Neutrophil Gelatinase-Associated Lipocalin Reflects the Severity of Renal Impairment in Subjects Affected by Chronic Kidney Disease

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Key Words
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Abstract
Neutrophil gelatinase-associated lipocalin (NGAL) is a small 25-kDa protein released from kidney tubular cells after harmful stimuli. It represents one of the most promising future biomarkers in the diagnostic field of acute kidney injury (AKI), as the increase in NGAL levels is a good predictor of a brief-term onset of AKI, notably anticipating the resulting increase in serum creatinine. However, recent studies also suggest a possible role for NGAL in chronic kidney disease (CKD). For this reason we evaluated serum (sNGAL) and urinary NGAL (uNGAL) in a cohort of CKD patients in order to verify the relationship with the severity of renal impairment. In CKD patients sNGAL, uNGAL and the fractional excretion of this protein were notably increased as compared to controls. Furthermore both sNGAL and uNGAL were correlated with serum creatinine and, inversely, with residual glomerular filtration rate (GFR): this last relationship was found to be even closer than that found between GFR and serum creatinine. Multivariate models validate these correlations as independent, confirming that in these patients NGAL is a better predictor of GFR than serum creatinine. The results confirm NGAL as an important biomarker in clinical nephrology, extending to CKD the pathophysiological role of this protein in tubular adaptations to renal damage.

Introduction

Clinical nephrology is discovering neutrophil gelatinase-associated lipocalin (NGAL), a small 25-kDa protein belonging to the lipocalin family, as one of the most promising biomarkers in the diagnostic field of acute kidney injury (AKI) [1]. This protein, initially found in activated neutrophils as an innate anti-bacterial factor, is released massively from kidney tubular cells after harmful experimental stimulations of various nature, activating specific iron-dependent pathways with the self-defensive intent to contrast oxidative stress and cellular apoptosis [2]. In patients undergoing treatments potentially detrimental to the kidney, such as contrast medium administration and cardiac surgery [3, 4], as well as in subjects with unstable nephropathies [5], the increase in NGAL levels is a good predictor of a brief-term onset of AKI, notably anticipating the resulting increase in serum creatinine levels and thus enabling the arrangement of preventive therapeutic measures in a timely manner. In parallel,
Recent studies have also reported altered NGAL levels in patients affected by some chronic kidney disease (CKD)-associated conditions, such as autoimmune [6], polycystic [7] and proteinuric diseases [8, 9], suggesting the possibility that under these circumstances NGAL production from tubular cells may reflect the entity of active renal damage that underlies the chronic impairment condition [2]. With the present pilot study, we aimed to evaluate serum (sNGAL) and urinary NGAL (uNGAL) levels in a heterogeneous cohort of patients with moderate CKD, in order to assess the eventual relationships between this tubular biomarker and the severity of renal impairment.

**Methods**

69 patients with CKD secondary to polycystic kidney disease (n = 20), idiopathic glomerulonephritis (n = 10), previous obstructive nephropathy (n = 15) and nephroangiosclerosis (n = 24) were studied. Subjects with cancer, infections, alterations in leucocyte count or formula, pathologic proteinuria (>150 mg/day) or severe glomerular filtration rate (GFR) reduction (≤20 ml/min) were excluded from the study in order to avoid potential confounding factors. 32 healthy subjects without a history of arterial hypertension, diabetes and neoplastic, cardiovascular, inflammatory, renal, lung or endocrine diseases were recruited as controls. The 2 groups were well matched with regard to age and sex.

Complete data from the study population are reported in table 1. NGAL was measured in blood and urine using a commercial available ELISA kit (Antibody Shop, Gentofte, Denmark) according to the manufacturer’s instructions. All measurements were made in triplicate and blinded manner. sNGAL levels are expressed in nanograms per milliliter, and uNGAL levels are expressed in nanograms per gram of creatinine.

Statistical analyses of data were made using the MedCalc (version 8.0) software and the GraphPad Prism (version 4.0) package. Data are presented as mean ± SD for normally distributed values and median (range) for non-normally distributed values. Differences between groups were established by Kruskal-Wallis analysis followed by Dunn’s test for nonparametric values. The Pearson correlation coefficient was employed to test correlations between variables. Before testing correlations, all non-normally distributed values were log-transformed to better approximate normal distributions. Afterwards multiple regression analyses were performed in order to assess independent relationships. Data are expressed as partial correlation coefficients (β) and p values. All results were considered significant if the p value was <0.05.

**Results**

When compared to healthy controls, CKD patients showed increased sNGAL (409.9 [52.6–1,215.4] vs. 26.2 [9.2–46.6] ng/ml; p < 0.005), uNGAL (195.3 [74.8–963.1] vs. 7.5 [3.3–12.9] ng/g creatinine; p < 0.004) and fractional excretion rate of NGAL (FeNGAL, calculated accord-
ing to the formula uNGAL/NGAL × serum creatinine/ urinary creatinine ×100), which was 3.15 [1.95–4.67] vs. 0.04 [0.01–0.09]; p < 0.001). Assessment of the Pearson coefficient reported univariate correlations between GFR (Cockcroft-Gault formula/MDRD formula) and, respectively, sNGAL (R = –0.739, p < 0.0001), uNGAL (R = –0.771, p < 0.0001), FeNGAL (R = 0.452, p < 0.001), and serum creatinine (R = –0.698, p < 0.005). Furthermore, significant correlations were also found between serum creatinine and, respectively, sNGAL (R = 0.445, p < 0.001) and uNGAL (R = 0.399, p < 0.001). Using GFR as a dependent variable in a multivariate model including its univariate correlates (r² = 0.799, p < 0.001), the relationships with serum creatinine (β = –0.580, p < 0.01), sNGAL (β = –0.645, p < 0.01) and uNGAL (β = –0.688, p < 0.005) remained significant, although not as close (fig. 1).

**Discussion**

The results reported here confirm that NGAL is an important biomarker in clinical nephrology, extending to CKD the pathophysiological role of this protein in tubular adaptations to renal damage. It can be hypothesized that a sustained production by the stressed kidney, with a compensatory defensive mechanism very similar to that previously described during acute injuries, is responsible for the increased sNGAL and uNGAL levels re-
ported in CKD patients as compared to controls. The parallel elevation in FeNGAL supports the hypothesis of an active tubular production, also excluding a passive consequence of reduced renal clearance capacity.

From this point of view, NGAL would thus represent the expression of how much active kidney damage lies beneath the overall condition of chronic renal impairment, rather than being a simple marker of decreased filtration such as serum creatinine, as recently proposed by Mori and Nakao [2].

Generally, it could not be excluded that extra-renal tissues may contribute importantly to increased NGAL levels. In fact several conditions associated with CKD, such as hypertension, endothelial dysfunction, atherosclerosis and even vascular damage, were previously seen to be strictly associated with enhanced circulating NGAL levels [10–12], expressing an increased local production rather than an altered systemic balance. However, in our patients no correlations were found between NGAL values and the number of white cells, systolic or diastolic blood pressure levels, erythrocyte sedimentation rate, or C-reactive protein (as indices of systemic inflammation). On the contrary, strong univariate correlations, confirmed as being independent in multivariate models, were found between both sNGAL and uNGAL levels and GFR: these relationships were also seen to be closer than that found between serum creatinine and GFR upon univariate as well as multivariate analyses. Thus, in these CKD patients NGAL levels well reflect the severity of renal impairment, independently predicting residual GFR even better than serum creatinine. Findings from the present study support recent observations from other authors, proposing NGAL as a useful, easy-to-detect biomarker of acute as well as chronic kidney damage. For example, Mitsnefes et al. [13] reported very similar observations in a cohort of children with CKD, where NGAL levels were correlated with estimated GFR (Schwartz formula) and even calculated GFR (ioversol clearance) better that serum creatinine and cystatin C, although these relationships were not tested by multivariate analyses.

Further studies on larger populations are required to validate our reports and to evaluate the potential utility of NGAL measurements in the progression and monitoring of specific CKD-associated conditions.

References