Urinary neutrophil gelatinase-associated lipocalin as graft dysfunction predictor at one year of kidney transplantation

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Abstract

Introduction: Recent studies suggest that urinary neutrophil gelatinase-associated lipocalin (uNGAL) is superior to creatinine for renal graft dysfunction early detection, but there are only few studies assessing its usefulness as long-term predictor of said function. **Objective:** To explore if uNGAL values within the first 48 hours after kidney transplantation predict graft function on the long term. **Method:** Prospective cohort, where uNGAL values were assessed at 2, 12, 24 and 48 hours post-kidney transplantation. **Results:** Seventy-nine kidney transplant recipients were evaluated. At one year of follow-up, 30.4% of patients had graft dysfunction. No statistically significant differences were found between the uNGAL values and the renal graft function at one year ($p = 0.65$); the multivariate analysis showed that no uNGAL value was a predictor marker of graft dysfunction at one year of kidney transplantation. **Conclusion:** The uNGAL values obtained within the first 48 hours post-transplant were not associated with graft dysfunction at one year of kidney transplantation.

KEY WORDS: Lipocalin. Graft loss. Kidney transplantation.

Introduction

Delayed graft function (DGF), defined as the need for renal replacement therapy during the first 7 days post-transplant or serum creatinine not decreasing $< 10\%$ for 3 consecutive days,¹ is one of the most common complications of kidney transplantation;² it originates due to the ischemia-reperfusion injury that occurs after kidney transplantation and has been shown to be a risk factor for acute rejection and renal graft acute nephropathy that may favor its loss within the first year.³⁻⁶ Different therapeutic approaches to prevent DGF have been explored; however, successful options in animal models have not been reproduced in humans^{3,7} owing to late identification of this injury,^{3,8-10} since current biomarkers for its diagnosis (which include serum creatinine

and urinary output) are poorly sensitive for its prediction.¹¹⁻¹⁴

There is a current need for more sensitive and specific markers that enable DGF and subclinical kidney injury early diagnosis,¹⁵ in order for therapeutic interventions that increase renal graft survival to be established.^{3,16} Recent studies suggest that urinary neutrophil gelatinase-associated lipocalin (uNGAL) could be superior to serum creatinine for DGF early detection, since its elevation can occur within the first 12 hours after kidney transplantation in patients with subclinical dysfunction.^{3,5,7,16-26} However, there are only few studies that have assessed uNGAL usefulness as a long-term predictor of renal graft function, and it is not clear whether its values can predict dysfunction.^{2,18,21,27,28}

At Hospital Pablo Tobón Uribe, in Antioquia, Medellín, Colombia, deceased donor transplants, where the

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prevalence of DGF is higher, are predominant, and a higher prevalence of subclinical kidney injury is therefore to be expected. We consider that uNGAL might be a good prognostic marker to predict renal function at one year of kidney transplantation. In one study previously conducted by our group, uNGAL value 48 hours post-kidney transplantation was found to be a DGF early predictor; however, it turned out not to be a better predictor when compared with serum creatinine.²⁹

In this article, we describe the results of a cohort of patients followed for one year with the purpose to explore whether uNGAL values within the first 48 hours after kidney transplantation predict long-term graft function.¹⁸

Method

This was a prospective cohort-type study. The cohort was initially established in order to assess the relationship of uNGAL values obtained at 1, 12, 24 and 48 hours from kidney transplantation and the presence or not of DGF.²⁹ Subsequently, for the performance of this second study, the cohort follow-up was continued for a one-year period after the kidney transplantation.

This study was carried out at the Pablo Tobón Uribe Hospital. Patients older than 12 years diagnosed with end-stage chronic kidney disease (CKD) who received a kidney transplantation between November 2012 and December 2013 were included. Patients with anuria within the first hour after kidney transplantation were excluded. Induction treatment used in this cohort consisted of basiliximab 20 mg on days 0 and 4 in low immune risk patients or 1 mg/kg/day of thymoglobulin for 3 to 5 days in patients with high immune risk and 3 methylprednisolone boluses. Maintenance treatment consisted of a triple immunosuppression regimen with prednisolone, an antiproliferative agent (mycophenolate or azathioprine) and a calcineurin inhibitor (tacrolimus or cyclosporine). All patients were followed-up during the year following the kidney transplantation by nephrologists blinded to the uNGAL values.

Primary outcome measure was defined as a glomerular filtration rate (GFR) < 60 mL/minute/1.74 m² body surface area (BSA) as estimated with the CKD-EPI equation³⁰ during the first year after the kidney transplantation. Serum creatinine values for the above estimate were measured during patient monthly follow-up during the first year post-kidney transplantation. As exposure variable, uNGAL values obtained at 1, 12, 24 and 48 hours post-kidney transplantation were used. Other assessed clinical parameters included gender,

age, chronic kidney disease (CKD) etiology, history of kidney transplantation and dialysis prior to transplant, cold and warm ischemia time, immunosuppressant treatment and presence of DGF; the latter was defined by the need for dialysis in the first week post-kidney transplantation or serum creatinine decrease $< 10\%$ for 3 consecutive days or a serum creatinine value > 3 mg/dL at day 5 post-kidney transplantation.

Data source, laboratory procedures

10 mL of urine were collected from each patient directly from the bag attached to the urinary catheter that was permanent during the first post-transplantation days; urine that was previously contained in the bag was previously discarded in order to collect fresh urine. Samples were taken at 1, 12, 24 and 48 hours post-transplant and sent to the hospital laboratory in less than 1 hour from collection, where they were centrifuged at 400 revolutions per minute and stored at -70°C until they were processed. uNGAL determination was performed by means of turbidimetric immunoassay, from the Sentinel Diagnostics manufacturer (REF 1P9300), on Abbott's Architect® i8000 platform. The samples were processed within a period of < 3 months since the take. Serum creatinine was routinely measured by enzymatic method, daily during the first week post-transplant, and monthly thereafter over the following 12 months.

Sample size and power

A time-to-event model was used, with uNGAL values within the first 48 post-transplant hours being regarded as the exposure variable and GFR < 60 mL/minute/1.73 m² BSA in the first post-transplant year as the outcome variable of interest.³¹

Given that a cohort with a defined sample size (79 patients) was analyzed, the power to detect the risk ratio (RR) for graft dysfunction was estimated according to the uNGAL values in the first 48 hours post-transplant. The assumptions used were: RR of 2;^{29,32,33} expected proportion of transplanted patients with graft dysfunction: 35 %.^{10,22} According to the above assumptions, and with an alpha error of 0.05, this cohort had a power of 0.881 to identify a RR of 2 with a GFR < 60 mL/minute/1.73 m² BSA during the first year post-transplantation in patients with elevated uNGAL values.

The patients were grouped according to the primary outcome measure: presence or not of graft dysfunction at one year of kidney transplantation; whether the

uNGAL values predicted renal graft dysfunction was explored in both groups.

Statistical analysis

Descriptive statistics was used, and the distribution of variables was verified by means of the Kolmogorov-Smirnov test. Quantitative variables were expressed as means and standard deviation or medians and interquartile ranges, according to data distribution; qualitative variables were analyzed as proportions. Dispersion diagrams were plotted between the uNGAL values of the first 48 hours and GFR by CKD-EPI one year post-kidney transplantation; subsequently, the correlation between both these variables was assessed using Pearson's correlation coefficient (r).

The discriminatory capacity of the different uNGAL values for the detection of renal graft dysfunction during the first year following transplantation was explored and a Kaplan-Meier survival analysis was carried out. A Cox proportional risks model was also constructed.

Ethical aspects

This study was approved by the Pablo Tobón Uribe Hospital ethics and research committee, and the regulations on ethical aspects of research in human subjects contained in resolution 008430, issued by the Ministry of Health of Colombia in 1993, were followed; in addition, the confidentiality of included patients' personal data was preserved. On the other hand, this study did not pose any risks for patients, since blood tests were part of the kidney transplantation protocol and the uNGAL samples were directly obtained from the collection bag attached to the permanent urinary catheter patients had after kidney transplantation.

Results

Between November 2012 and December 2013, 83 kidney transplantations were carried out at Pablo Tobón Uribe Hospital; 2 patients were excluded owing to anuria in the first hours and 2 because they were younger than 12 years, with 79 patients being left. One patient died during follow-up; however, his GFR was normal at the moment of death, and he was therefore included in the group of patients. All patients received deceased donor kidney transplantation, but none included any of the extended criteria (donor > 60 years of age or between 50 and 60 years of age with at least two of the following criteria: history of high blood pressure, serum creatinine

levels > 1.5 mg/mL or with stroke as cause of death). Most patients ($n = 44$, 55.7 %) were males, mean age was 39.6 ± 4.18 years; for 91.1 % ($n = 72$) it was their first transplantation, and 82.3 % ($n = 65$) were on dialysis prior to the transplantation, with a median of 23.5 months (interquartile range [IQR] = 4-48). Previous transfusions had been received by 51.9 % ($n = 41$), and cold ischemia time had a mean of 15.04 ± 5.84 hours. DGF occurred in 13 patients (16.5 %), with 5 of them (6.3 %) requiring dialysis during the first week. At one-year follow-up, 24 patients (30.4 %) had renal graft dysfunction; demographic characteristics, grouped by presence or not of renal graft dysfunction are shown in Table 1.

No correlation was found between 1 year post-transplant GFR by CKD-EPI and uNGAL values at 1, 12, 24 and 48 hours ($r = 0.14$, $p = 0.2$; $r = 0.05$, $p = 0.66$; $r = 0.06$, $p = 0.61$; $r = 0.11$, $p = 0.34$, respectively); neither was another pattern of behavior observed (Fig. 1).

Figure 2 shows the ROC curves and their respective areas under the curve for each uNGAL value at 1, 12, 24 and 48 hours post-transplant with regard to renal graft dysfunction outcome at one year (0.569 [95% CI = 0.424-0.715], 0.542 [95% CI = 0.406-0.678], 0.55 [95% CI = 0.398-0.702] and 0.55 [95% CI = 0.408-0.692], respectively).

uNGAL values behavior

Seventy-five percent ($n = 59$) of patients had an uNGAL drop higher than 50 % in the first 48 hours, 15 % ($n = 12$) had a ≤ 50 % decrease, and in 8 patients (10 %) uNGAL values were increased with regard to the first measurement. The percentage of patients free of renal graft dysfunction at 6 and 12 months post-transplant according to uNGAL levels behavior was 88 and 71 % in the group with > 50 % drop; 74 and 65 % in the group with < 50 % decrease, and 62 and 62 %, respectively, in the group where uNGAL values were increased (Fig. 3) (log-rank test $p = 0.65$).

The Cox proportional risk model according to uNGAL behavior 48 hours post-transplant showed no statistically significant association with renal graft dysfunction occurrence one year after transplantation (Table 2).

Among the patients with graft dysfunction ($n = 24$), 16.7 % ($n = 4$) had previously developed DGF, in comparison with 16.4 % (9/54) in patients with GFR > 60 mL/minute/1.73 m² BSA ($p = 0.6$).

Discussion

In this cohort of 79 patients with kidney transplantation, all from deceased donors, uNGAL values in the

Table 1. Descriptive analysis grouped by renal graft dysfunction outcome during the first year post-transplant

Variable	GFR $\leq$ 60 mL/minute (n = 24)		GFR > 60 mL/minute (n = 55)	
	n	%	n	%
Male gender	8	33.3	36	65.5
History of previous transfusions	19	79.2	46	83.6
Second transplantation	0	0	7	12.7
Previous dialysis	19	79.2	46	83.6
Induction with basiliximab	5	20.8	20	36.4
Induction with thyroglobulin	19	79.2	35	63.5
Delayed renal graft function	4	16.7	9	16.4
Need for dialysis in the first week after kidney transplantation	1	4.2	4	7.3
Acute rejection in the first year following transplantation	10	41.7	6	10.9
	Mean $\pm$ SD		Mean $\pm$ SD	
Age at transplantation (years)	40.67 $\pm$ 15.07		39.07 (13.88)	
Donor's age (years)	35.67 $\pm$ 15.78		27 $\pm$ 11.28	
Donor's creatinine (mg/dL)	0.98 $\pm$ 0.34		0.84 $\pm$ 0.27	
Cold ischemia time (hours)	14.54 $\pm$ 6.08		15.13 $\pm$ 5.80	
Warm ischemia time (hours)	33.75 $\pm$ 7.11		32.81 $\pm$ 7.51	
DR incompatibility	1.5 $\pm$ 0.5		1.2 $\pm$ 0.6	
HLA total incompatibility	4.9 $\pm$ 0.8		4.2 $\pm$ 1	
Post-kidney transplantation GFR (mL/minute/1.73 m ² BSA)				
At 5 days	56.93 $\pm$ 26.62		58.59 $\pm$ 32.52	
At 6 months	53.37 $\pm$ 24.25		68.22 $\pm$ 19.56	
	Median (IQR)		Median (IQR)	
Post-kidney transplantation creatinine (mg/dL)				
Day 1	7.73 (5.4-13.6)		8.8 (6.6-12.6)	
Day 2	5.16 (3.34-6.41)		6.1 (3.32-8.79)	
Day 3	1.68 (0.94-2.22)		1.7 (1.14-2.59)	
Day 4	1.34 (0.92-1.89)		1.4 (1.05-1.9)	
Day 5	1.25 (0.97-1.77)		1.19 (0.95-1.68)	
Day 30	1.27 (1.1-2.16)		1.27 (0.99-1.52)	
Post-kidney transplantation uNGAL (ng/mL)				
At 1 hour (n = 79)	301.5 (131-1180.4)		677 (270-1203)	
At 12 hours (n = 78)*	185.9 (74.6-546.5)		279.9 (87.1-686.9)	
At 24 hours (n = 78)*	115.3 (24.65-494.3)		212 (60.6-446.3)	
At 48 hours (n = 77)	47.65 (14.35-189.6)		59 (22.3-215.8)	

GFR=glomerular filtration rate, IQR=interquartile range.

*One female patient had thrombosis and renal graft loss at first hour of transplantation and only taking the first uNGAL sample was possible.

first 48 post-transplant hours were not shown to be predictors of graft insufficient function at 12 months of follow-up. Neither was a correlation found between uNGAL values and GFR at one year of kidney transplantation or could a cutoff point be established for uNGAL that was able to discriminate patients with graft dysfunction.

Currently, there is no biomarker that enables early predicting renal graft long-term result. uNGAL has been proposed as a useful biomarker for DGF early detection,^{19,28,34-36} however only few studies have assessed its role as a long-term biomarker of renal graft dysfunction.³⁷ We considered useful to explore whether uNGAL values in kidney transplantation recipients

Figure 1. uNGAL values dispersion plots in the first 48 hours post-transplant and glomerular filtration rate values estimated by the CKD-EPI formula at 1 year of kidney transplantation.

Figure 2. uNGAL first 48-hour values ROC curves.

with no evidence of DGF can predict long-term renal graft dysfunction; this might suggest that subclinical renal injury not diagnosed by serum creatinine values but rather by uNGAL elevated levels might have an impact on long-term renal graft function, regardless of

the presence or not of DGF and would enable treatment strategies to be established in order to prevent or limit renal damage before it becomes irreversible.⁵

Different studies have suggested that uNGAL values measured within the first 48 hours after kidney

Table 2. Cox proportional risk model to predict renal graft dysfunction at 1-year post-transplant according to uNGAL behavior

	RR	95% CI	p
uNGAL* decrease > 50	1	Reference	
uNGAL* decrease < 50	1.412	0.474-4.205	0.535
uNGAL* increase	1.59	0.466-5.439	0.459

*During the first 48 hours after kidney transplantation.

RR=risk ratio, CI=confidence interval.

Figure 3. Kaplan-Meier curve for graft survival estimation according to uNGAL behavior in the first 48 hours.

transplantation predict long-term renal graft function. Hall et al. measured the uNGAL values at 0, 6, 12, 18, 24 and 48 hours post-deceased donor kidney transplantation in 92 patients. The patients with uNGAL values higher than percentile 66 had higher serum creatinine levels 3 months after kidney transplantation ($p = 0.004$).³ The same group carried out another study in 153 patients with kidney transplantation who had 24 renal graft losses and 13 dysfunctions during the 1-year follow-up. When uNGAL values were compared according to the presence or not of renal graft dysfunction, uNGAL values were higher in patients with this outcome in comparison with those without it (uNGAL 911 ng/mL [percentiles 25-75 = 133-3143], in contrast with 370 ng/mL [percentiles 25-75 = 29-2381], respectively [$p = 0.002$]). In the logistic regression analysis, uNGAL values measured on day 1 after kidney transplantation were significantly associated with renal graft poor function (OR = 6; 95 % CI = 1.5-24).²⁷ Choi et al., in a study carried out in 69 patients with kidney transplantation, found that uNGAL values were significantly higher in those patients with renal graft dysfunction one year after kidney transplantation; furthermore, in the multivariate logistic regression analysis, uNGAL values at post-transplantation day 2 were independent predictors of poor graft function (OR = 1.003; 95 % CI = 1.001-1.005);¹⁸ however, after two

years' follow-up, day 0, 2 and 6 post-kidney transplantation uNGAL values were not significantly associated with poor graft function.²⁸

Fonseca et al. assessed 42 patients; in the linear regression analysis they found that uNGAL values at day 7 post-transplant adjusted for a history of acute rejection and re-hospitalization were independently associated with serum creatinine values (regression coefficient = 0.138; 95 % CI = 0.041-0.235).²¹ Conversely, Rahizamdeh et al. observed that uNGAL values measured at days 1, 3 and 7 post-kidney transplantation were not associated with kidney dysfunction one year after transplantation,² which are similar findings to those in our study.

One possible explanation for the differences between the results of previous studies and ours is the type of outcome measure assessed. Hall et al. used a GFR < 30 mL/min/1.73 m² BSA at one year of transplantation to define graft dysfunction,³ Fonseca et al. used serum creatinine values at one year of kidney transplantation as outcome measure. Another important difference is the type of population. The presence of donors with extended criteria (> 60 years of age, or between 50 and 60 years of age with at least two of the following criteria: high blood pressure, creatinine levels > 1.5 mg/dL or stroke as cause of death) is associated with higher risk for renal graft loss.³⁸ This is explained because renal response to the ischemia-reperfusion injury after kidney transplantation is a balance between repair and fibrosis processes: in the presence of severe injury, progressive fibrosis and renal graft scarring takes place; in mild cases or in the presence of suitable quality grafts, renal epithelial cell recovery is achieved without importantly affecting the graft function.

In our cohort, there were no extended criteria donors; all of them were young and had no associated comorbidities or kidney dysfunction during the process of death (donor serum creatinine prior to extraction: 0.9 ± 0.4 mg/dL), which might explain the lower frequency of subclinical injury or the successful recovery of renal grafts from ischemia-reperfusion injury. In contrast, the percentage of donors with extended criteria in Hall and Choi studies was 18 and 30.9 %, ^{3,18} respectively. In addition, donors' age in the different studies ranged from 42 to 51 years,^{3,18,21,27} whereas mean age of our population's donors was 29.6 ± 14.18 years.

Among the limitations of our study, the sample size and the low proportion of patients with the outcome of interest stand out, which suggests that there was no sufficient power to find statistically significant differences and to estimate the effect of potential confounding variables such as cold ischemia time, the induction

therapy used and patient adherence to the treatment protocols, which may influence on kidney function on the short and long term.

Conclusion

In our cohort of patients with kidney transplantation from donors that met optimal criteria, uNGAL values in the first post-transplant hours were not associated with long-term graft dysfunction. Further investigations are required in order to identify the true role played by uNGAL values in long-term graft function, taking into account other populations at risk for DGF and kidney dysfunction.

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Conflicts of interest

We declare that none of the authors has any conflicts of interest.

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