and endothelial dysfunction [28, 29]. Serum uric acid levels are also correlated with ADMA levels, and a recent study found serum uric acid to be an independent predictor of ADMA [13, 28, 29]. Given these findings, we hypothesized that uric acid and ADMA in combination may play a synergistic, predictive role in the occurrence of new CVE.

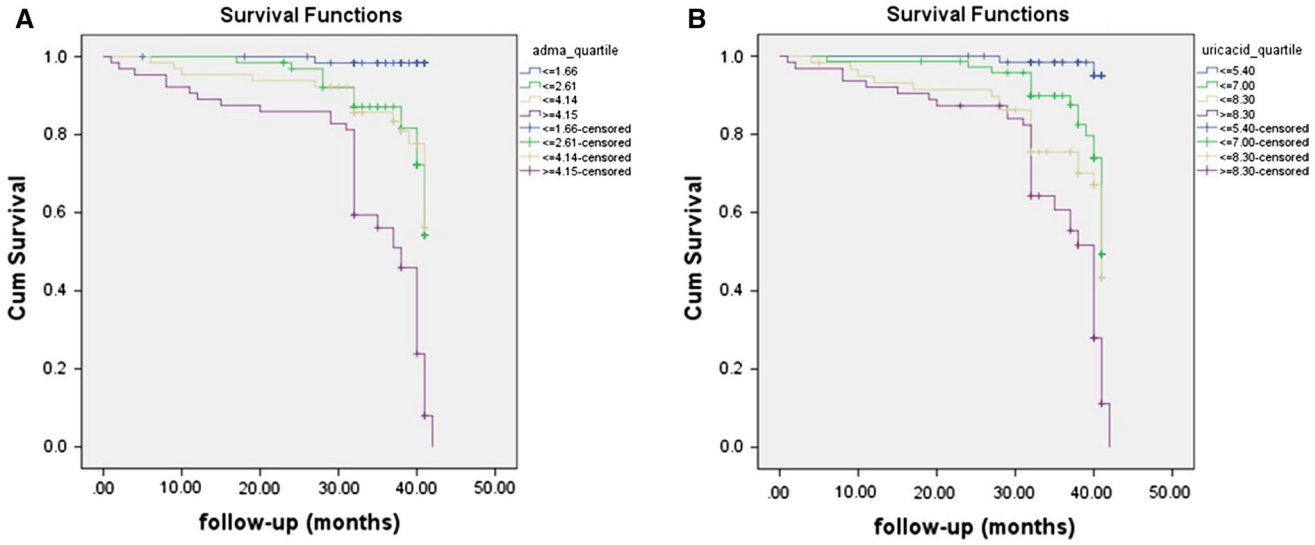

Fig. 1 Kaplan–Meier analysis for cardiovascular events according to quartiles of ADMA (**a**) and uric acid (**b**) ($p < 0.001$ for both)

Table 5 Performance of the models for predicting cardiovascular mortality and fatal and non-fatal cardiovascular events

	All-cause mortality		Cardiovascular events		Composite outcome	
	Model 1	Model 2	Model 1	Model 2	Model 1	Model 2
Discrimination						
Δ C statistics, 95 % CI	Reference	0.003[a] (−0.027 to 0.034)	Reference	0.015[b] (−0.030 to 0.061)	Reference	0.009[c] (−0.008 to 0.027)
Calibration						
AIC	223.68	222.48	803.59	775.52	849.63	825.89
BIC	235.46	236.62	828.59	805.52	875.17	856.55
H–L	$\chi^2 = 12.96$ $p = 0.16$	$\chi^2 = 5.34$ $p = 0.80$	$\chi^2 = 12.25$ $p = 0.19$	$\chi^2 = 16.60$ $p = 0.06$	$\chi^2 = 13.91$ $p = 0.13$	$\chi^2 = 11.90$ $p = 0.22$
Reclassification						
IDI, 95 % CI	Reference	0.035[a] (−0.011 to 0.113)	Reference	0.023[b] (−0.075 to 0.146)	Reference	0.018[c] (−0.054 to 0.110)
NRI, 95 % CI	Reference	0.235[a] (−0.155 to 0.492)	Reference	0.171[b] (0.025 to 0.383)	Reference	0.163[c] (0.020 to 0.334)

C statistic for model 1 was 0.882 for all-cause mortality, 0.789 for cardiovascular events, and 0.924 for the composite outcome

Model 1—age, gender, smoking status, diabetes, systolic blood pressure, log HDL, log total cholesterol, log eGFR, log proteinuria, log hsCRP

Model 2—model 1 + log uric acid + log uric acid

[a] Comparison with model 1 for all-cause mortality

[b] Comparison with model 1 for cardiovascular events

[c] Comparison with model 1 for composite outcome

Our study clearly shows that the patients with high levels in both biomarkers are at an increased risk of CVE, independently of traditional and renal-specific risk factors. However, we demonstrate for the first time that the addition of both biomarker into baseline prediction models for cardiovascular death or fatal and non-fatal CVE does not improve the prediction abilities of those models. While speculative, there are several potential explanations. First, uric acid or ADMA may not be as specific as eGFR with respect to cardiovascular risk prediction, and it is also possible that the relationship of uric acid or ADMA with CVE may simply reflect their association with eGFR levels. Indeed, a multivariate analysis by the Framingham heart study failed to demonstrate a relationship between uric acid and future cardiovascular disease [30]. However, this explanation may not be satisfactory since there are numerous studies showing that uric acid is an independent risk factor for CVE [18, 31, 32] and because mechanistically uric acid has been shown to block endothelial function in both cell culture and animal models [7, 9, 33–37].

Second, it is also possible that uric acid and ADMA lie in the same causal pathway. Indeed, the levels of uric acid

and ADMA were found to be correlated. The possibility that uric acid may be upstream of ADMA is suggested by a recent study in which the rs734553 polymorphism in the urate transporter, SLC2A9, the strongest genetic marker of uric acid levels discovered so far, interacts with ADMA in determining the risk of CKD progression in CKD patients [17]. Indeed, in a double-blind study allopurinol decreased ADMA levels in chronic heart failure patients [29]. In contrast, endothelial dysfunction may also drive up uric acid levels, as noted by studies showing that mice lacking endothelial nitric oxide synthase develop hyperuricemia and features of metabolic syndrome [38, 39].

The study has some limitations. Cause and effect cannot be inferred, although the longitudinal nature does demonstrate that both uric acid and ADMA are risk factors for CVE in subjects with CKD. Indeed, there are now some studies showing that lowering uric acid can reduce CVE in subjects with CKD [40, 41]. Second, the measurements were taken at only one time point. Third, the patients were recruited from one center and the results cannot be generalized. Finally, our sample size and number of outcomes were relatively small, but we performed additional statistical adjustments to overcome this limitation. Nevertheless, this is the only study to our knowledge that combines ADMA and uric acid to investigate their interaction in a longitudinal study evaluating endothelial function and all-cause mortality and/or CVE in subjects with CKD.

In conclusion, both uric acid and elevated ADMA are associated with cardiovascular death and/or fatal and non-fatal CVE in subjects with CKD. Combining the two measures does not increase predictability for none of the outcomes. Nevertheless, these studies continue to emphasize the importance of endothelial function and of two key markers, ADMA and uric acid, in influencing cardiovascular risk in subjects with CKD.

Compliance with ethical standards

Conflict of interest There is no conflict of interest between authors.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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