Neutrophil Gelatinase-Associated Lipocalin (NGAL) – a potential biomarker for early diagnosis of acute rejection in renal transplantation

A. Moise[1, 2], B. Şerbănescu[2, 3], C. Gîngu[3], I. Constantinescu[1, 2]

[1] *Centrul de Imunogenetică şi Virusologie, Institutul Clinic Fundeni, Bucureşti*

[2] *Universitatea de Medicină şi Farmacie „Carol Davila", Bucureşti*

[3] *Centrul de Uronefrologie şi Transplant Renal, Institutul Clinic Fundeni, Bucureşti*

Abstract

Background: Early diagnosis of kidney allograft dysfunction and treatment of subclinical rejection is crucial to the management and long-term survival of the transplanted kidney. in our study, we sought to assess whether NGAL represented a novel, sensitive marker of kidney function in adult patients with kidney transplantations.

Material and methods: We have evaluated thirty-eight patients (23 men and 15 women) who underwent kidney transplantation in 2012 in Fundeni Clinical Institute. The study design consist into two groups according to donor source: first group (A) have included patients who had living-related donor and the second group (B) recipients who received kidney harvested from cadaveric donor. Plasma NGAL concentrations was performed from samples collected prior transplantation and then on days 3, 10 and 30 after transplantation, using commercial kits (AntibodyShop, Gentofte, Denmark). Serum creatinine was assessed at the same time, using an automated analyzer Dimension (Siemens, Germany).

Results: Pretransplant, NGAL values are significantly higher, closely correlated with serum creatinine. Posttransplantation, higher levels of NGAL were obtained on days 3 and 10, especially in group B. No significant difference between the two groups have been noted one month after transplantation. Also, five recipients had elevated NGAL plasma levels (>350ng/ml) a few days before the acute rejection occurrence.

Conclusions: NGAL could be considered a sensitive biomarker of renal function. Routine measurement of serum/urinary NGAL could increase the chances of early diagnosis of acute rejection, even subclinical rejection, allowing promptly institution of therapeutic measures for the protection of renal function, thereby improving prognosis.

Key words: acute rejection, kidney allograft dysfunction, lipocalin, NGAL, renal transplant

Corespondenţă: Bogdan Şerbănescu
Institutul Clinic Fundeni, Centrul de Chirurgie Urologică şi Transplant Renal
Şoseaua Fundeni nr. 258, Sector 2, 022328, Bucureşti
Tel./Fax: 021-300.75.70.
E-mail: bgdnserbanescu@gmail.com

Background

Great improvements in surgical techniques and harvested kidneys preservation, the progress achieved in the pretransplant immunological assessment in both, the recipient and the donor, in order to establish the best histocompatibility matching, the availability of new, more potent immunosuppressive therapies, have made kidney transplantation the most effective therapeutic choice for patients with end-stage renal disease.

Acute kidney injury (AKI) is a common medical problem among kidney transplant recipients, which may have a significant impact on patient and allograft survival. Early diagnosis of kidney allograft dysfunction and treatment of subclinical rejection is crucial to the management and long-term survival of the transplanted kidney [1-3]. Acute kidney injury in allografts can result from several different etiologies: acute tubular necrosis manifested as delayed graft function (DGF) or slow graft function, acute rejection (AR) or drug toxicity. Allograft function after kidney transplantation is commonly monitored by measuring serum creatinine concentration. However, serum creatinine concentrations are greatly affected by numerous nonrenal factors, including gender, age, body weight, muscle metabolism and protein intake [4]. On the other hand, increase of serum creatinine in acute graft disfunction is a late sign of kidney damage, associated or not with other symptoms such as reduced urine output. A marked reduction in glomerular filtration rate can be present and up to 50% of kidney function has already been lost before serum creatinine increase. Rise of creatinine level can be relevant for rejection and reduction of nephron mass, provided that other causes of graft dysfunction are excluded (infection, dehydration, ureteric obstruction, immunosuppressants toxicity, renal artery stenosis).

Currently, an allograft biopsy remains the „gold standard" for assessing the cause of impaired kidney function. Limitations of the allograft biopsy include the risk of bleeding, infections, injury to the adjacent viscera, and the possibility of sampling error leading to an inadequate diagnosis.

In this context, in the last years, clinical and laboratory scientists have been searching for noninvasive tools that would allow early and accurate diagnosis of acute kidney allograft dysfunction without performing a kidney biopsy [5]. More than 20 different biomarkers, including neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18), kidney injury molecule-1(KIM-1), cystatin C or liver fatty acid-binding protein (L-FABP), have been assessed in recent years, in human populations and clinical results are promising [6-15].

Considering these data, in our study, we sought to assess whether NGAL represented a novel, sensitive marker of kidney function in adult patients with kidney transplantations.

Material and methods

We have evaluated thirty-eight patients (23 men and 15 women) who underwent kidney transplantation in 2012 in Fundeni Clinical Institute. The study design consist into two groups according to donor source: first group (A) have included patients who had living-related donor and the second group (B) recipients who received kidney harvested from cadaveric donor. Histocompatibility matching was assessed by group-specific HLA genotyping for class I (A and B) and class II (DRB1) loci. In both groups, in most cases, HLA matching for A, B and DRB1 loci was 50% (for each locus tested, the pairs had a common allele - three HLA-mismatch- 3MM). In group B, four pairs had less than 50% compatibility (4MM), while in group A there was a case where recipient and donor were brothers and they had 100% compatibility. Concerning pretransplant anti-HLA immunization in four recipients in group A and two in group B cytotoxic antibodies screening was positive but PRA (Panel Reactive Antibody) was up to 10% and antibodies had no specificity against donor HLA antigens (crossmatch test performed by ELISA were negative in all cases). The distribution by sex, age, HLA compatibility, hemodialysis period into two groups is detailed in Table 1.

Table 1. *Baseline characteristics of the two groups (SD – standard deviation)*

Characteristics	**Group A (Living donor) n=20**	**Group B (Cadaveric donor) n=18**
Sex		
M	10	13
F	10	5
Mean age (years)±SD	35,5 ± 6,75	37 ± 7,25
HLA compatibility		
4 MM	0	4
3MM	17	14
2MM	2	0
0MM	1	0
Cytotoxic antibodies		
Class I	1	0
Class II	1	1
Class I and II-a	2	1
Mean time of hemodialysis (months) ±SD	20 ± 10,4	5,2 ± 3,7

After transplantation, all patients received anti-CD25 monoclonal antibodies as induction treatment and then, triple therapy with Tacrolimus (T), mycophenolate mofetil (MMF), and prednisolone (Pred) was adopted as the immunosuppressive maintenance protocol.

Plasma NGAL concentrations was performed from samples collected prior transplantation and then on days 3, 10 and 30 after transplantation, using commercial kits (AntibodyShop, Gentofte, Denmark). The assay is a sandwich ELISA performed in microwells coated with a monoclonal antibody against human NGAL. Bound NGAL is detected with another monoclonal antibody labeled with biotin and the assay is developed with horseradish peroxidase (HRP)-conjugated streptavidin and a color-forming substrate. The optical density is measured with a spectrophotometer, the value is fitted on a calibration curve and we can get NGAL concentration.

Serum creatinine was assessed at the same time, using an automated analyzer Dimension (Siemens, Germany).

Results

Prior transplantation, the serum creatinine range was between 5.6 and 15.1 mg/dL (mean 9.95 mg/dL, normal range = 0,9 – 1,1 mg/dL) and NGAL range between 295 and 678 ng/mL (mean 392 ng/mL, normal range = 37 – 106 ng/mL). Posttransplantation, higher levels of NGAL were obtained on days 3 and 10, especially in group B, in recipients who received kidney from cadaveric donor. No significant difference between the two groups have been noted one month posttransplantation (see Table 2).

Table 2. *Time course changes in plasma NGAL and serum creatinine in patients undergoing kidney transplantation (M – means, SD – standard deviation)*

Group	Plasma NGAL (ng/mL) - M±SD			Serum creatinine (mg/dL) - M±SD		
	day 3	day 10	day 30	day 3	day 10	day 30
Group A	387,3±192,4	312,3±167	163,7±137,7	2,04±0,7	1,76±0,83	1,48±0,39
Group B	478,8±210	358,7±167	171,6±122,2	2,90±1,99	1,73±0,96	1,36±0,24

Discussion

An ideal biomarker for acute kidney injury (AKI) would be highly sensitive and specific, responding consistently and rapidly to injury, with levels that correlate to severity, as well as having biological stability and a reliable, quick and cost effective assay for detection [16,17].

Human Neutrophil Gelatinase Associated Lipocalin (NGAL) is one of the most extensively studied novel biomarkers both in AKI and in kidney transplant dysfunction. NGAL is a novel 25-kD protein of human neutrophils, that is in part covalently complexed with neutrophil gelatinase. It is freely filtered by the glomerulus and completely absorbed by the proximal tubule.

In patients with normal renal function, NGAL is almost undetectable in either urine or plasma, yet animal studies clearly demonstrated that NGAL is markedly elevated early following ischemic injury [18]. The early appearance of urinary NGAL in animals with AKI, hours to days before other biomarkers has established NGAL as an early biomarker following various acute and chronic kidney injuries [19-21].

In our study, we have get results that overlap those in the literature. Pretransplant, NGAL values are significantly higher (average about three times higher than the maximum of normal value) reflecting chronic renal impairment and a reduction in glomerular filtration rate. NGAL plasma levels were closely correlated with serum creatinine. Posttransplant, in most cases, NGAL plasma levels gradually decreased, but they have still remained over normal value during the first two weeks. NGAL normal levels occurred just one month posttransplantation. Moreover, Malyszko et al. have been reported similar results [22].

The much higher NGAL plasma levels on the third day, in patients from group B, with cadaveric donors, may be explained by more extensive renal damage, in these cases, due to prolonged cold ischemia time, compared with recipients of allograft from living donors (mean 11h vs. 30 min.). Two patients in group B had delayed graft function (DGF), with the maintenance of elevated serum creatinine concentrations (up to 5.7 and 6.5 mg/dL) until the fifth and respectively seventh day. In fact, in these recipients, have recorded the highest NGAL plasma levels on the third day (735.4 and respectively 801.3 ng/mL).

Also, five of eight recipients (two in group A and three in group B) who, on tenth day posttransplantation, had serum NGAL concentrations over 300ng/mL, in the following days showed increases of serum creatinine. One of the patients was the one who had four HLA mismatches and the others were among anti-HLA class II presensitized recipients. In the absence of biopsy, based on the promptly response to methylprednisolone pulse-therapy, clinical symptoms and laboratory features have been interpreted as acute rejection.

In conclusion, NGAL could be considered a sensitive biomarker of renal function. Its plasma and urine levels are greatly increased in patients with chronic renal failure and in acute kidney allograft dysfunction after kidney transplantation being strongly correlated with serum creatinine levels. Our results, like other studies, show that, in renal transplantation, NGAL appears to be an early marker of acute rejection. For these reasons, routine measurement of serum/urinary NGAL at least weekly in the first three months posttransplantation, when there is a higher risk of acute rejection, could be included in the monitoring protocol of the kidney allograft recipients toghether with serum creatinine and cytotoxic antibodies. In this way, could increase the chances of early diagnosis of acute rejection, even subclinical rejection, allowing promptly intervention of therapeutic measures for the preservation of renal function and thus, improving prognosis and quality of life of the transplanted patients.

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Rezumat

Introducere: Diagnosticul precoce al disfuncţiei alografei renale şi tratamentul rejetului subclinic reprezintă un obiectiv crucial în managementul şi supravieţuirea pe termen lung a rinichiului transplantat. În studiul nostru, am căutat să vedem în ce măsură NGAL poate reprezenta un marker sensibil al funcţiei renale la pacienţi adulţi transplantaţi renal.
Material şi metode: Studiul a fost efectuat pe 38 de pacienţi (23 de bărbaţi şi 15 femei) care au fost transplantaţi renal în Institutul Clinic Fundeni în anul 2012, dintre care 20 au avut donator viu înrudit (lotul A) iar la 18, transplantul a fost efectuat cu rinichi prelevaţi de la donatori în moarte cerebrală (lotul B). Determinarea concentraţiei plasmatice a NGAL a fost realizată din probe recoltate pretransplant şi apoi posttransplant în zilele 3, 10 şi 30, folosind kit-uri comerciale (AntibodyShop, Gentofte, Danemarca). În acelaşi timp a fost măsurată şi concentraţia serică a creatininei folosind analizorul automat Dimension (Siemens, Germania).
Rezultate: Valorile NGAL sunt semnificativ crescute pretransplant în corelaţie strânsă cu valorile creatininei serice. Posttransplant, valori mai mari ale NGAL s-au obţinut în zilele 3 şi 10, mai ales în lotul B. La o lună posttransplant nu se observă diferenţe semnificative între cele două loturi. De asemenea, la cinci primitori s-au înregistrat nivele crescute peste 350ng/ml cu câteva zile înaintea apariţie rejetului acut.
Concluzii: NGAL poate fi considerat un biomarker sensibil al funcţiei renale. Determinarea periodică a concentraţiilor plasmatice/urinare a NGAL ar putea creşte şansele unui diagnostic cât mai precoce al rejetului acut, încă din stadiu subclinic, permiţând astfel instituirea unor măsuri terapeutice prompte pentru protecţia funcţiei renale, îmbunătăţind astfel prognosticul.

Cuvinte cheie: disfuncţie de grefă renală, lipocalin, NGAL, rejet acut, transplant renal