

High Urinary Aspartate Aminotransferase in the Late Posttransplant Period Predicts Rapid, Progressive Decline in Kidney Allograft Function

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Abstract

Objectives: Scant information is available on factors for predicting the rate of decline in kidney allograft function beyond 1 year posttransplant. We investigated whether urinary enzymes (alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, N-acetyl- β -D-hexosaminidase, and γ -glutamyl transpeptidase) in the late postoperative period can predict the decline in estimated glomerular filtration rate.

Materials and Methods: In 79 kidney allograft recipients 1 to 17 years after kidney transplant, we assessed a value of urinary enzymes single measurement for predicting the slope of estimated glomerular filtration rate, rapid decline in estimated glomerular filtration rate (> 5 mL/min/1.73 m²/y), and significant decline in estimated glomerular filtration rate ($\geq 25\%$ from baseline) during a 2-year period.

Results: At baseline, patients with estimated glomerular filtration rate < 60 mL/min/1.73 m² ($n = 54$) differed from those with estimated glomerular filtration rate ≥ 60 mL/min/1.73 m² ($n = 25$) only in their lower median urinary alanine aminotransferase:creatinine ratio (expressed as U/L:mmol/L): 0.055 versus 0.222 ($P = .011$). Higher urinary activity of aspartate aminotransferase at baseline predicted the negative-slope value for estimated glomerular filtration rate (beta, -0.279 ; standard error, 0.131; $P = .037$) and decline in estimated glomerular filtration rate of > 5 mL/min/1.73 m²/year (odds ratio, 2.06; 95%

confidence interval, 1.10-3.83; $P = .023$) over 2 years. It also predicted the drop in estimated glomerular filtration rate $\geq 25\%$ after 1 year (odds ratio, 2.62; 95% confidence interval, 1.07-6.37; $P = .034$) and 2 years (odds ratio, 2.75; 95% confidence interval, 1.12-6.73; $P = .027$). Combined with time after transplant, urinary aspartate aminotransferase had good power for predicting an estimated glomerular filtration rate decrease $\geq 25\%$ after 2 years of follow-up.

Conclusions: Higher urinary activity of aspartate aminotransferase in the late posttransplant period is useful for identifying transplant patients who are at risk for progressive loss of graft function.

Key words: Biomarkers, Glomerular filtration rate, Graft dysfunction, Renal transplantation, Urinary enzymes

Introduction

Kidney transplant confers the highest survival benefit among renal replacement therapies.¹ However, at present, despite excellent 1-year kidney allograft (KAG) survival, many patients who receive transplants develop chronic allograft dysfunction; thus, the preservation of allograft function in the late period after transplant has become the main challenge.¹ Identification of patients at risk for progressive decline in graft function is a prerequisite for developing strategies for saving graft function and improving graft survival.^{2,3} However, little information is available on factors that can predict the rate of decline in kidney function beyond the first year after transplant.^{2,4,5}

Percutaneous needle biopsy is considered to be a criterion standard for diagnosing allograft pathology and can provide prognostic information.^{5,6} However, biopsy is an invasive procedure with some risk of complications, so it cannot be repeated frequently. Consequently, noninvasive biomarkers are needed for kidney transplant patients to better understand

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pathogenesis of disease, assess immune risk, detect early injuries to the graft, make differential diagnoses, and guide therapeutic decision-making.^{7,8}

It seems logical that urinary biomarkers carry more useful information about the KAG's state than serum biomarkers, since the urine specimen provides a representative sample of the entire KAG.⁸ Several urinary biomarkers, including enzymes of tubular origin, denote injury to the appropriate epithelial cells' compartment. These biomarkers have shown diagnostic and prognostic value in native kidney diseases in clinical and experimental settings.⁹⁻¹⁴ Thus, such enzymes as N-acetyl- β -D-hexosaminidase (NAG),¹⁵⁻¹⁸ γ -glutamyl transpeptidase (GGT),^{16,19-21} and alkaline phosphatase (AP)^{19,20} have been proposed for in-depth assessment of the KAG state. However, most published studies on these biomarkers in KAG recipients were limited to the early posttransplant period^{16,18,20,22,23} and focused on the diagnosis of acute tubular necrosis,^{16,18,20,22,23} acute graft rejection,^{16,20,23} or nephrotoxicity.^{17,23} Only a few studies were dedicated to the problem of chronic graft injury.^{15,21} Although several papers have described the prognostic significance of urinary enzymes in the KAG setting,^{15,21} their endpoint was graft failure,²¹ and the pattern of progression to graft failure was not studied.¹⁵ Meanwhile, investigating the association between urinary enzymes and the rate of progression toward graft failure would be of interest, because the rate of decline in glomerular filtration rate (GFR) might be an early indicator of allograft injury and a strong predictor of graft survival.^{2,24}

In the present study, we tested the hypothesis that measuring urinary enzymes (alanine aminotransferase [ALT], AP, aspartate aminotransferase [AST], GGT, and NAG) in the late posttransplant period could predict decline in GFR during 1- to 2-year follow-up.

Materials and Methods

Study population

During the 4-month study period (September to December 2012), a total of 90 white patients who received a KAG in the Zaporizhzhia transplantation center between January 1995 and September 2011 and were willing to participate were recruited. Of these, a total of 79 patients (47 men and 32 women, aged 16-64 y) who fulfilled the inclusion criteria were enrolled in the study. The inclusion criteria of the study were

the following: an adult KAG recipient, male or female, with a primary transplant from a related or deceased donor, with allograft survival of ≥ 1 year and estimated GFR (eGFR) ≥ 15 mL/min/1.73 m². The exclusion criteria were receiving regular dialysis, or having acute kidney injury, diseases of the immune system, solid tumors, or clinical signs of acute infections.

Induction immunosuppression consisted of anti-interleukin-2 receptor antibodies and was used in recipients who underwent transplant surgery after 2005. All recipients received triple maintenance immunosuppressive therapy consisting of a calcineurin inhibitor (cyclosporine or tacrolimus), an antiproliferative agent (mycophenolate mofetil or azathioprine), and a steroid. All participants gave their written informed consent. This research study was approved by the local ethics committee and carried out in accordance with the ethical standards outlined in the Declaration of Helsinki (2013 version) and the Declaration of Istanbul.

Laboratory methods

Serum was obtained from venous blood taken in the morning from patients in a fasting condition. Simultaneously, freshly voided morning urine samples were collected and centrifuged at 3000 rpm for 15 minutes. We measured the serum concentration of urea and creatinine using the urease and Jaffe methods. Urine specific gravity was measured with a urinometer. We measured urine protein level using the pyrogallol red-molybdate method, and urine creatinine concentration using the Jaffe method. Urinary ALT, AP, AST, and GGT were measured in supernatants of fresh urine using enzymatic methods. All kits were supplied by Filisit-Diagnostics (Dnipro, Ukraine). For measuring NAG, aliquots of urine supernatant were frozen and stored at -40°C . The activity of NAG was measured using a colorimetric method. When NAG is present in the urine sample, it hydrolyzes the substrate 4-nitrophenyl-N-acetyl- β -D-glucosaminide (Sigma-Aldrich, Darmstadt, Germany). This reaction releases p-nitrophenol, which is measured by spectrophotometry. The absorbance was measured using a spectrophotometer PD-303UV (Apel Co., Ltd., Saitama, Japan). The resulting levels of total protein (mg/L) and enzymes (U/L) in the urine spot sample were normalized to urinary creatinine (mmol/L). We considered 15 mg/mmol to be the threshold of normalized proteinuria.²⁴ For GFR estimation, we

used a 4-variable equation derived from the Modification of Diet in Renal Disease Study.²⁵

Risk factors and outcomes

In this study, we assessed the predictive value of a cross-sectional measurement of urinary ALT, AP, AST, GGT, and NAG. Archival case records and outpatient data cards were used to obtain more information on major risk factors and evolution of renal function in allograft recipients. Data were collected on kidney recipients' age, sex, and presence of chronic arterial hypertension, which was defined as a regular intake of antihypertensive drugs. We also obtained transplant-related information: donor source, type of immunosuppressive regimen, initial graft function, acute rejection episodes, and time after transplant. At enrollment, each patient's arterial pressure was measured in a sitting position after a 10-minute rest period.

For linear regression analyses, initial allograft function was classified on a scale of 0 to 3 as follows: immediate function (0 points); slow function, ie, serum creatinine reduction < 70 from transplant to day 7 (1 point); delayed function, ie, need for dialysis ≥ 1 time in the first 7 days after transplant (2 points); and delayed function that required > 1 dialysis procedure (3 points). Acute graft rejection was defined as the need for treatment, with or without biopsy confirmation. Patients enrolled in this study were followed up for 2 years until death, return to dialysis, or the study conclusion in December 2014. In the course of follow-up, GFR was estimated annually.

During year 2 of follow-up, a total of 3 patients with a functioning graft died, and 5 grafts failed. For 5 patients who returned to dialysis, eGFR was 10 mL/min/1.73 m². The annualized change (slope) in eGFR (mL/min/1.73 m²/y) over a 2-year period was calculated for each patient, comprising 3 eGFR values, using the linear mixed-effects model with varying intercept and slope. We determined the proportion of patients whose eGFR decreased $\geq 25\%$ from baseline after 1 and 2 years of follow-up and the proportion of patients with rapid decline in eGFR (> 5 mL/min/1.73 m²/y), since both measures indicate progressive loss of kidney function.²⁴ The endpoints of the study were the slope of eGFR, decline in eGFR > 5 mL/min/1.73 m²/year, and drop in eGFR $\geq 25\%$ from baseline after 1 and 2 years of follow-up.

Statistical analyses

Normally distributed data are expressed as the mean $\pm$ standard deviation; the results were compared using the *t* test. Continuous nonparametric data are expressed as the median (interquartile range). For comparison, we used the Mann-Whitney *U* test; and the Spearman's rank correlation coefficient (ρ) was calculated. Frequency data are expressed as percentages, and for comparison, we applied the chi-square test. To identify predictors of the eGFR slope, linear regression was used. To identify independent predictors of a significant drop in eGFR after 1 and 2 years of follow-up $\geq 25\%$ from baseline²⁴ and rapid decline in eGFR of > 5 mL/min/1.73 m²/year,²⁴ we used univariate logistic regression analysis. Next we performed multivariate analysis with a backward stepwise selection method, based on the probability of the likelihood-ratio statistic, using only those predictive variables that demonstrated individual *P* values < .05. We also calculated area under the receiver operating characteristic curves (AUCs). We did so to assess the ability of each variable considered significant by simple logistic regression to identify patients with a drop in eGFR $\geq 25\%$ after 1 and 2 years of follow-up, and patients with rapid decline in eGFR > 5 mL/min/1.73 m²/year, from those who did not meet these criteria. We used logistic regression models to estimate combinations of urinary enzymes and other variables of interest and evaluated the discriminatory abilities of the combinations using the AUCs. For statistical analyses, we used Statistica 7.0 (StatSoft Inc., Tulsa, OK, USA), IBM SPSS Statistics software for Windows (version 19.0, IBM Corp., Armonk, NY, USA), and MedCalc bvba software (version 14.8.1, MedCalc, Ostend, Belgium). Statistical significance was set at *P* < .05.

Results

Baseline characteristics of patients

According to the definition of chronic kidney disease,²⁴ we categorized transplant patients into "normal GFR" and "low GFR" subsets defined as eGFR ≥ 60 mL/min/1.73 m² and < 60 mL/min/1.73 m². The demographics and clinical characteristics of patients are listed in Table 1. Most patients received a kidney from a deceased donor and received cyclosporine-based immunosuppressive drugs. No significant differences in sex, age, type of

immunosuppressive drug regimen, time after transplant, frequency of impairment of initial graft function, or acute rejection were observed between the 2 groups. Only 1 patient had late acute rejection (at 3.5 y), whereas the remaining patients had early acute rejection episodes (≤ 3 mo posttransplant). The mean arterial pressure and the percentage of patients regularly receiving antihypertensive therapy were significantly higher in the low GFR group. The differences between the main laboratory parameters of graft status (eg, serum creatinine, eGFR, normalized proteinuria) were highly significant (Table 1).

Urinary enzyme levels and their correlations with clinical variables

Levels of urinary enzymes in the 2 patient cohorts at enrollment are shown in Table 2. The ALT activity in urine was significantly lower in the low GFR group. The activities of AST, GGT, AP, and NAG in urine at enrollment were not significantly different in the compared groups. Table 3 summarizes the cor-

relations of urinary enzymes with markers of chronic kidney disease at baseline. As can be seen in Table 3, none of the enzymes correlated significantly with serum creatinine. Meanwhile, all enzymes demonstrated weak correlations with normalized proteinuria. In addition, the activity of NAG in the urine correlated with a history of acute graft rejection ($\rho = .311$, $P = .010$) and with the age of the recipient ($\rho = .273$, $P = .022$). The level of proteinuria correlated with the mean blood pressure ($\rho = .488$, $P < .001$) and with episodes of previous acute graft rejection ($\rho = .264$, $P = .027$). Correlations between urinary enzymes are shown in Table 4. The activity of most enzymes in urine weakly correlated with each other, except for AST, which did not correlate with AP and GGT. We observed moderate correlations between AP and GGT, and between AP and NAG.

The evolution of allograft function and predictive variables

After 1 year of follow-up, mean eGFR did not significantly change in the total patient population: it fell slightly to 49.2 ± 19.7 mL/min/1.73 m² from 50.4 ± 19.7 mL/min/1.73 m² at baseline ($P = .289$). Eight of the patients (15%) from the low GFR group

Table 1. Baseline Characteristics of the Study Population (N = 79)

Parameter	Normal eGFR, ≥ 60 mL/min/ 1.73 m ² (n = 25)	Low eGFR, < 60 mL/ min/1.73 m ² (n = 54)	P Value*
Recipient age at baseline, y	36 $\pm$ 12 [†]	39 $\pm$ 11	.248
Recipient sex, n (%)			
Male	13 (52) [‡]	34 (63)	.356
Female	12 (48)	20 (37)	
Calcineurin inhibitor, n (%)			
Cyclosporine	22 (88)	41 (76)	.214
Tacrolimus	3 (12)	13 (24)	
Antiproliferative agent, n (%)			
Azathioprine	3 (12)	7 (13)	.905
Mycophenolate mofetil	22 (88)	47 (87)	
Anti-IL-2 receptor antibodies, n (%)	17 (68)	33 (61)	.555
Type of donor, n (%)			
Deceased	20 (80)	45 (83)	.718
Living related	5 (20)	9 (17)	
Impaired initial function, n (%)	2 (8)	11 (20)	.168
Previous acute rejection, n (%)	4 (16)	15 (28)	.255
Time after transplant, mo	78.7 $\pm$ 48.9	88.8 $\pm$ 56.2	.444
Serum creatinine, μ mol/L	107.9 $\pm$ 19.5	181.6 $\pm$ 49.3	< .001
eGFR at baseline, mL/min/1.73 m ²	74.5 $\pm$ 9.7	39.0 $\pm$ 10.9	< .001
Serum urea, mmol/L	6.3 $\pm$ 1.6	11.3 $\pm$ 3.3	< .001
Proteinuria/creatinine, mg/mmol	3.6 (2.7-7.9) [§]	11.8 (4.5-21.8)	.005
Proteinuria/creatinine > 15 mg/mmol, n (%)	1 (4)	17 (32)	.007
Diuresis, mL/d	1484 $\pm$ 258	1485 $\pm$ 340	.693
Urine specific gravity	1014 $\pm$ 4	1007 $\pm$ 4	< .001
Mean arterial pressure at baseline, mm Hg	102 $\pm$ 10	111 $\pm$ 9	< .001
Treated hypertension at baseline, n (%)	7 (28)	33 (61)	.006

Abbreviations: eGFR, estimated glomerular filtration rate; IL-2, interleukin 2

*Considered significant if $P < .05$.

[†]Mean $\pm$ standard deviation; t test used for comparison.

[‡]Numbers (percentages): chi-square tests used for comparison.

[§]Median (interquartile range): Mann-Whitney U test used for comparison.

Table 2. Activity of Enzymes in Urine of Kidney Allograft Recipients at Enrollment (N = 79)

Urinary Enzymes Normalized to Creatinine	Normal eGFR, ≥ 60 mL/min/ 1.73 m ² (n = 25)	Low eGFR, < 60 mL/min/ 1.73 m ² (n = 54)	P Value*
ALT/Cr, U/L/mmol/L	0.222 (0.055-0.464) [†]	0.055 (0.000-0.151)	.011
AP/Cr, U/L/mmol/L	6.412 (3.992-12.177)	5.313 (3.048-10.457)	.244
AST/Cr, U/L/mmol/L	0.109 (0.013-0.313)	0.145 (0.062-0.347)	.558
GGT/Cr, U/L/mmol/L	0.905 (0.677-2.106)	1.359 (0.650-1.806)	.850
NAG/Cr, U/L/mmol/L	1.062 (0.582-1.770)	1.174 (0.578-1.760)	.593

Abbreviations: ALT, alanine-aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; Cr, creatinine; eGFR, estimated glomerular filtration rate; GGT, γ -glutamyl transpeptidase; NAG, N-acetyl- β -D-hexosaminidase

*Considered significant if $P < .05$.

[†]Median (interquartile range): Mann-Whitney U test used for comparison.

Table 3. Correlation of Urinary Enzymes with Markers of Chronic Kidney Disease in Allograft Recipients at Enrollment (N = 79)*

Urinary Enzymes Normalized to Creatinine	Serum Creatinine, μ mol/L	Pr/Cr, mg/mmol
ALT/Cr	$\rho = -.136$, $P = .267$	$\rho = .293$, $P = .014$
AP/Cr	$\rho = -.005$, $P = .968$	$\rho = .313$, $P = .008$
AST/Cr	$\rho = .200$, $P = .077$	$\rho = .382$, $P = .001$
GGT/Cr	$\rho = -.007$, $P = .957$	$\rho = .335$, $P = .012$
NAG/Cr	$\rho = .207$, $P = .086$	$\rho = .580$, $P < .001$

Abbreviations: ALT, alanine-aminotransferase; AP, alkaline phosphatase; AST, aspartate-aminotransferase; Cr, creatinine; GGT, γ -glutamyl transpeptidase; NAG, N-acetyl- β -D-hexosaminidase; Pr/Cr, proteinuria in spot urine normalized to creatinine; ρ , Spearman's rank correlation coefficient

*Considered significant if $P < .05$.

and 2 of the patients (8%) from the normal GFR group ($P = .397$) demonstrated a decline in eGFR $\geq 25\%$ from baseline after 1 year. Eight patients with significant decrease in GFR ($\geq 25\%$) had chronic allograft dysfunction, but only in 2 cases was the diagnosis confirmed histologically. Of these, 1 patient had chronic, active T-cell-mediated rejection, and the second patient had chronic nephrotoxicity combined with hypertensive nephropathy. In 2 other patients, we also observed progression of heart failure from New York Heart Association functional class II to class III, which might have caused worsening graft function. Six patients (11%) from the low GFR group showed an increase in eGFR $\geq 25\%$, whereas such patients were absent in the normal GFR group ($P = .083$). The presumed causes of improving graft function were conversion of immunosuppressive regimens (from cyclosporine to tacrolimus in 2 patients, and from mycophenolate mofetil to everolimus in 2 patients), effective antibiotic treatment of urinary tract infection (1 patient), and increase in renal perfusion after coronary artery bypass (1 patient).

Table 5 details logistic regression analyses of the value of urinary biomarkers and selected laboratory and clinical parameters for predicting eGFR decline by $\geq 25\%$ during 1- and 2-year follow-up. Higher normalized proteinuria and higher urinary activity

of AST were individually predictive of decline in eGFR $\geq 25\%$ after 1 year of follow-up, whereas only AST retained its predictive value in a multivariate model.

The ability of normalized proteinuria and urinary AST activity to show a drop in eGFR $\geq 25\%$ during follow-up was determined by AUC analysis. As shown in Table 6, urinary AST and normalized proteinuria were both significant predictors of decline in eGFR $\geq 25\%$ after 1 year with fair discriminating ability. The combination of AST and proteinuria did not improve the predictive properties of the model (Table 6).

During year 2 of follow-up, a total of 3 deaths in patients with a functioning graft occurred, and a total of 5 grafts failed. The cause of allograft failure was verified histologically in 3 patients and found to be chronic, active antibody-mediated rejection (1 patient), chronic pyelonephritis (1 patient), and de novo glomerulonephritis (1 patient). After 2 years of follow-up, mean eGFR in the total patient population ($n = 76$) had significantly decreased to 46.3 ± 20.6 mL/min/1.73 m² from 50.4 ± 19.7 mL/min/1.73 m² at baseline ($P = .002$). This decline was also significant compared with eGFR after 1 year ($P < .001$). Nine patients (18%) from the low GFR group and 3

Table 4. Internal Correlation Between Urinary Enzymes in Allograft Recipients at Enrollment ($N = 79$)*

	ALT/Cr	GGT/Cr	AP/Cr	NAG/Cr
AST/Cr	$\rho = .476$ $P < .001$	$\rho = .101$ $P = .462$	$\rho = -.026$ $P = .833$	$\rho = .387$ $P < .001$
ALT/Cr		$\rho = .413$ $P = .002$	$\rho = .481$ $P < .001$	$\rho = .599$ $P < .001$
GGT/Cr			$\rho = .601$ $P < .001$	$\rho = .555$ $P = .001$
AP/Cr				$\rho = .658$ $P < .001$

Abbreviations: ALT, alanine-aminotransferase; AP, alkaline phosphatase; AST, aspartate aminotransferase; Cr, creatinine; GGT, γ -glutamyl transpeptidase; NAG: N-acetyl- β -D-hexosaminidase; ρ , Spearman's rank correlation coefficient

*Considered significant if $P < .05$.

Table 6. Area Under the Curve for Potential Markers of eGFR Decline $\geq 25\%$ After 1 and 2 Years of Follow-up*

Predictive Variables	AUC	CI	P Value
eGFR decline $\geq 25\%$ after 1 year of follow-up ($n = 10$)			
Pr/Cr	0.784	0.623-0.945	.004
AST/Cr	0.724	0.557-0.891	.040
Pr/Cr + AST/Cr	0.770	0.597-0.943	.032
eGFR decline by $\geq 25\%$ after 2 years of follow-up ($n = 12$)			
Time posttransplant	0.700	0.553-0.847	.016
AST/Cr	0.700	0.551-0.850	.030
Time posttransplant + AST/Cr	0.804	0.647-0.960	.004

Abbreviations: AST/Cr, urinary aspartate aminotransferase normalized to urinary creatinine; AUC, area under the receiver operating characteristic curve; CI, 95% confidence interval; eGFR, estimated glomerular filtration rate; Pr/Cr, proteinuria in spot urine normalized to urinary creatinine

*Only variables that significantly influenced eGFR decline in a univariate analysis were included. Considered significant if $P < .05$.

Table 5. Significant Predictors of eGFR Decline $\geq 25\%$ After 1 and 2 Years of Follow-up*

Predictive Variables	Univariate Logistic Regression			Multivariate Logistic Regression		
	OR	CI	P Value	OR	CI	P Value
eGFR decline $\geq 25\%$ after 1 year of follow-up ($n = 10$)						
Pr/Cr	1.04	1.01-1.07	.009			
AST/Cr	2.62	1.07-6.37	.034	2.62	1.07-6.37	.034
eGFR decline by $\geq 25\%$ after 2 years of follow-up ($n = 12$)						
AST/Cr	2.35	1.11-4.98	.026	2.75	1.12-6.73	.027
Time posttransplant (y)	1.02	1.00-1.03	.009	1.02	1.00-1.04	.026

Abbreviations: AST/Cr, urinary aspartate aminotransferase normalized to creatinine; CI, 95% confidence interval; eGFR, estimated glomerular filtration rate; OR, odds ratio; Pr/Cr, proteinuria in spot urine normalized to creatinine

*Only variables that significantly influenced eGFR decline in univariate analysis were included. Considered significant if $P < .05$.

patients (12%) from the normal GFR group had a decrease in eGFR $\geq 25\%$ compared with baseline ($P = .417$). We did not observe any new intercurrent events during this period. Only 3 patients (6%) from the low GFR group showed an increase in eGFR $\geq 25\%$ compared with baseline, whereas such patients were absent in the normal GFR group ($P = .192$).

With respect to graft function after 2 years of follow-up, higher urinary activity of AST and longer time posttransplant were both significantly associated with higher odds ratios of a $\geq 25\%$ eGFR drop (Table 5). Both urinary AST and time posttransplant exhibited fair discriminatory power for predicting eGFR decline $\geq 25\%$ after 2 years of follow-up (Table 6). The combination of urinary AST and time posttransplant yielded an AUC of 0.804, indicating good predictive power for a $\geq 25\%$ eGFR drop after 2 years (Table 6). The AUC of this combined model was higher than the AUC for each predictor alone, but not significantly ($P > .05$).

The eGFR slope in the overall group of patients was -2.0 ± 5.7 mL/min/1.73 m²/year (median, -2.0 mL/min/1.73 m²/y). In the normal GFR group, the slope was -3.9 ± 6.9 mL/min/1.73 m²/year (median, -3.0 mL/min/1.73 m²/y), which did not significantly differ from the low GFR group: -1.2 ± 4.9 mL/min/1.73 m²/year (median, -1.3 mL/min/1.73 m²/y) ($P = .101$). Nine patients (17%) in the low GFR group and 6 patients (24%) in the normal GFR group lost GFR at a rate of > 5.0 mL/min/1.73 m²/year ($P = .440$). Higher urinary activity of AST was the only predictor of a more negative slope for eGFR using linear regression analysis (beta, -0.279 ; standard error, .131; $P = .037$). None of the other variables, including eGFR at baseline, correlated with the magnitude of eGFR slope. Higher urinary activity of AST was also the only predictor of rapid decline in eGFR of > 5 mL/min/1.73 m²/year in logistic regression analysis (OR, 2.06; 95% confidence interval [CI], 1.10-3.83; $P = .023$). Urinary AST demonstrated fair discriminatory power for predicting eGFR decline by > 5 mL/min/1.73 m²/year (AUC, .705; CI, 0.554-0.885; $P = .018$).

Given the observed performance of AST as a predictive biomarker, patients were divided into quartiles of normalized urinary AST activity at baseline: ≤ 0.060 U/L/mmol/L, 0.061-0.145 U/L/mmol/L, 0.146-0.324 U/L/mmol/L, and ≥ 0.325 U/L/mmol/L. We observed AST activity in the highest quartile in

14 patients (26%) from the low GFR group, and in 5 patients (20%) from the normal GFR subset ($P > .05$).

Discussion

Identifying patients at risk for progressive decline in KAG function in the late posttransplant period using urinary enzyme tests might enable individualized care without risk of adverse effects when performing testing. The principal finding of our study is the association between urinary levels of AST in the late posttransplant period and subsequent decline in KAG function within a short time frame, irrespective of graft function at baseline. During 2-year follow-up, urinary AST activity predicted with fair accuracy significant decline in eGFR $\geq 25\%$ after 1 and 2 years and rapid decline in eGFR > 5 mL/min/1.73 m²/year. Urinary AST activity also predicted negative-slope values of eGFR. In conjunction with time after transplant, urinary AST had good power for predicting an eGFR drop $\geq 25\%$ after 2 years. To our knowledge, this is the first report about the prognostic significance of urinary AST in the late period after kidney transplant.

Patients were randomized at enrollment into low GFR and normal GFR subsets, according to the Kidney Disease: Improving Global Outcomes definition.²⁴ The only difference in urinary enzyme activity was lower ALT activity in the low GFR group. Given that tubular epithelium is a main source of urinary enzymes,²³ the most likely cause of low urinary ALT activity in these patients was tubular atrophy characteristic of chronic renal allograft dysfunction.²⁶ As discussed by Jung and colleagues,²³ patients with a greatly reduced GFR generally show subnormal excretion of enzymes, owing to smaller renal cellular mass. However, although ALT activity correlated poorly with other enzymes, the latter's activity was not reduced in the group with low GFR, which might indicate the relative preservation of the renal parenchyma. It is also worth considering that different enzymes have demonstrated varying abilities to measure renal dysfunction.²³

During 2 years of follow-up, we observed different patterns of changes in allograft function: worsening, stabilization, or even improvement. The evolution of graft function is a complex issue that has been discussed extensively in the literature,^{24,5} including in our previous paper.²⁷ Nevertheless, by

the end of this study, the average eGFR in the whole cohort had decreased significantly compared with baseline. Patients in the whole population, as well as in the 2 subgroups, demonstrated a negative average slope of eGFR. Fifteen patients (20%) had rapid decline in eGFR of 5 mL/min/1.73 m²/year; in addition, 10 patients (13%) and 12 patients (16%) displayed significant decline in eGFR $\geq$ 25% from baseline after 1 and 2 years of follow-up.

Although median urinary AST level did not differ significantly between the patient subgroups at enrollment, urinary AST was the only enzyme that predicted the value of the negative slope in eGFR, the significant decline in eGFR $\geq$ 25% from baseline after 1- and 2-year follow-up, and the rapid decline in eGFR of > 5 mL/min/1.73 m²/year after 2 years. For each 1-unit increase in urinary AST activity, the odds ratios were 2.62, 2.75, and 2.06 for a significant drop in eGFR after 1 and 2 years and for rapid decline in eGFR for 2 years. Moreover, urinary AST showed a fair discriminatory power for predicting $\geq$ 25% decline in eGFR after 1 and 2 years and for rapid decline in eGFR during 2-year follow-up. When we studied the distribution of data on AST activity across patient subgroups, we observed that participants in the highest quartile of AST (≥ 0.325 U/L/mmol/L) belonged with equal frequency to the normal and low GFR groups.

Aspartate aminotransferase is a mitochondrial and cytosolic enzyme of epithelial cells predominantly of the distal and, to a lesser extent, of the proximal tubules of the nephron.²³ Taking into account that little urinary enzymatic activity derives from the serum,²³ higher AST activity in the urine of some patients at enrollment could have been a sign of ongoing deep, irreversible damage to the tubular epithelial cells.²⁸⁻³¹ Observed correlations between AST and ALT activity, NAG, and proteinuria confirm this hypothesis. Our findings also confirm data from the literature that enzymuria intensity is generally more likely to indicate kidney pathologic process activity, rather than kidney function.²³ Some patients can have both acute and chronic processes under way in their KAG that presumably lead to opposite changes in urinary enzyme activity. Thus, enzymuria can be low, high, or unchanged depending on the ratio of damaged, sclerotic, hypertrophic, or hypermetabolic nephrons.²³ Data from the literature also suggest that patients simultaneously displaying acute and chronic changes in their KAG, for example,

interstitial infiltrate on a background of fibrosis, have worse subsequent KAG function and morphology.^{6,26,32,33}

Several other reasons can explain the absence of coordinated increase in urinary enzyme activity in patients with low GFR in this study. First, patients with acute tubular injury were excluded from the study. Second, different patients may have had opposite changes in enzyme activity: an increase or a decrease. As long as we demonstrated coordination among brush border membrane enzymes, lysosomal enzymes, mitochondrial enzymes, and cytosolic enzymes, we suppose that among our patients were those with low as well as high urinary enzyme excretion.²³ Third, rises in enzyme activity might be smaller in chronic kidney injury than in acute kidney injury.²³ Indeed, in some patients, atrophic processes in the KAG might be advanced in the late posttransplant period, as evidenced by a decrease in ALT levels. Thus, apparently some patients had active tubular injury at enrollment (though not reaching the threshold for diagnosis of acute kidney injury), which worsened their prognosis. Although we did not assess threshold AST levels, our results show that elevated urinary AST excretion, even in the absence of decreased renal function, must be taken as a serious sign of graft injury and should guide diagnostic biopsy. To our knowledge, scant published evidence has shown that biomarkers measured during times of clinical quiescence can predict transplant outcomes.³⁴ Our results are in line with the conception of Halloran and associates that progression of KAG dysfunction can be explained only as a consequence of ongoing disease and injury in the graft.³⁵ Presumably, high urinary AST is one of the manifestations of stereotyped active injury–repair response in the graft. As demonstrated by Halloran and colleagues,³⁵ the active injury–repair response in the KAG correlates most strongly with subsequent functional disturbances and progression to graft failure.

The present results have further clinical and theoretical implications. Our findings, along with data from the literature^{2,4} imply that eGFR alone is not an accurate predictor of kidney graft failure, as we discussed in our earlier paper.²⁷ In this regard, urinary AST, which provides additional information about injuries to the graft, might improve the predictive strength of eGFR. Urinary AST also seems superior to other urinary enzymes for differentiating

KAG recipients who subsequently lose KAG function from those who do not. The AST test provides a quick and accurate assessment using a commonly available, cost-effective, colorimetric-based assay, which can be readily implemented in clinical laboratories.^{9,19} The association of elevated urinary AST with decline in eGFR might have considerable clinical impact, since the latter surrogate marker is directly related to inferior allograft survival rates.² Finally, urinary AST itself can be a surrogate marker for the progression of KAG dysfunction. In this way, our findings expand on the results of previous studies that looked at predictive variables for a drop in GFR in transplant recipients.

We also identified proteinuria in the late post-transplant period as a predictor of definite $\geq 25\%$ decline in allograft function after 1 year of follow-up and whose negative influence on KAG function and survival is well recognized.^{5,36-39} However, in our study, the influence of proteinuria was significant only in the univariate model, before adjusting for AST. Thus, urinary AST was superior to nonselected proteinuria for predicting progressive decline in KAG function. In our patients, proteinuria correlated with both history of acute rejection and mean arterial pressure, so it could have had both immunologic and nonimmunologic causes.³⁶⁻³⁸ Low-grade proteinuria, which we observed in our patients, was associated mostly with interstitial and tubular damage.^{36,37,40} It is known that proteinuria itself can injure tubular epithelial cells and stimulate them to synthesize chemokines, which contribute to inflammation in the tubulointerstitium.^{40,41} In turn, interstitial inflammation strongly correlates with reduced allograft survival. The correlations between proteinuria and the full range of tubular enzymes (particularly NAG and AST) point to an association of proteinuria with ongoing injury in the tubulointerstitial compartment of the KAG and lead us to believe that measuring enzymuria enhances the diagnostic significance of proteinuria alone. We can hypothesize that tubular damage induced by proteinuria might manifest as an increase in urinary AST activity, which needs further study. In any case, our results imply that higher urinary AST levels in the late posttransplant period are a surrogate marker for chronic KAG injury.

Longer time posttransplant along with higher urinary AST predicted $\geq 25\%$ decline in eGFR after 2 years of follow-up, which can be attributed to a larger cumulative burden of graft injury over time.³⁵ Regarding the effect of the "transplant era," as

reported by Kasiske and associates,⁴² the rate of kidney graft function decline has significantly improved in recent years owing to improvements in patient care and treatment strategies. Time post-transplant and urinary AST, when considered separately, had fair power for identifying patients with $\geq 25\%$ decline in eGFR after 2 years. Used in conjunction, these variables further enhanced the quality of patient prognosis and displayed good predictive performance. Thus, when combined with time posttransplant, urinary AST can predict a significant drop in GFR with high sensitivity and specificity.

Our study had several shortcomings. First, it was a single-center study carried out on a small group of white patients and restricted to only 2 years of follow-up. Thus, our conclusions might have only a preliminary character and should be confirmed by a study in a larger cohort representative of the general kidney transplant population. After that, prospective interventional trials could be undertaken to determine whether monitoring strategies based on urinary AST in the late period posttransplant could improve long-term KAG outcomes. Second, we did not measure GFR directly but relied on eGFR using the Modification of Diet in Renal Disease equation, which nonetheless is allowed in the recommendations of recent guidelines.²⁴ Creatinine measurements were not calibrated with respect to a reference laboratory. However, the effect of possible calibration errors would have equally influenced the results of measurements in all patients and therefore cannot explain the observed differences in eGFR values. Third, we could only assume the cause of KAG dysfunction and could not differentiate between glomerular and tubulointerstitial diseases because allograft biopsy was performed in only 5 individual patients. It is therefore possible that combining urinary AST measurement with biopsy results could improve the diagnosis and prediction of kidney transplant pathology in future.

Conclusions

This study demonstrates that a higher urinary level of AST in the late posttransplant period independently predicts decline in KAG function within a short time frame, irrespective of graft functioning at the time of testing. Higher urinary AST activity at baseline independently related to several measures

of deterioration in KAG function over 2 years as a negative slope in eGFR and a rapid decline in eGFR of $> 5.0 \text{ mL/min/1.73 m}^2/\text{year}$, as well as decline in eGFR $\geq 25\%$ after 1 and 2 years of follow-up. With respect to its discriminative characteristics, urinary AST predicted with fair accuracy an eGFR drop of $\geq 25\%$ after 1 and 2 years and a decline in eGFR of $> 5 \text{ mL/min/1.73 m}^2/\text{year}$ over 2 years. When combined with time after transplant, urinary AST has good power for predicting eGFR drop $\geq 25\%$ after 2 years. Thus, higher urinary AST is a risk factor for progression of KAG dysfunction. These observations highlight the potential importance of elevated urinary AST in the late posttransplant period for identifying transplant patients who are at risk for progressive loss of graft function and might benefit from early allograft biopsy and subsequent therapeutic interventions.

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