IJKD KIDNEY DISEASES

The Role of Urinary Angiotensinogen in Kidney Interstitial Inflammation and Renal Prognosis

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Introduction. Urinary angiotensinogen (uAGT) has been described as a novel biomarker of acute kidney injury (AKI) and chronic kidney disease (CKD). Renal interstitial inflammatory cell infiltration is a common renal pathological feature of AKI and CKD. However, the correlation between uAGT and renal interstitial inflammatory cell infiltration is unknown. The aim of this study was to analyze the expression of uAGT, its relationship with interstitial inflammatory cell infiltration, and prognosis in patients with renal insufficiency.
Methods. The expression of uAGT, urinary kidney injury molecule 1 (uKIM-1), and urinary neutrophil gelatinase-associated lipocalin (uNGAL) were examined by enzyme-linked immunosorbent assay (ELISA) at baseline and kidney pathology was evaluated at the same time.
Results. Sixty-five patients with renal insufficiency and 12 healthy controls were enrolled. uAGT, uKIM-1, and uNGAL levels were significantly higher compared with healthy participants. uAGT showed the strongest correlation with interstitial inflammatory cell infiltration ($r = 0.366$, $P < .05$). uAGT level was able to identify interstitial inflammatory cell infiltration with greater accuracy (AUC = 0.664, $P < .05$) than other urinary biomarkers. After a median follow-up of 22 months, 15 patients reached the composite renal endpoint. Kaplan meier survival curves followed by multivariate cox proportional hazards regression analysis showed that uAGT (> 166.8 ng/mg creatinine) independently predicted higher risk of the endpoint.
Conclusion. uAGT may be used as a non-invasive biomarker of interstitial inflammatory cell infiltration and a strong predictor of renal prognosis in patients with renal insufficiency.

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INTRODUCTION

Renal tubulointerstitial fibrosis is the last stage of almost all progressive chronic kidney diseases.[1,2] Moreover, the severity of tubulointerstitial involvement in most renal diseases is associated with the renal prognosis.[3,4] Renal interstitial inflammation serves as a primer that triggers tissue fibrogenesis and correlates with the extent of fibrosis. Renal fibrosis could be effectively ameliorated by inhibition of renal inflammation by some methods.[5,6] It is thus important to evaluate the level of renal interstitial inflammation. The gold standard for determining the degree of tubulointerstitial lesion is renal biopsy. However, because of its contraindications and invasiveness, it cannot be used as a routine and dynamic detection

method. Therefore, finding noninvasive biomarkers that are closely related to interstitial inflammatory cell infiltration is of great value.

Recently, some biomarkers including kidney injury molecule 1 (KIM-1) and neutrophil gelatinase–associated lipocalin (NGAL) have been examined in kidney diseases. These studies showed that urinary KIM-1 (uKIM-1) level reflects tissue KIM-1, and it is significantly associated with fibrosis and inflammation in different human renal diseases.[7] Moreover, uKIM-1 also has prognostic value in acute kidney injury (AKI),[8,9] chronic kidney disease (CKD),[10,11] and transplant recipients,[12,13] indicating the role of uKIM-1 as a biomarker in the kidney disease. Unfortunately, the prognostic value of uKIM-1 was not as strong as other biomarkers.[14,15] Similarly, NGAL has been validated as a specific, sensitive, and early predictor of AKI after cardiac surgery,[16] contrast media administration,[17] septic shock,[18] and even renal transplantation.[19] Urinary NGAL (uNGAL) also reflects the severity of kidney disease and predicts progression in CKD patients.[20] Studies showed that uNGAL is associated with tubular atrophy and interstitial fibrosis in CKD patients and higher uNGAL concentration predicts faster progression to ESRD. Unfortunately, there are few studies regarding the association between NGAL and interstitial inflammatory cell infiltration, although neutrophils and macrophages may both be present in the interstitium.[20,21]

In recent years, urinary angiotensin (uAGT) is considered to be a new biomarker for the prognosis of AKI. uAGT is mainly derived from proximal renal tubules and has nothing to do with blood circulating AGT. It is the only substrate of RAS rate-limiting enzyme renin, and eventually produces angiotensin II (Ang II), the main effector molecule of RAS.[22] Activation of the intrarenal RAS plays an important role in the process of CKD. Experiments have shown that locally generated AngII through the induction of non-infectious inflammation injures target organs.[23] Notably, inhibition of AngII can elicit renal protection via attenuation of renal inflammation.[24] uAGT has thus been proposed as a marker of RAS activity in kidney and a predictive index for the progression of CKD.[25,26] Therefore, based on these reports; uAGT may be a useful non-invasive biomarker of renal interstitial inflammatory cell infiltration and a strong predictor for renal prognosis in renal insufficiency patients.

In the current study, we explore the expression of uAGT, uKIM-1, and uNGAL; and their relationship with interstitial inflammatory cell infiltration and prognosis in patients with renal insufficiency.

MATERIALS AND METHODS

Study Design

This study was conducted at the First Affiliated Hospital of Zhengzhou University from December 2014 to April 2016. It was a prospective study performed at a single center. The expression of uAGT, uKIM-1, and uNGAL was examined by enzyme-linked immunosorbent assay (ELISA) at baseline and the pathology of the kidney was evaluated at the same time. The patients were then followed for 22 months. The relationship between the biomarkers and renal interstitial inflammatory cell infiltration was analyzed at baseline. The relationship of the biomarkers at baseline with the endpoint was also analyzed. The study was approved by the review board of The First Affiliated Hospital of Zhengzhou University and all participants provided written informed consent.

Patients

Chinese patients at the nephrology department of the First Affiliated Hospital of Zhengzhou University with various degrees of renal impairment were screened from December 2014 to April 2016.

The inclusion criteria were as follows: age ≥ 18 years, serum creatinine ≥ 115 μmol/L (enzymatic method, normal range 20 to 115 μmol/L) or eGFR < 60 mL/min/ 1.73m^2 (eGFR calculated by CKD-EPI equation[27]). All patients underwent a renal biopsy.

The exclusion criteria were as follows: renal insufficiency caused by prerenal and postrenal diseases, patients in CKD stage 5, renal biopsy specimen containing less than 10 glomeruli, patients receiving an ACE inhibitor or Ang II type 1 receptor blocker, and renal pathology demonstrating subacute tubulointerstitial nephropathy accompanied by subacute tubulointerstitial nephropathy.

According to their different clinicopathological conditions, we divided patients with renal insufficiency into two groups: AKI (AKI was defined as any of the following: increase in SCr

by x 0.3 mg/dL (X26.5 lmol/L) within 48 hours or increase in SCr to x 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days, or Urine volume < 0.5 mL/kg/h for > 6 hours) and CKD according to KDIGO 2012 (CKD was defined as abnormalities of kidney structure or function, present for more than 3 months, with implications for health). The criteria was as follows: albuminuria (AER ≥ 30 mg/24 hours, ACR ≥ 30 mg/g [≥ mg/mmol]), urine sediment abnormalities, electrolyte and other abnormalities due to tubular disorders, abnormalities detected by histology, structural abnormalities detected by imaging, history of kidney transplantation, eGFR < 60 mL/min/ 1.73m^2).

Laboratory Parameters and Definitions

Blood samples were taken in the fasting state in the morning and the first urine specimen of the day was also collected. The biochemical parameters including hemoglobin, blood urea nitrogen (BUN), serum creatinine (sCr), and urinary protein (UP) were measured at baseline in all patients and healthy participants; according to standard methods in the clinical laboratory. eGFR was assessed using the CKD-EPI formula. Urine samples were stored at -80^C until analyzed.

uAGT (catalog number 27412; Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan), uKIM-1 (catalog number CSB-E08807h; Cusabio Biotech Co., Ltd., Wuhan, China), and uNGAL (catalog number DLCN20; R&D Systems Co., Ltd., Minneapolis, MN, USA) levels were determined by ELISA, according to the manufacturer's instructions. Urinary creatinine was assessed by enzymatic method as a correcting factor. All specimens were diluted to obtain concentration for the optimal density according to the ELISA kit instruction. Coefficients of variation for the uAGT, uKIM-1, and uNGAL assays were 5.0%, < 8%, and 3.7%; respectively, for intra-assay variation; and 2.7%, < 10%, and 6.5% for inter-assay variation. The enzymatic reactions were quantified in an automated microplate photometer. All measurements were made in triplicate and in a blinded manner. uAGT, uKIM-1, and uNGAL are expressed as nanograms per milliliter. They were also measured in a small group of 12 healthy participants with normal serum creatinine, well matched with renal insufficiency patients for age and gender.

Prospective Follow-up and Progression End Point

After the baseline assessments, patients were followed prospectively until the end of the observation period or the progression endpoint was reached. The progression endpoint was defined as a decline in eGFR of ≥ 50% from baseline, ESRD, or death.

Renal Biopsy and Pathological Classification

The renal tissue specimens were processed for light microscopy, immunofluorescence, and electron microscopy. We assessed pathological injury by semiquantitative Katafuchi score.[28] All scoring was performed by at least two independent renal pathologists who were unaware of the clinical status of each patient. The index of glomerular sclerosis was determined according to the percentage of obliterated glomeruli due to global sclerosis as follows: 0, no global sclerosis; 1, global sclerosis in less than 10% of glomeruli; 2, global sclerosis in more than 10% and less than 25% of glomeruli; 3, global sclerosis in over 25% and less than 50% of glomeruli; 4, global sclerosis in more than 50% of glomeruli. The severity of interstitial cell infiltration and interstitial fibrosis were semi quantitatively determined as follows: 0, none; 1, occupying less than 25% of cortical area of biopsy specimen; 2, occupying between 25% and 50% of cortical area; 3, occupying over 50% of cortical area. The index of tubular atrophy was graded as follows: 0, none; 1, involving less than 25% of tubules; 2, involving between 25% and 50% of tubules; 3, involving over 50% of tubules. Glomerular, tubular, and interstitial lesions were scored as 0, absent; 1, mild; 2, moderate; 3 or 4, severe.

Statistical Analyses

SPSS 22.0 software was used for all analyses. Data are presented as the mean ± SD, median (interquartile range), or percentage frequency, as appropriate. Differences between groups were established by unpaired t-test for normally distributed values and by Mann-Whitney U test for nonparametric values. Dichotomized values were compared using the x^2 test. Pearson or Spearman correlation coefficients were used as appropriate to test correlations between eGFR and other variables. Before multiple linear regression was tested, all non-normally distributed values were

log-transformed to better approximate normal distributions. To distinguish different pathologic lesions, receiver operating characteristics (ROC) curve analysis of urinary biomarkers was carried out to determine the area under the curve (AUC). Kaplan-Meier curves were generated to assess renal survival. Adjusted risk estimates for the progression endpoint were calculated using a Cox proportional hazards regression model. A *P* value of $P < .05$ was considered as a statistically significant difference.

RESULTS

Baseline Characteristics

Sixty five patients were enrolled totally in this study. Patients with renal insufficiency (42 with AKI, 23 with CKD) included 20 women and 45 men with ages ranging from 20 to 72 years old. Healthy participants included 6 women and 6 men with ages ranging from 27 to 61 years old. The main baseline characteristics of the participants are summarized in Table 1.

Correlation of eGFR

Upon correlation analysis, the eGFR was directly correlated with hemoglobin (r = 0.478, $P < .001$) and an inverse correlation existed between eGFR, BUN (r = -0.54, $P < .001$), and uNGAL (r = -0.454, $P < .001$) (Table 2). Using eGFR as a dependent variable in a multiple linear regression model, adjusted for age; hemoglobin, blood urea nitrogen (BUN), Ln uAGT, Ln uKIM-1, and Ln uNGAL; only the associations with hemoglobin (β = 0.299, $P < .05$), BUN (β = -0.402, $P < .001$), Ln uAGT (β = 0.244, $P < .05$), and Ln uNGAL (β = -0.379, $P < .05$) were significant. Of note, this model explained approximately 48% of the total variance of eGFR. Table 3 provides a summary of these statistics. The subgroups of AKI and CKD group showed the similar results with the whole group (Tables 4 to 7).

Table 2. Pearson or Spearman Correlation Between eGFR and Clinical Parameters in Renal Insufficiency Patients

Clinical Parameters	Detection Value	r or p	*P*
Age, y	42.60 ± 14.08	-0.217	> .05
SBP, mmHg	139 ± 20	-0.056	> .05
DBP, mmHg	88 ± 15	0.047	> .05
Hemoglobin, g/L	116.5 ± 27.5	0.478	< .001
BUN, mmol/L	13.1 ± 6.9	-0.54	< .001
UP, g/d	2.2 (1, 7.6)	0.135	> .05
uAGT, ng/mg Cr	206.5 (68.5, 581.0)	-0.225	> .05
uKIM-1, ng/mg Cr	1.9 (1, 3.6)	-0.222	> .05
uNGAL, ng/mg Cr	81.1 (10.5, 362)	-0.454	< .001

SBP, systolic blood pressure; DBP, diastolic blood pressure; UP, urinary protein

Table 1. Comparison of Main Clinical Parameters Between All Patients and Healthy Subjects

Clinical Parameters	All Patients (n = 65)	Healthy Subjects (n = 12)	AKI (n = 42)	CKD (n = 23)	*P*
Men (%)	45 (75)	6 (50)	31 (74)	14 (60.9)	> .05
Age, y	42.60 ± 14.08	42.83 ± 11.18	44.24 ± 14.92	39.6 ± 12.2	> .05
Hemoglobin, g/L	116.5 ± 27.5	140.9 ± 14.9	119.5 ± 30.6	111.2 ± 20.3	< .001
BUN, mmol/L	13.1 ± 6.9	4.7 ± 0.9	14.3 ± 7.7	10.9 ± 4.5	< .001
sCr, umol/L	172 (139, 255)	77 (55, 86)	157 (134, 302)	182 (156, 225)	< .001
eGFR, mL/ (min×1.73m^2)	36.6 ± 17.7	101.3 ± 10.7	36.7 ± 20.4	36.2 ± 11.7	< .001
uAGT, ng/mg Cr	206.5 (68.5, 581)	0.14 (0.04, 2.79)	154.5 (22.1, 507)	231.3 (86, 779.7)	< .001
uKIM-1, ng/mg Cr	1.9 (1, 3.6)	0.7 (0.4, 1.2)	2 (1, 3.4)	1.9 (1.1, 3.8)	< .001
uNGAL, ng/mg Cr	81.1 (10.5, 362)	2.6 (0.8, 15.3)	91.8 (20.7, 692.9)	30.7 (8.3, 187.6)	< .001

BUN, blood urea nitrogen; sCr, serum creatinine; eGFR, estimated glomerular filtration rate; uAGT, urinary angiotensinogen; uKIM-1, urinary Kidney Injury Molecule 1; uNGAL, urinary Neutrophil Gelatinase-Associated Lipocalin

Table 3. Multiple Linear Regression Analysis of eGFR at Baseline in Renal Insufficiency Patients

Variable	B	Std. Error	β	t	*P*
Age, y	-0.175	0.120	-0.139	-1.453	> .05
Hemoglobin, g/L	0.200	0.078	0.299	2.552	< .05
BUN, mmol/L	-1.036	0.260	-0.402	-3.991	< .001
Ln uAGT, ng/mg Cr	2.591	1.247	0.244	2.078	< .05
Ln uKIM-1, ng/mg Cr	0.234	2.246	0.012	0.104	> .05
Ln uNGAL, ng/mg Cr	-3.136	1.111	-0.379	-2.824	< .05

Table 4. Pearson or Spearman Correlation Between eGFR and Clinical Parameters in AKI (n = 42)

Clinical Parameters	Detection Value	r or p	*P*
Age, y	44.24 ± 14.92	-0.312	< .05
SBP, mmHg	134 ± 20	-0.066	> .05
DBP, mmHg	86 ± 17	0.057	> .05
Hemoglobin, g/L	119.5 ± 30.6	0.567	< .001
BUN, mmol/L	14.3 ± 7.7	-0.541	< .001
UP, g/d	1.7 (0.3, 8.7)	0.103	> .05
uAGT, ng/mg Cr	154.5 (22.1, 507)	-0.118	> .05
uKIM-1, ng/mg Cr	2 (1, 3.4)	-0.173	> .05
uNGAL, ng/mg Cr	91.8 (20.7, 692.9)	-0.558	< .001

SBP, systolic blood pressure; DBP, diastolic blood pressure; UP, urinary protein

Table 5. Pearson or Spearman correlation between eGFR and clinical parameters in CKD (n = 23)

Clinical Parameters	Detection Value	r or p	*P*
Age, y	39.61 ± 12.16	0.126	> .05
SBP, mmHg	148 ± 17	-0.012	> .05
DBP, mmHg	91 ± 9	0.003	> .05
Hemoglobin, g/L	111.2 ± 20.3	0.064	> .05
BUN, mmol/L	10.9 ± 4.5	-0.658	< .05
UP, g/d	2.5 (1.5, 6.4)	-0.165	> .05
uAGT, ng/mg Cr	231.3 (86, 779.7)	-0.396	> .05
uKIM-1, ng/mg Cr	1.9 (1, 3.8)	-0.361	> .05
uNGAL, ng/mg Cr	30.7 (8.3, 187.6)	-0.253	> .05

SBP, systolic blood pressure; DBP, diastolic blood pressure; UP, urinary protein

Urinary Biomarkers and Renal Pathological Lesions

The strongest correlation was shown between uAGT and interstitial inflammatory cell infiltration (r = 0.366, $P < .05$) in renal insufficiency patients (Table 8). The results of the subgroups of AKI and CKD were consistent with the whole group (Table 9 and 10). Notably, uAGT could reflect the severity of infiltration (Table 11). ROC curve analysis showed that uAGT level was able to identify interstitial inflammatory cell infiltration with greater accuracy (AUC = 0.664, $P < .05$) than other pathological lesions and urinary biomarkers (Figure 1).

Associations with Survival

During the observation period (median follow-up of 22 months, range from 0 to 34 months), 15 patients (23%) reached the renal end point. ROC analysis showed an AUC for age, sBP, and uAGT of 0.693 (95% CI: 0.534 to 0.852, $P < .05$), 0.732 (95% CI: 0.596 to 0.868, $P < .05$), 0.687 (95% CI: 0.550 to 0.823, $P < .05$); respectively. The best cut-off level was found to be 51.5 years for age (sensitivity 53.3%, specificity 78%), 143.5 mmHg for SBP (sensitivity 73.3%, specificity 70%), and was 166.8 ng/mg Cr for uAGT (sensitivity 86.7%, specificity 58%).

Kaplan-Meier curves were generated to assess renal survival. Kaplan-Meier survival curves in patients with age, sBP, and uAGT level above the optimal cut-off experienced a significantly faster progression to the endpoint. These were also found in patients with glomerular sclerosis, moderate or severe interstitial inflammatory cell infiltration, interstitial fibrosis, and tubular atrophy. Among them, patients with uAGT values above 166.8 ng/mg Cr experienced a significantly faster

Table 6. Multiple Linear Regression Analysis of eGFR at Baseline in AKI

Variable	B	Std. Error	β	t	*P*
Age, y	-0.407	0.131	-0.300	-3.115	< .05
Hemoglobin, g/L	0.139	0.086	0.198	1.616	> .05
BUN, mmol/L	-1.024	0.273	-0.384	-3.755	< .05
Ln uAGT, ng/mg Cr	5.258	1.369	0.435	3.840	< .05
Ln uKIM-1, ng/mg Cr	0.399	2.576	0.017	0.155	> .05
Ln uNGAL, ng/mg Cr	-5.707	1.333	-0.598	-4.280	< .001

Table 7. Multiple Linear Regression Analysis of eGFR at Baseline in CKD

Variable	B	Std.Error	β	t	*P*
Age, y	0.239	0.180	0.251	1.329	> .05
Hemoglobin, g/L	-0.019	0.131	-0.035	-0.149	> .05
BUN, mmol/L	-1.669	0.516	-0.644	-3.235	< .05
Ln uAGT, ng/mg Cr	-0.802	2.273	-0.114	-0.353	> .05
Ln uKIM-1, ng/mg Cr	2.635	3.729	0.239	0.706	> .05
Ln uNGAL, ng/mg Cr	-2.083	2.021	-0.351	-1.030	> .05

Table 8. Pearson or Spearman Correlation Between Urinary Biomarkers and Pathological Lesions in Renal Insufficiency Patients

Pathological Lesions	uAGT		uKIM-1		uNGAL	
	γ	*P*	γ	*P*	γ	*P*
Glomerular Sclerosis	0.242	> .05	0.079	> .05	-0.231	> .05
Interstitial Inflammatory Cell Infiltration	0.366	< .05	0.302	< .05	0.206	> .05
Interstitial Fibrosis	0.184	> .05	0.023	> .05	-0.316	< .05
Tubular Atrophy	0.244	> .05	0.155	> .05	-0.21	> .05

Table 9. Pearson or Spearman Correlation Between Urinary Biomarkers and Pathological Lesions in AKI

Pathological Lesions	uAGT		uKIM-1		uNGAL	
	γ	*P*	γ	*P*	γ	*P*
Glomerular Sclerosis	0.047	> .05	-0.053	> .05	-0.188	> .05
Interstitial Inflammatory Cell Infiltration	0.307	< .05	0.262	> .05	0.376	< .05
Interstitial Fibrosis	-0.031	> .05	-0.146	> .05	-0.363	< .05
Tubular Atrophy	0.134	> .05	0.197	> .05	-0.141	> .05

Table 10. Pearson or Spearman Correlation Between Urinary Biomarkers and Pathological Lesions in CKD

Pathological Lesions	uAGT		uKIM-1		uNGAL	
	γ	*P*	γ	*P*	γ	*P*
Glomerular Sclerosis	0.380	> .05	0.271	> .05	0.005	> .05
Interstitial Inflammatory Cell Infiltration	0.676	< .001	0.594	< .05	0.327	> .05
Interstitial Fibrosis	0.485	< .05	0.344	> .05	0.124	> .05
Tubular Atrophy	0.396	> .05	0.230	> .05	0.119	> .05

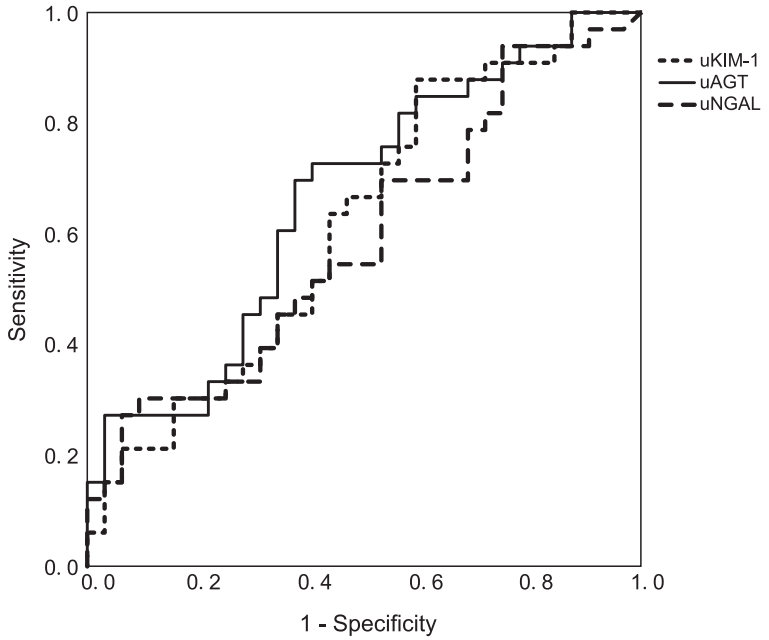

Figure 1. ROC curve analysis showed that uAGT level was able to identify interstitial inflammatory cell infiltration with greater accuracy than other pathological lesions and urinary biomarkers.

progression to the renal endpoint ($P < .05$), with a mean follow-up time to progression of 24.6 months (95% CI: 20.5 to 28.6) compared with 30.9 months (95% CI: 29.5 to 32.3) for uAGT below the cut-off (Figure 2).

A Cox proportional hazards regression model indicated that age, acute or chronic renal insufficiency, uAGT, and interstitial inflammatory cell infiltration predicted higher risk of the progression to endpoints independently (Table 12).

Table 11. Relationship Between Clinical Characteristics and Histologic Characteristics in Renal Insufficiency Patients

	Glomerular Sclerosis		Interstitial Inflammatory Cell Infiltration		Interstitial Fibrosis		Tubular Atrophy	
	None (n = 33)	Present (n = 32)	None Mild (n = 32)	Moderate Severe (n = 33)	None Mild (n = 44)	Moderate Severe (n = 21)	None Mild (n = 45)	Moderate Severe (n = 20)
Men, %	23 (70)	22 (69)	25 (78)	20 (61)	31 (70)	14 (67)	31 (69)	14 (70)
Age, y	43.09 ± 14.51	42.09 ± 13.84	42.34 ± 13.24	42.85 ± 15.06	42.77 ± 14.32	42.24 ± 13.92	42.58 ± 14.37	42.65 ± 13.79
sCr, umol/L	177 (135, 435)	190 (142, 229)	142 (131, 255)	200 (159, 315)**	160 (135, 315)	200 (156, 239)	166 (134, 322)	198 (158, 229)
eGFR, mL/min×1.73m^2	36.02 ± 20.56	37.17 ± 14.52	43.02 ± 18.47	30.35 ± 14.69**	37.5 ± 20	34.68 ± 11.79	37.24 ± 20.08	35.13 ± 10.99
UP, g/d	1.7 (0.5, 8.1)	3.9 (1.9, 7.6)*	4 (0.5, 10.7)	2.3 (1.6, 5.3)	3.5 (0.7, 9.8)	2.5 (1.7, 5.4)	2.3 (0.7, 8.8)	3.2 (1.7, 7.5)
uAGT, ng/mg Cr	192 (21, 613)	262 (118, 671)	147 (43, 581)	288 (115, 1005)*	239 (61, 630)	235 (115, 780)	223 (64, 597)	262 (108, 926)
uKIM-1, ng/mg Cr	2.2 (1.2, 3.6)	2.4 (1.2, 5.1)	2.1 (1, 3.5)	2.4 (1.3, 6.3)	2.3 (1.3, 3.7)	2.1 (1.1, 6.3)	2.3 (1.3, 3.6)	2.2 (1, 7.1)
uNGAL, ng/mg Cr	183 (27, 1033)	37 (11, 178)	57 (16, 356)	91 (16, 1018)	130 (23, 1011)	39 (10, 174)	97 (24, 693)	39 (14, 206)

* $P < .05$
** $P < .001$

DISCUSSION

Our results demonstrated that uAGT, uKIM-1, and uNGAL levels were significantly higher compared with those measured in healthy participants, indicating that they can be used as non-invasive biomarkers of renal damage. This is in accordance with recent investigations. Studies performed in patients with AKI and CKD showed that uAGT levels were significantly increased compared with healthy controls.[29-31] Importantly, uKIM-1 is non-detectable in normal kidneys but has strong expression after various types of renal injury.[7] uNGAL has also been validated as a specific, sensitive, and early predictive of AKI after cardiac surgery,[16] contrast administration,[17] septic shock,[18] and even renal transplantation.[19] Furthermore, it reflects the severity of kidney disease and predicts progression in CKD patients as well.[20]

In our study, the eGFR was directly correlated with hemoglobin, whereas significant inverse correlations were evident with BUN, and uNGAL. Using eGFR as a dependent variable in a multiple linear regression model, adjusted by age, hemoglobin, BUN, Ln uAGT, Ln uKIM-1, and Ln uNGAL; only the associations with hemoglobin, BUN, Ln uAGT, and Ln uNGAL remained significant. These results are in accord with earlier research of Kobori *et al.* and Bolignano *et al.* suggesting uAGT and uNGAL may be indicators of the severity of renal injury.[20,30,32,33] However, our study failed to associate eGFR with uKIM-1 and this is in discord with the research of van Timmeren *et al.* Earlier studies have shown that increased uKIM-1 could indicate either injury or the repair response to injury. Thus, the uKIM-1 level may not be able to differentiate the injury process from recovery of AKI in patients accurately. Therefore, the result of our study seems to be reasonable.

Our results showed that uAGT is correlated with interstitial inflammatory cell infiltration and could reflect its severity. ROC curve analysis showed that uAGT level was able to identify interstitial inflammatory cell infiltration with greater accuracy than other urinary biomarkers and pathological lesions. This has not been reported in previous studies. uKIM-1 also has a correlation with interstitial inflammatory cell infiltration. This is in accord with the research of van Timmeren *et al.* which demonstrated that uKIM-1 is associated with inflammation and renal function in various renal diseases.[7,21] However, uKIM-1 was not significantly increased in patients with moderate

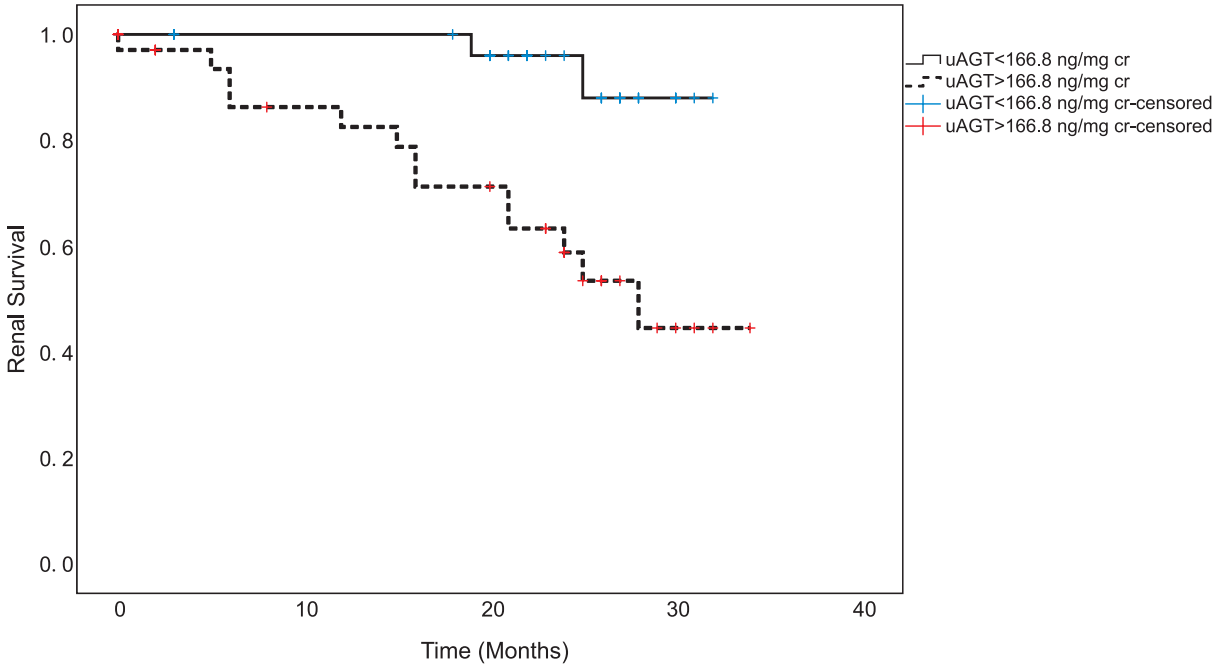

Figure 2. The patients with uAGT values above 166.8 ng/mg Cr experienced a significantly faster progression to the renal endpoint, with a mean follow-up time to progression of 24.6 months compared with 30.9 months for uAGT below the cut-off.

Table 12. Cox Proportional Hazards Regression Analysis for Progression End Point of Renal Insufficiency Patients

Variable	HR	95% CI	x^2	*P*
Age	36.96	5.60 - 244.16	14.05	< .001
A or C Renal Insufficiency	5.10	1.18 - 22.12	4.73	< .05
uAGT	36.50	4.30 - 310.06	10.86	< .05
Interstitial Inflammatory Cell Infiltration	18.40	2.03 - 166.89	6.70	< .05

HR, hazard ratio; CI, confidence interval; uAGT, urinary AGT; A, acute; C, chronic.

or severe interstitial inflammation compared with patients with absent or mild interstitial inflammation. uNGAL level was associated with interstitial fibrosis rather than interstitial inflammatory cell infiltration. This is also in accord with the research of Nickolas *et al.* which verified that uNGAL was significantly correlated with eGFR and interstitial fibrosis and failed to correlate with interstitial inflammatory cell infiltration in CKD patients.[21] Therefore, uAGT performs better than both uKIM-1 and uNGAL in demonstrating renal interstitial inflammatory cell infiltration.

Patients who reached the composite endpoint presented with significantly increased age, sBP, and uAGT level at baseline compared with patients with renal survival. Considering progression endpoint status as variable, ROC analysis showed a significant AUC for age, sBP, and uAGT level; respectively. Kaplan-Meier survival curves in patients with age, sBP, and uAGT level above the optimal cut-off experienced a significantly faster progression to the composite endpoint. These were also found in patients with glomerular sclerosis, moderate or severe interstitial inflammatory cell infiltration, interstitial fibrosis and tubular atrophy. Results from the multivariate cox proportional hazards regression analysis showed that age (> 51.5 years), chronic kidney disease, uAGT (> 166.8 ng/mg Cr), and moderate or severe interstitial inflammatory cell infiltration predicted a higher risk of the progression endpoint independently in patients with renal insufficiency. These results have not been reported in previous studies.

The possible mechanism of infiltration of inflammatory cells in the interstitium by AGT could be the intrarenal RAS caused interstitial inflammatory cell infiltration. There is substantial evidence that AngII present in renal tissues is generated locally from AGT delivered to the kidney as well as from AGT locally produced by proximal tubule cells.[22,34,35] urinary AngII was proposed previously

as a biomarker of intrarenal AngII activity. AGT is the only known substrate for renin, and the level of AGT in humans and rats is close to the Km value for renin.[36] Therefore, changes in either AGT (substrate) or renin (enzyme) could influence RAS activity. The activation of intrarenal RAS plays a crucial role in the development of renal injury. Increases in intrarenal RAS components, particularly AngII, in parallel with the severity of fibrotic renal damage, have been demonstrated in chronic progressive nephropathy in rats and humans.[37] Experiments have also shown that target organs are injured by locally produced AngII through the induction of noninfectious inflammation.[23] AngII is a proinflammatory cytokine and chemokine, and can activate the expression of a series of inflammatory factors. Monocyte chemotactic factor 1 (MCP-1/CCL2) is considered to be one of the most important chemokines induced by AngII,[38] and researchers have shown that uMCP-1 levels have a strong correlation with interstitial inflammatory cell infiltration in drug-induced tubulointerstitial nephritis.[39] Lai *et al.* found that interstitial inflammatory cell infiltration in IgA nephropathy is secondary to activation of the RAS by in situ hybridization.[40] Numerous animal and clinical studies have demonstrated that uAGT level correlates with intrarenal AGT and AngII, and have shown it to be an indicator of intrarenal RAS activity.[37,41] The strong correlation between uAGT and interstitial inflammatory cell infiltration in renal insufficiency patients was demonstrated in our study.

The study also had limitations. First, it was a single-center study, and the cohort of patients was relatively small. Confirmation in a broader cohort is indispensable to verify the general validity to our report. Second, treatment factors were not examined sufficiently during follow-up, and our analyses were not adequately adjusted for these factors. Further in-depth investigations should be undertaken to verify these findings.

CONCLUSION

uAGT may be used as a non-invasive biomarker of interstitial inflammatory cell infiltration and is a strong predictor for renal prognosis in patients with renal insufficiency.

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DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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