



Original article

Neutrophil gelatinase-associated lipocalin (NGAL) in heart transplant recipients after conversion to everolimus therapy



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ABSTRACT

Background: Due to the lack of nephrotoxic activity, proliferation signal inhibitors (PSI) such as everolimus are recommended for immunosuppression after heart transplantation, but the assessment of renal function in patients receiving PSI has led to conflicting results. We examined renal integrity and function using neutrophil gelatinase-associated lipocalin (NGAL) and conventional markers [plasma creatinine, cystatin C, urine albumin, α_1 -microglobulin (α_1 M)] in heart transplant patients, who underwent conversion to everolimus due to allograft vasculopathy, graft rejection episodes, or renal function deterioration, and in patients maintained on calcineurin inhibitors (CNI).

Methods: This cross-sectional study included 121 consecutive heart transplant recipients: 44 patients received CNI-free immunosuppressive therapy with everolimus and 77 patients received CNI. Renal parameters were determined in plasma and urine samples using standard enzymatic or immunochemical methods.

Results: Heart transplant recipients receiving everolimus therapy had significantly lower NGAL concentrations in plasma [median (95% CI): 128 (97–176) ng/mL vs. 252 (224–283) ng/mL, $p < 0.001$] and urine [median (95% CI): 6.4 (4.5–7.6) ng/g vs. 15.7 (10.2–25.9) ng/g creatinine, $p < 0.001$]. In contrast, no significant differences were observed between everolimus- and CNI-treated groups with regard to creatinine and cystatin C, as well as urine albumin and α_1 M levels. Significant correlations were noted between plasma NGAL and creatinine ($r = 0.42$, $p < 0.001$), cystatin C ($r = 0.44$, $p < 0.001$), N-terminal brain natriuretic propeptide ($r = 0.31$, $p < 0.01$) and indicators of chronic inflammation [lipoprotein-associated phospholipase A₂ (Lp-PLA₂), $r = 0.31$, $p < 0.01$] and soluble CD40 ligand (sCD40L, $r = 0.22$, $p < 0.05$), and between urinary NGAL and α_1 M ($r = 0.21$, $p < 0.05$). Multiple regression analysis indicated that cystatin C and Lp-PLA₂ were the best predictors of plasma NGAL.

Conclusion: The present study documents reduced plasma and urinary NGAL levels in the absence of differences in conventional renal parameters in patients on CNI-free immunosuppressive therapy with everolimus. These results support favorable effects of everolimus on renal integrity in heart transplant recipients.

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Introduction

Cardiac transplantation is now an established treatment for patients with end-stage heart failure. The introduction of

calcineurin inhibitors (CNI) into immunosuppressive therapy regimens has significantly improved the survival rate among heart transplant recipients. However, the long-term outcome after heart transplantation is adversely affected by CNI-induced nephrotoxicity, which leads to progressive renal deterioration and ultimately to end-stage renal disease. Proliferation signal inhibitors (PSI), such as everolimus or sirolimus, exert immunosuppressive effects by blocking the growth factor-driven proliferation of T and B cells [1]. The major advantage of PSI over CNI is

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their lack of intrinsic nephrotoxic activity. Accordingly, maintenance protocols that partially or fully replace CNI with PSI have been reported to alleviate renal impairment [2–4] and references therein). However, the conversion from CNI to PSI in heart transplant recipients is not uniformly effective, with a lack of improvement or even deterioration of kidney function observed in a few studies [2–4].

The overwhelming majority of previously published reports have used plasma creatinine concentrations and/or calculated creatinine clearance for the assessment of renal status after heart transplantation. Unfortunately, neither variable reliably reflects renal function, the marked deterioration of which can occur prior to an increase in serum creatinine levels. Neutrophil gelatinase-associated lipocalin (NGAL), also known as siderocalin, lipocalin-2, or LCN2, is a 25-kDa glycoprotein with bactericidal properties, which was initially isolated from activated neutrophils and subsequently identified in the epithelial cells of the alimentary and respiratory tracts, cardiomyocytes, and renal tubular cells [5,6]. Upregulated NGAL production has been observed in several inflammatory conditions, including inflammatory bowel disease, chronic obstructive pulmonary disease, and myocarditis [5–7]. In the setting of acute renal failure, serum and urine NGAL levels are elevated from 7- to 16-fold and from 25- to 100-fold, respectively [8]. Consequently, NGAL has been suggested as a sensitive biomarker of acute kidney injury [5,6,8]. However, increased NGAL concentrations have also been reported in chronic kidney disease, which presumably reflects the protracted damage to tubular cells irrespective of the glomerular filtration rate (GFR) [5,9–11]. Therefore, the determination of NGAL levels may aid the early detection of renal function changes that occur alongside chronic pathological conditions and can be overlooked using conventional indicators such as creatinine, cystatin C, or estimated GFR (eGFR).

Few previous observational studies have reported increased plasma NGAL concentrations in adult or infant heart transplant recipients [12–14]. However, the effect of CNI to PSI conversion in the course of the immunosuppressive therapy after heart transplantation on plasma or urine NGAL levels has not been examined to date. This current study reports the novel findings of reduced plasma and urine NGAL concentrations in heart transplant recipients who received a CNI-free immunosuppressive maintenance regimen that included everolimus.

Materials and methods

Patients, study design, and sample collection

The study was of a cross-sectional design. Ethics board approval was gained for the study protocol, and written informed consent was obtained from all patients. The examined cohort included 121 patients after cardiac transplantation recruited at the Department of Cardiothoracic Surgery, University Hospital Münster, between January 1990 and December 2010. Excluded patients included those temporarily treated in other cardiac surgery centers as well as heart–lung transplants, repeat transplants, pediatric transplants (<18 years), patients within their first year after transplantation, patients with a history of diabetes that required insulin, and patients with a diagnosed malignancy. All patients underwent yearly monitoring for cardiac allograft vasculopathy (CAV) with dobutamine stress echocardiography and single photon emission computed tomography. In case of a suspicious result in either examination, coronary angiography was subsequently performed. For the present study, patients were examined and blood and urine samples were taken during 2011 in consecutive visits to the outpatients department at the Department of Cardiology. At the time of examination, all patients were free

from acute rejection, acute heart failure, and clinical signs of infection. Initially, all patients received the maintenance immunosuppressive therapy, which consisted of CNI [tacrolimus or cyclosporine A (CsA)], mycophenolate mofetil, and/or a low-dose steroid (2–5 mg prednisolone daily). The steroid was withdrawn only if patients developed osteoporosis or diabetes. Beginning in April 2004, CNI was successively replaced by everolimus in 44 patients. The indications for conversion to everolimus included CAV (6%), repetitive episodes of graft rejection (6%), and a deterioration in renal function (88%). Serum creatinine levels before conversion to everolimus averaged 1.75 (1.50–1.99) mg/dL and decreased slightly but not significantly thereafter. At the time of the study, patients had been receiving everolimus for at least 6 months. The target trough levels for CsA, tacrolimus, and everolimus were 120–150 ng/mL, 6–8 ng/mL, and 4–6 ng/mL, respectively. Statins (with or without ezetimibe) were routinely given to all patients.

Blood and urine sample collection

Blood samples were collected from patients by venipuncture during control visits. Samples were centrifuged immediately after blood collection and plasma or sera were aliquoted and stored at -70°C until analysis. Overnight urine samples were collected into vials containing 5.0 mmol/L EDTA to prevent oxidation and were kept at -70°C until analysis.

Analytical procedures

Standard clinical chemistry parameters, including serum creatinine, cystatin C, and C-reactive protein (CRP) and urinary creatinine were determined at the Center for Laboratory Medicine using enzymatic, photometric, or immunoturbidimetric assays on a routine laboratory analyzer (ADVIA 1800, Siemens Healthcare Diagnostics, Eschborn, Germany). All interassay coefficients of variation (iCVs) were <6.8%. eGFR was calculated by converting creatinine (eGFR_{crea}) or cystatin C (eGFR_{cyst}) values in plasma using the modification of diet in renal disease (MDRD) formula [15], or the formula described by Hoek et al. [16]. Plasma concentrations of troponin I (TnI), N-terminal brain natriuretic propeptide (NT-proBNP), and interleukin-6 (IL-6) were measured using chemiluminescence immunoassays on laboratory analyzers (ADVIA Centaur XP or Immulite 2500, both Siemens). All iCVs were <12%. Urine concentrations of albumin and α_1 -microglobulin ($\alpha_1\text{M}$) were determined on a BN-II System nephelometer (Siemens) with iCVs <7.7%. Measurements of NGAL concentrations in the plasma and urine were performed using a turbidimetric immunoassay (BioPorto, Gentofte, Denmark) adapted on the ADVIA 1800 laboratory analyzer. The detection limit of the assay was 12 ng/mL and the iCVs were 3.6% and 2.5% at low (198 ng/mL) and high (497 ng/mL) concentrations, respectively. Lipoprotein-associated phospholipase A₂ (Lp-PLA₂) activities in the plasma samples were determined using a colorimetric assay (Cayman Chemical, Ann Arbor, MI, USA) using 2-thio-platelet activating factor as a substrate. Lp-PLA₂ activity was calculated from the slope of the kinetic absorption curve and expressed in $\mu\text{mol} \times \text{min}^{-1} \times \text{mL}^{-1}$. The iCVs were 5.1% for a low control and 3.8% for a control close to the sample mean. Soluble CD40 ligand (sCD40L) concentrations in plasma were determined using a commercially available ELISA kit (IBL, Hamburg, Germany).

Statistical analysis

Exploratory statistics were performed using the MedCalc Statistical Software version 12.7.7 (MedCalc Software bvba, Ostend, Belgium). The level of dependence between two categorical

variables was analyzed by the Chi-square test. The distribution of continuous variables was assessed for normality using the Shapiro–Wilk test. The Student’s *t*-test (2-sided) or Mann–Whitney *U*-test (2-sided) was used to compare groups with normally or non-normally distributed data, respectively. Spearman’s rank correlation was calculated to examine correlations between numerical variables. Stepwise multiple regression analysis was used to determine independent factors that affected NGAL concentrations in plasma and urine. Non-normally distributed data were Ln-transformed. The local significance level (*p*) was set to 0.05. For descriptive purposes, the relative rates (in percent) were provided for categorical variables and means (±SD) or medians (95% CI) were calculated for normally or non-normally distributed continuous variables, respectively.

Results

The general characteristics of groups treated with everolimus or CNI are shown in Table 1. Both groups did not differ significantly with regard to age and sex, as well as body mass index, systolic and diastolic blood pressure, and left ventricular ejection fraction (LVEF). Comparable percentages of patients in each group suffered from type 2 diabetes mellitus. The mean time elapsed from cardiac transplantation for the entire study population was 8.5 ± 5.6 years and there was no significant difference between both groups. The everolimus-treated group had received this treatment for 3.8 ± 1.8 years. The indications for transplantation were mainly coronary heart disease and dilated cardiomyopathy in the everolimus- and CNI-treated groups, respectively. Similar rates of patients in each group received mycophenolate mofetil and/or low-dose steroid therapy in addition to everolimus or CNI (CsA or tacrolimus). The observed trough concentrations of CsA, tacrolimus, and everolimus were within the target ranges. None of the patients examined was receiving sirolimus therapy or anti-oxidative drug therapy at the time of the study. No significant differences with respect to lipid-lowering, anti-hypertensive, or anti-coagulant therapy were present between both groups.

The distributions of variables that reflected renal function and integrity in heart transplant patients receiving immunosuppressive therapy with everolimus or CNI are shown in Table 2. No significant differences between the groups were detected with regards to plasma creatinine and cystatin C levels, as well as eGFR_{crea} and eGFR_{cyst}. Likewise, urinary α₁M concentrations and the urinary albumin/creatinine ratio, which reflect tubular and glomerular kidney injury, respectively, did not significantly differ between the everolimus- and CNI-treated groups. However, patients who received everolimus presented with significantly

Table 1
Baseline characteristics of the study population.

	Everolimus (n = 44)	CNI (n = 77)	p
Age (years)	57 ± 14	56 ± 15	0.75
Female gender	8 (18)	19 (25)	0.54
Body mass index (kg/m ²)	25.4 ± 4.6	24.9 ± 4.2	0.51
Diabetes	10 (22)	12 (16)	0.46
Pre-transplant condition			
- Ischemic cardiopathy	21 (48)	48 (62)	0.17
- Dilated cardiomyopathy	18 (40)	19 (25)	0.15
- Other	5 (12)	10 (13)	0.95
Time since transplant (years)	8 ± 4	9 ± 6	0.99
Time on everolimus (years)	3.8 ± 1.8	-	-
Systolic pressure (mmHg)	121 ± 10	124 ± 9	0.31
Diastolic pressure (mmHg)	77 ± 7	79 ± 8	0.19
Left ventricular ejection fraction (LVEF, %)	61.5 ± 12.1	62.1 ± 6.9	0.69
Medication			
- Everolimus	44 (100)	-	-
- Tacrolimus	-	56 (73)	-
- Cyclosporine	-	21 (27)	-
- Mycophenolate mofetil	32 (72)	60 (80)	0.67
- Azathioprine	1 (2)	5 (6)	0.61
Steroids	43 (98)	67 (87)	0.11
Lipid lowering	43 (98)	74 (96)	0.96
Anti-hypertensive	43 (98)	74 (96)	0.96
Observed trough concentrations			
- Everolimus (ng/mL)	5.2 ± 2.1	-	-
- Tacrolimus (ng/mL)	-	6.9 ± 4.1	-
- Cyclosporin A (ng/mL)	-	133.7 ± 71.5	-

According to treatment groups: data are given as mean ± SD or rates [relative rates (%) are shown in parentheses]. CNI (calcineurin inhibitors) include cyclosporine A or tacrolimus. Medication with steroids includes prednisone or prednisolone. Medication with lipid-lowering drugs includes simvastatin or fluvastatin or pravastatin or atorvastatin combined or not with ezetimibe. Medication with anti-hypertensive drugs includes β-blockers, angiotensin-converting enzyme inhibitors, sartans, or Ca²⁺-channel blockers.

lower plasma NGAL concentrations and urinary NGAL/creatinine ratios compared to the group that received CNI. Conventional biomarkers of systemic inflammation, such as plasma CRP and IL-6 levels, were comparable between both groups examined in this study. In contrast, heart transplant recipients that received everolimus showed significantly lower levels of plasma Lp-PLA₂ activity and sCD40L concentrations. These parameters reflect chronic inflammatory processes that are dependent on macrophage activation. Biomarkers that reflect cardiac integrity and function (TnI, NT-proBNP) were not significantly different between the everolimus- and the CNI-treated groups.

Table 2
Renal, cardiac, and inflammation markers in the study population.

	Everolimus (n = 44)	CNI (n = 77)	p
NGAL in plasma (ng/mL)	128 (97–176)	252 (224–283)	<0.001
NGAL in urine (ng/mg creatinine)	6.4 (4.5–7.6)	15.7 (10.2–25.9)	<0.001
Creatinine (mg/dL)	1.38 (1.27–1.53)	1.48 (1.28–1.71)	0.36
eGFR _{crea} (mL/min/1.73 m ²)	48.4 (43.8–57.5)	55.5 (44.5–59.6)	0.52
Cystatin C (mg/L)	1.72 (1.40–2.19)	1.72 (1.48–2.02)	0.90
eGFR _{cyst} (mL/min/1.73 m ²)	43.3 (32.3–53.1)	43.4 (35.4–50.0)	0.63
Albumin (mg/g creatinine)	69.5 (37.4–112.3)	56.9 (29.3–64.9)	0.65
α ₁ -Microglobulin (mg/L)	15.6 (11.4–24.3)	17.7 (12.9–24.9)	0.51
CRP (mg/dL)	0.68 (0.56–0.74)	0.64 (0.57–0.82)	0.34
IL-6 (ng/mL)	5.01 (3.83–6.55)	4.06 (3.35–4.90)	0.13
sCD40L (ng/mL)	0.81 (0.44–0.98)	1.21 (0.82–1.67)	<0.05
Lp-PLA ₂ (μmol/mL/min)	0.008 (0.007–0.009)	0.010 (0.009–0.011)	<0.001
NT-proBNP (mg/mL)	601 (396–1083)	737 (507–972)	0.79
Troponin I (ng/mL)	0.005 (0.002–0.008)	0.006 (0.004–0.010)	0.35

Data are given as median (95% CI). CNI (calcineurin inhibitors) include cyclosporine A or tacrolimus. NGAL, neutrophil gelatinase-associated lipocalin; eGFR, estimated glomerular filtration rate; Crea, creatinine; Cyst, cystatin C; CRP, C-reactive protein; IL, interleukin; sCD40L, soluble CD40 ligand; Lp-PLA₂, lipoprotein-associated phospholipase A₂; NT-proBNP, N-terminal brain natriuretic propeptide.

To further assess the reciprocal relationships between renal function and integrity markers, correlations between parameters that reflected renal function, systemic and local inflammation, as well as cardiac biomarkers were examined. As shown in Fig. 1, plasma NGAL concentrations significantly correlated with plasma levels of creatinine, cystatin C, $eGFR_{Crea}$, $eGFR_{Cyst}$, sCD40L, and NT-proBNP, as well as Lp-PLA₂ activity. In contrast, the urinary NGAL/creatinine ratio correlated solely with the urinary α_1 M concentration. Parameters that tended to correlate with NGAL ($p < 0.10$) were subsequently included in the stepwise multiple regression analysis. For circulating levels of NGAL, the best predictor was plasma cystatin C ($\beta = 0.32$, $p < 0.01$), which accounted for 11% of the plasma NGAL concentration. This was followed by Lp-PLA₂ ($\beta = 0.28$, $p < 0.01$), which explained 8% of the plasma NGAL concentration. Similar results were obtained when $eGFR_{Cyst}$ was substituted for plasma cystatin C. No predictors of urinary NGAL could be identified in the multiple regression analysis.

Discussion

As a result of the lack of intrinsic nephrotoxic activity, the use of PSI such as everolimus has been frequently recommended for immunosuppressive therapy after heart transplantation. However, the results of clinical studies that compared PSI with CNI in heart transplant recipients have been supportive, neutral, or even negative with regard to adverse effects on renal function after patients were switched to PSI-based regimens [2–4]. Prompted by the ongoing controversy regarding the conversion of CNI to PSI in heart transplant maintenance therapy, we assessed renal integrity and functional status in heart transplant patients who underwent conversion to everolimus or were maintained on CNI therapy. We assessed levels of NGAL, a sensitive indicator of early tubular injury, as well as conventional renal biomarkers (urine albumin, α_1 M, plasma creatinine, cystatin C, $eGFR_{Crea}$, and $eGFR_{Cyst}$). The novel finding was observed of lower NGAL concentrations in both the urine and plasma of patients receiving CNI-free therapy with everolimus. Importantly, reduced NGAL levels in everolimus-treated heart transplant recipients were determined in the absence of significant differences between both groups with respect to plasma creatinine, cystatin C, and $eGFR$, as well as urinary concentrations of albumin and α_1 M. These results could be interpreted as an indication of the beneficial effects exerted by everolimus, which were not adequately reflected by changes in conventional markers of renal integrity and function.

NGAL expression is markedly upregulated within the thick ascending limb of Henle and the collecting duct during ischemic or toxic renal injury, and the resulting release of NGAL into the urine accounts for the major fraction of urinary NGAL [5,6,9,17]. Accordingly, increased NGAL excretion into the urine has been observed in a variety of renal diseases (e.g. polycystic kidney disease, immunoglobulin A nephropathy, human immunodeficiency virus nephropathy), in which it closely reflects the magnitude of renal tubular injury [18–20]. In the setting of chronic heart failure (CHF), elevated levels of urinary NGAL paralleled by the increased excretion of kidney injury molecule-1 and N-acetyl- β -D-glucosaminidase, sensitive and specific markers of tubular damage, have been observed in several studies [21–24]. Importantly, increased NGAL excretion could be used to predict unfavorable outcomes, even in patients with normal renal function as assessed by plasma creatinine levels, $eGFR$, and the concentration of urinary albumin [23]. These findings were corroborated by the results of the present study, which failed to observe correlations between the conventional indices of renal function and NGAL concentrations in urine and to identify predictors of urinary NGAL in the multiple regression analysis. Given the capability of urinary NGAL to specifically predict kidney injury and the close relationship between NGAL excretion and the extent of tubular damage, it is reasonable to assume that patients that received CNI-free therapy with everolimus have a lower risk of developing renal impairment.

In a major contrast to NGAL excretion in urine plasma, NGAL levels were found to be closely related to indicators of renal function (serum creatinine, cystatin C levels, $eGFR_{Crea}$, and $eGFR_{Cyst}$), as well as markers of heart function (NT-proBNP) and chronic inflammation (Lp-PLA₂ and sCD40L). The distinct origins of excreted and circulating NGAL may account for these differing findings. Whereas NGAL concentrations in the urine are directly determined by tubular damage, the direct contribution of kidney injury to circulating NGAL has been questioned; indeed, other tissues and cells may represent its main source in plasma [5,6,25]. For example, NGAL expression in cardiomyocytes is upregulated by several fold in the failing myocardium in response to hypoxia or pro-inflammatory factors [26]. Accordingly, correlations between plasma NGAL levels and CHF severity, as reflected by New York Heart Association class, LVEF, and NT-proBNP levels,

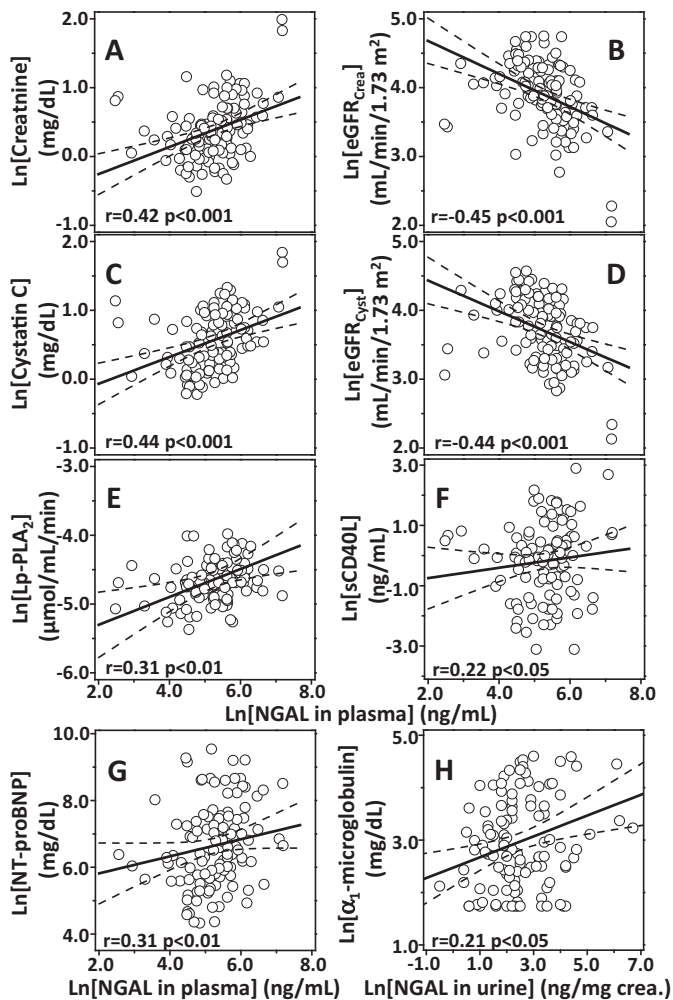


Fig. 1. Correlations between neutrophil gelatinase-associated lipocalin (NGAL) concentrations in serum and urine and renal, cardiac, and inflammatory markers in everolimus- and calcineurin-inhibitor (CNI)-treated heart transplant recipients. Correlations between variables were examined using the Spearman rank correlation test. Non-parametric data are presented after Ln-transformation. Solid lines: correlation; dotted lines: confidence intervals. (A) Creatinine; (B) estimated glomerular filtration rate for creatinine ($eGFR_{Crea}$); (C) cystatin C; (D) estimated glomerular filtration rate for cystatin C ($eGFR_{Cyst}$); (E) lipoprotein-associated phospholipase A₂ (Lp-PLA₂); (F) soluble CD40 ligand (sCD40L); (G) N-terminal brain natriuretic propeptide (NT-proBNP); and (H) α_1 -microglobulin.

have been reported previously and the latter finding is recapitulated by the findings of the present study [26–29]. In addition, activated inflammatory cells, such as neutrophils and macrophages, may represent an important source of circulating NGAL. The close relationship between NGAL concentrations in plasma (but not urine) and indicators of systemic inflammation, such as tumor necrosis factor α , have been described in diverse clinical settings that include CHF and ischemic cerebrovascular disease [26,29,30]. The present investigation extends these findings by showing the novel association between plasma NGAL levels and markers of chronic inflammatory processes, such as Lp-PLA₂ and sCD40L. Both Lp-PLA₂ and sCD40L are independent risk predictors of coronary ischemic disease, in which they are believed to reflect the magnitude of macrophage activation in atherosclerotic lesions. In this context, it is worth observing that increased plasma NGAL levels have been reported in coronary ischemic disease and that NGAL can be detected within atherosclerotic plaques [31,32]. The close relationship between sCD40L, Lp-PLA₂, and NGAL levels revealed in the present study may indicate common upstream mechanisms, which locally regulate these factors during the pathogenesis of atherosclerotic vasculopathy and which may be somewhat distinct from mechanisms that govern the expression of systemic inflammation markers, such as IL-6 and CRP.

No definitive conclusion can be drawn from the present study as to which mechanisms might account for the lower levels of urinary and plasma NGAL observed in heart transplant recipients after conversion to everolimus. Although it is conceivable that the discontinuation of nephrotoxic CNI therapy directly limits the extent of tubular damage, the contribution of other, indirect mechanisms cannot be dismissed. The development of renal impairment as a consequence of diverse pathological processes in the myocardium and coronary vasculature culminating in cardiac dysfunction, a condition known as cardiorenal syndrome, has been described in CHF, in which it is accompanied by elevated plasma NGAL levels [33,34]. Everolimus has been previously demonstrated to prevent endomyocardial remodeling and reduce development of vascular lesions in both heart transplant recipients and in animal models of CAV and atherosclerosis [35–39]. In addition, this compound was found to inhibit the infiltration of macrophages into vascular wall and the release of pro-inflammatory factors, such as chemokines and Lp-PLA₂ [39,40]. Based on these findings and the results of the present study, it is tempting to hypothesize that everolimus helps to sustain renal integrity indirectly via limiting inflammatory processes in the heart and thereby counteracting the development of cardiac dysfunction and cardiorenal syndrome.

Several limitations of the present study have to be acknowledged. Firstly, as the patient recruitment was confined to a single transplantation center, only a moderate number of heart transplant recipients could be included in the study. The power of the study is therefore limited and a thorough statistical analysis of confounding factors is precluded. Secondly, the study was performed in a cross-sectional design and, with the exception of creatinine, no information was available regarding parameters of renal function prior to the initiation of everolimus therapy. Accordingly, the present findings do not constitute unequivocal evidence that established NGAL as an early indicator of renal deterioration during CNI-based immunosuppression. Nevertheless, the present findings clearly demonstrate reduced plasma and urinary NGAL levels in everolimus-treated patients irrespective of the time elapsed from their conversion to CNI-free therapy. Consequently, they mandate the establishment of further prospective observational studies that will aim to appraise the utility of NGAL as a marker of early kidney injury in patients who receive post-transplant immunosuppressive therapy. Additional research will also be necessary to fully elucidate the direct and/or indirect

mechanisms that underlie the beneficial renal effects of everolimus in patients after heart transplantation.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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