Endocr (2009) 36:45–51
DOI 10.1007/s12020-009-9187-x

CASE REPORT

Changes of serum and urine neutrophil gelatinase-associated lipocalin in type-2 diabetic patients with nephropathy: one year observational follow-up study

Yi-Hua Yang · Xiao-Jie He · Shen-Ren Chen · Ling Wang · En-Min Li · Li-Yan Xu

Received: 15 January 2009 / Accepted: 27 March 2009 / Published online: 24 April 2009

Abstract We initiated the present work to explore whether neutrophil gelatinase-associated lipocalin (NGAL) could be used to predict the progression of diabetic nephropathy in type-2 diabetic patients. Seventy-four type-2 diabetic patients were divided into normo-, micro- and macro-albuminuria groups according to their 24 h-urinary albumin excreting rate. Serum and urine NGAL, and other clinical parameters were detected. Patients were followed and measurements were repeated 1 year later. An increased tendency of urine NGAL and a decreased tendency of serum NGAL were detected, from normo-albuminuria group to macro-albuminuria group. Serum NGAL was found to rise after follow-up. Moreover, urine NGAL was found to be correlated positively with cystatin C, urea nitrogen, and serum creatinine (SCr), and inversely with glomerular filtration rate (GFR), while serum NGAL correlated negatively with cystatin C and urea nitrogen, at both baseline and follow-up levels. The results indicate that NGAL correlates closely with renal function. Both serum and urine NGAL are sensitive for predicting the progression of type-2 diabetic nephropathy but they may change differently. Serum NGAL may be more useful in early detection and urine NGAL may be more meaningful in renal function assessment.

Keywords Neutrophil gelatinase-associated lipocalin · Diabetic nephropathy · Renal function

Yi-Hua Yang and Xiao-Jie He contributed equally to the work. Shen-Ren Chen, En-Min Li and Li-Yan Xu are co-corresponding authors.

Y.-H. Yang · X.-J. He · S.-R. Chen (✉)
Department of Endocrinology and Metabolism of the Second Affiliated Hospital, Shantou University Medical College, Shantou 515041, Guangdong, China
e-mail: chen-shenren@163.com

L. Wang · E.-M. Li
Department of Biochemistry and Molecular Biology, Shantou University Medical College, Shantou, Guangdong, China

L. Wang · L.-Y. Xu
Institute of Oncologic Pathology, The Key Immunopathology Lab of Guangdong Province, Shantou University Medical College, Shantou, Guangdong, China

Introduction

Diabetic nephropathy is one of the most common microvascular complications of diabetes mellitus, greatly affecting the life quality and survival of the patients. As global prevalence of type-2 diabetes is steadily increasing, the number of patients with diabetic nephropathy is expanding day by day. Diabetic nephropathy is now the leading cause of end stage renal disease (ESRD), a disease that is described as a medical catastrophe of worldwide dimensions [1]. Therefore, the prevention of the disease, or at least the postponement of its progression, has emerged as a key issue.

Adverse outcomes of renal failure can be prevented or delayed through early detection and treatment [2]. For quite a long time, the impaired renal function of patients with diabetic nephropathy is mainly reflected by laboratory detection of SCr and blood urea nitrogen (BUN), both of which are not sensitive enough to illustrate early change of renal function, when active management is important. Recently, cystatin C and transferrin, two kinds of small-molecular proteins, have been proposed to be more sensitive and more reliable in assessing renal function [3, 4]. But both were controversial and need further verification. Therefore, the searching of sensitive indicator for the disease remains to be a hot-topic.

Neutrophil gelatinase-associated lipocalin (NGAL), a member of lipocalin family, was originally identified as 25-kDa protein covalently associated with human matrix metalloproteinase 9 (MMP-9) from human neutrophils [5]. It is stored mainly in the specific granules of neutrophils, but also expresses at very low-levels in several human tissues, including kidney, trachea, lungs, stomach, and colon [6]. NGAL is found to possess diverse functions such as transporting, activating MMP-9, inducing apoptosis, regulating immune response and so on.

Recently, researches have proved that NGAL can trigger nephrogenesis by stimulating the conversion of mesenchymal cells into kidney epithelia [7]. NGAL also plays a renoprotective role through enhancing tubule cell proliferation in kidney injury, especially in ischemia-reperfusion injury [8]. Moreover, latest discoveries show that NGAL is a marker closely associated with obesity, insulin resistance, and hyperglycemia in human beings [9]. Therefore, we set up this study to investigate the possible association of NGAL and type-2 diabetic nephropathy since NGAL relates so tightly with kidney and diabetes.

Research design and methods

Subjects

A total of 74 Chinese type-2 diabetic patients (according to the 1999 WHO criteria) were recruited at the Department of Endocrinology and Metabolism, of the Second Affiliated Hospital of Shantou University, in 2007. The study was approved by the Ethics Committee and conducted in accordance with the Helsinki Declaration as revised in the year 2000.

Additional exclusion criteria were: patients who complicated with ketoacidosis, fever, infection, surgery, or trauma within 6 months; patients who were suffering from systemic, renal, cardiac, or hepatic diseases; patients with evidences of malignant tumors.

The eligible patients were divided into three groups according to their 24 h-urinary albumin excreting rate: normo-albuminuria group (group 1, $n = 26$, UAER < 30 mg/24 h), micro-albuminuria group (group 2, $n = 24$, 30 mg/24 h $\leq$ UAER < 300 mg/24 h) and macro-albuminuria group (group3, $n = 24$, UAER > 300 mg/24 h). The clinical features of the study subjects are summarized in Table 1.

Measurements

All the assays were unchanged during the study period. 24 h-urinary albumin excreting rate was measured in sterile urine by radioimmunoassay. Urinary transferrin was detected by enzyme-linked immunosorbent assay. Levels of SCr and urea nitrogen were determined by a fully automated kinetic method on an automatic biochemistry analyzer. Fasting blood glucose (FBG) was measured by a glucose oxidase method. Hemoglobin A_{1c} levels were measured using affinity chromatography. Serum cystatin C was measured by a particle-enhanced nephelometric immunoassay and high-sensitive C reactive protein was using immunoturbidimetry.

Assessment of renal function

The modification of diet in renal disease (MDRD) study equation for Chinese people was used to estimate glomerular filtration rate (GFR) as follows [10]: MDRD GFR (ml/min/1.73 m^2) $= 186.3 \times SCr^{-1.154} \times Age^{-0.203} \times (0.742$ for women) $\times$ (1.233 for Chinese).

Enzyme-linked immunosorbent assay for serum and urine NGAL

First-voided morning urine sample was collected for urine NGAL detection. Both serum and urine NGAL were detected by using a commercially available sandwich enzyme-linked immunosorbent assay kit (ELISA Starter Accessory Package, Bethyl Lab, USA). The assay was done mainly according to the manufacturers' instructions. Briefly, Microtiter plates were coated overnight at 4°C with a mouse monoclonal antibody raised against human NGAL (HYB211-02, AntibodyShop, Denmark) at 4°C. All subsequent steps were performed at room temperature. Plates were blocked with buffer containing 1% bovine serum albumin (BSA), coated with 100 µl primitive samples (serum or urine) or standards NGAL (expressed in P. pastoris and affinity purified by our laboratory), and incubated with a biotinylated monoclonal antibody against human NGAL (HYB211-01B, AntibodyShop, Denmark) followed by avidin-conjugated horseradish peroxidase (HRP, Vector Laboratory, USA). The substrate 3,3,5,5-tetramethyl-benzidine (TMB, from Bethyl Lab ELISA Starter Accessory Package, USA) was added for color development, and the enzymatic process was halted by adding 100 ul sulfuric acid 15 min later. Finally, light absorption was measured by a microplate reader (ELX 800, Bio-Tek, USA) under 450 nm wavelength (reference wavelength: 630 nm). All measurements were made in duplicate. For urine NGAL the average recovery was $96.2 \pm 2\%$, and for serum NGAL the average recovery was $97.3 \pm 2\%$. Assays for both serum and urine NGAL demonstrated near linearity with the squared correlation coefficient $R^2 = 0.991$. The intra-assay coefficients of variation were 2.0 (range: 1.3–4.0%) and 3.0% (range: 1.2–4.0%) in urine and serum, respectively. Inter-assay

Table 1 Baseline characteristics of the study groups

Characteristics	Group 1	Group 2	Group 3
Number (*n*)	26	24	24
Percentage of men (%)	46.2	41.7	54.2
Age (years)	60.12 ± 12.56	59.96 ± 10.67	64.50 ± 10.62
Diabetes duration (years)	8.96 ± 6.21	11.10 ± 6.80	11.92 ± 5.88Δ
Systolic blood pressure (mmHg)	126.04 ± 16.24	145.50 ± 25.83*	157.63 ± 23.69*
Diastolic blood pressure (mmHg)	76.58 ± 11.52	78.92 ± 12.38	83.17 ± 14.32
Total cholesterol (mmol/l)	4.78 ± 0.80	5.57 ± 1.51Δ	6.25 ± 1.76$^{\Delta}$
White blood cell ($\times 10^9$/l)	6.66 ± 1.61	7.93 ± 3.10	7.69 ± 2.00

$^{\Delta}$ $P < 0.05$

* $P < 0.01$ and ** $P < 0.001$ vs. group 1

variation was 9.0 (range: 6.6–18.2%) and 8.3% (range: 2.1–11.3%) in urine and serum, respectively.

Follow-up

The follow-up period lasted for 1 year, until the end of 2008 or death. The participants, during the follow-up period, underwent the standardized physical examinations, biochemical measurements and received treatments based on the standard strategies for diabetes, hypertension, and hyperlipidemia as usual. One year later, samples of the above patients were collected renewedly and assays were repeated.

Statistical analysis

All data are expressed as means ± SD, unless stated otherwise. In all cases, $P < 0.05$ was considered significant (two-tailed). For multiple comparisons, normally distributed variables were investigated by the one-way ANOVA followed by the unpaired Student-Newman-Keuls test, whenas non-normally distributed variables were performed by the Kruskal–Wallis test followed by the Mann–Whitney test. For matched data, paired *t*-test or Wilcoxon test was employed, which depended on the distribution too. A χ^2 test was used to compare noncontinuous variables. The relationship between NGAL and other laboratory parameters were analyzed by two-sided Pearson or Spearman correlation test. Data were stored and processed by using a commercially available program (SPSS for Windows, version 10.0, SPSS, Chicago, IL).

Results

Of 74 individuals into the study, six patients discontinued the follow-up and could not be located. Four patients died during the follow-up period. The cause of death was related to coronary heart disease in one patient, cerebrovascular accident in one and renal failure in two. Thus, the analysis of the outcome was determined for 64 patients (follow-up rate: 86%).

Table 1 shows the baseline characteristics of the study groups. There were no significant differences with regard to age, sex, diastolic blood pressure, and white blood cell among the subjects. Compared to the normo-albuminuria group, patients with macro-albuminuria had longer duration of diabetes. The total cholesterol levels in micro- and macro-albuminuric groups were significantly higher than normo-albuminuria group. Micro-albuminuria group had higher systolic blood pressure level compared to normo-albuminuria patients, and the difference of the pressure between macro-albuminuria group and normo-albuminuria group was more significant.

MDRD GFR in group 1 rose up after follow-up

The patients were followed prospectively for 1 year, without any intervention. MDRD GFR decreased from normo- and micro-albuminuric groups to macro-albuminuria group. MDRD GFR in normo-albuminuria group after follow-up was higher than at baseline, but no differences for the two levels were drawn from the other two groups (Table 2).

Table 2 GFR (ml/min/1.73 m^2) at baseline and follow-up levels

Groups	Baseline	Follow-up
Group 1	99.30 ± 20.25	109.98 ± 27.64*
Group 2	101.52 ± 34.45	109.74 ± 74.70
Group 3	53.38 ± 40.9*$^{\#}$	55.68 ± 42.31$^{\blacktriangle\Delta}$

For multiple comparisons, * $P < 0.001$ vs. group 1 and $^{\#}$ $P < 0.001$ vs. group 2 at baseline level; $^{\blacktriangle}$ $P < 0.01$ vs. group 1 and $^{\Delta}$ $P < 0.01$ vs. group 2 at follow-up level. For paired comparisons (follow-up groups vs baseline groups), * $P < 0.05$ vs. group 1 at baseline level

Comparisons of serum and urine NGAL before and after follow-up

Serum NGAL descended from normo-albuminuria group to macro-albuminuria group, and ascended after follow-up

Statistical analysis showed a decline tendency of serum NGAL from normo-albuminuria group to macro-albuminuria group, at both baseline and follow-up levels: NGAL in normo-albuminuria group were higher than micro-albuminuria group and macro-albuminuria group; NGAL in micro-albuminuria group were higher than macro-albuminuria group. Furthermore, serum NGAL at follow-up level was higher than baseline level, in each group.

The above messages are told from Table 3.

Urine NGAL ascended from normo- and micro-albuminuric groups to macro-albuminuria group

Levels of urine NGAL in macro-albuminuria group were higher than normo-albuminuria group and micro-albuminuria group, although no differences were found between normo- and micro-albuminuric groups, no matter at baseline or follow-up level. Moreover, no statistical changes were found in the paired groups before and after follow-up.

We got the information from Table 3 too.

Serum creatinine and blood urea nitrogen declined after follow-up

Table 4 shows the levels of some commonly used clinical parameters for patients with diabetic nephropathy. No significant differences with regard to FBG, hemoglobin A_{1c} (A_{1c}), transferrin (Tf), cystatin C (Cys C) and high-sensitive C-reactive protein (hsCRP) were found between the two paired groups (before and after follow-up) except BUN and SCr. Levels of blood urea nitrogen in normo-albuminuria group declined after follow-up, so did the levels of serum creatinine in normo-albuminuria group and macro-albuminuria group.

Associations

Urine NGAL correlated negatively with MDRD GFR

A negative correlation between urine NGAL and MDRD GFR was found at the two levels, no correlation between serum NGAL and MDRD GFR was found, as shown in Table 5.

Serum NGAL correlated negatively with cystatin C and urea nitrogen

Among the study parameters, serum NGAL was found negatively and weakly correlated with cystatin C and urea

Table 3 NGAL (ug/l) at baseline and follow-up levels

Groups	Baseline		Follow-up	
	Serum NGAL	Urine NGAL	Serum NGAL	Urine NGAL
Group 1	22.59 ± 45.74	1.56 ± 1.89	52.93 ± 73.02$^{\#}$	1.91 ± 2.08
Group 2	7.62 ± 5.91^	2.65 ± 2.94	17.41 ± 15.90$^{\wedge\blacktriangle}$	2.01 ± 1.78
Group 3	4.32 ± 3.89$^{\bigstar\dagger}$	7.22 ± 5.94$^{\Delta\star}$	13.38 ± 21.65$^{\bigstar\dagger *}$	4.48 ± 3.13$^{\Delta\star}$

For multiple comparisons, $^{\wedge}$ $P < 0.05$ vs serum NGAL in group 1; $^{\bigstar}$ $P < 0.05$ vs. serum NGAL in group 2; † $P < 0.001$ vs serum NGAL in group 1; $^{\Delta}$ $P < 0.01$ vs urine NGAL in group 1; * $P < 0.01$ vs urine NGAL in group 2

For paired comparisons, $^{\#}$ $P < 0.05$ vs serum NGAL in group 1, $^{\blacktriangle}$ $P < 0.05$ vs serum NGAL in group 2; * $P < 0.05$ vs serum NGAL in group 3

Table 4 Comparisons of other clinical parameters

		FBG (mmol/l)	A_{1C} (%)	BUN (mmol/l)	SCr (umol/l)	Tf (mg/l)	Cys C (mg/l)	hsCRP (mg/l)
Baseline	DN_1	9.49 ± 3.66	8.02 ± 2.35	5.49 ± 1.25	80.50 ± 16.56	0.24 ± 0.31	0.83 ± 0.28	9.49 ± 3.66
	DN_2	10.90 ± 4.50	9.00 ± 2.50	6.25 ± 2.78	84.25 ± 30.49	2.45 ± 3.26	1.32 ± 1.60	10.90 ± 4.50
	DN_3	9.00 ± 3.52	7.92 ± 2.28	13.34 ± 9.08	247.04 ± 200.22	4.03 ± 2.25	2.18 ± 1.57	9.00 ± 3.52
Follow-up	DN_1	8.08 ± 2.86	7.73 ± 2.77	4.42 ± 1.11*	74.9 ± 16.84$^{\#}$	0.71 ± 1.47	0.84 ± 0.25	8.08 ± 2.86
	DN_2	9.39 ± 4.10	7.9 ± 1.97	6.24 ± 2.77	88.52 ± 37.62	7.69 ± 19.09	1.01 ± 0.30	9.39 ± 4.10
	DN_3	9.15 ± 4.12	7.98 ± 1.83	12.04 ± 9.71	225.19 ± 184.63$^{\Delta}$	10.65 ± 14.30	2.08 ± 1.37	9.15 ± 4.12

* $P < 0.01$ vs. BUN in group 1 at baseline level

$^{\#}$ $P < 0.05$ vs SCr in group 1 at baseline level

$^{\Delta}$ $P < 0.05$ vs SCr in group 3 at baseline level

Table 5 The correlations between NGAL and other clinical parameters

Parameters	Serum NGAL				Urine NGAL			
	r (before)	*P* (before)	*r* (after)	*P* (after)	*r* (before)	*P* (before)	*r* (after)	*P* (after)
GFR (ml/min/1.73 m^2)	–	>0.05	–	>0.05	−0.57	<0.001	−0.38	<0.01
FBG (mmol/l)	–	>0.05	–	>0.05	–	>0.05	–	>0.05
A_{1C} (%)	–	>0.05	–	>0.05	–	>0.05	–	>0.05
BUN (mmol/l)	−0.29	<0.01	−0.43	<0.01	0.72	<0.001	0.46	<0.001
SCr (umol/l)	–	>0.05	–	>0.05	0.57	<0.001	0.55	<0.001
Tf (mg/l)	–	>0.05	–	>0.05	–	>0.05	–	>0.05
Cys C (mg/l)	−0.27	<0.05	−0.33	<0.05	0.56	<0.001	0.56	<0.001
hsCRP (mg/l)	–	>0.05	–	>0.05	–	>0.05	–	>0.05

before: before follow-up

after: after follow-up

nitrogen. No correlation between serum NGAL and other parameters was found.

Urine NGAL correlated positively with cystatin C, urea nitrogen, and serum creatinine

A positive correlation was found between urine NGAL and cystatin C, urea nitrogen and serum creatinine at both baseline and follow-up levels. No significant correlation was found between urine NGAL and FBG, A_{1C}, Tf and hsCRP. Serum NGAL did not correlate with urine NGAL (Table 5).

Discussion

An increasing body of evidence indicates that NGAL correlates tightly with kidney. In physiological process, NGAL can induce the formation of kidney epithelia by stimulating the conversion of mesenchymal cells into proximal tubule epithelia [7]. This is because NGAL can work as an iron-transporting protein to deliver Fe by forming a complex with iron-binding siderophores (NGAL: siderophore: Fe^{2+}), and Fe is crucial for cell growth and development [8]. In pathological process, accumulating evidences have suggested that NGAL relates tightly with series of renal dysfunctions. NGAL is one of the most robustly expressed proteins in the ischemic or nephrotoxic injury of kidney in both animals [11, 12] and human beings [13], and serum NGAL has been reported to be a sensitive and specific biomarker for early identification of acute kidney injury following cardiac surgery [14] and a novel biomarker in children with chronic kidney diseases [15].

Some studies have successfully investigated that the mRNA expression of NGAL is significantly higher in diabetic/obese mice or obese human beings, and associated closely with insulin resistance and hyperglycemia, which implies that NGAL may play an important role in type-2 diabetes [9].

In this study, serum NGAL was found stepping down from normo-albuminuria group to macro-albuminuria group. However, urine NGAL seemed to change in an opposite way for NGAL in macro-albuminuria group owned the highest level. The results kept consistent 1 year later. An adequate explanation for this phenomenon is lacking, but we do have reasons to deduce that serum NGAL may increase in the very early stage of diabetic nephropathy and drop down as the disease develops, since serum NGAL changes in this way in a variety of renal dysfunctions such as ischemia-reperfusion injury, drug-induced acute interstitial nephritis, kidney transplantation and so on. On the other hand, as the disease progresses, functions of kidney went poorer and poorer, excretion of NGAL in urine might increase day after day, and the absorption function of nephric tubule might decrease, thereby, urine NGAL reached its highest level in macro-albuminuria group.

Diabetic nephropathy does not develop within the first few years of diabetes and the disease condition can be improved by the well controlled diet, blood glucose, blood fat, blood pressure and so on. From a clinical perspective, the putative process of renal function is very strongly associated with GFR, serum creatinine, and blood urea nitrogen. In our follow-up study, MDRD GFR rose up, serum creatinine and blood urea nitrogen decreased in normo-albuminuria group 1 year later, which implied improved conditions for the patients. At the same time, we found serum NGAL increased in the same group, which may strongly support that the increasing of serum NGAL is a good sign for the disease.

Moreover, the correlation analysis highlighted the relationship between NGAL and other indexes. Urine NGAL correlated negatively with MDRD GFR, which is widely used as a gold standard in assessing renal function. Urine

NGAL was also found correlating positively with cystatin C, serum creatinine, and blood urea nitrogen; all of the three are major parameters in evaluating renal impairment. A correlation between serum NGAL and cystatin C or urea nitrogen was found too, although weakly. The correlation analysis may strongly imply that NGAL is an important biomarker associating with renal function.

Our results may suggest that serum NGAL is more useful in detecting the early stage of diabetic nephropathy since the content of serum NGAL changed more sharply than urine NGAL. Serum NGAL changed from normo-albuminuria group to micro-albuminuria group and macro-albuminuria group, and from baseline level to follow-up level, while no statistical differences could be found for urine NGAL between the normo- and micro-albuminuric groups or between the baseline and follow-up levels. But on the other hand, urine NGAL may be more informative for assessing renal function since it relates with the assessing markers more tightly and extensively.

In our study, the exact mechanisms for the changes of NGAL remain unclear. We speculate that NGAL in diabetic nephropathy is produced principally by the injured tubule cells to prevent kidney from early injury for many reasons. First, since injury of renal tubule is unavoidable in the process of diabetic nephropathy, therefore, repair mechanisms must be started by the body. As an iron-transporting protein, NGAL may be expressed by the damaged tubule to induce regeneration since iron is necessary for re-epithelialization. Besides, the complex of NGAL/siderophore/Fe^{2+} can up-regulate heme oxygenase 1 (HO-1), which could limit oxidant-mediated apoptosis of renal tubule cell death through limiting iron-driven oxidant stress [8]. Second, it is well known that the pathological changes of diabetic nephropathy involve accumulation of extracellular matrix, which is degraded mainly by matrix metalloproteinases (MMPs). The activities of MMPs depend on metal ions and limited by tissue inhibitor of metalloproteinase-1(TIMP-1), and NGAL is probably a universal activator of the MMPs family [16]. Studies have found that the metabolic disorder in the process of diabetic nephropathy usually destroys the balance of MMPS/TIMP: the degradation ability of MMP-9 declines [17], and the expression of TIMP-1 is up-regulated [18], therefore, extracellular matrix accumulates. NGAL is capable of protecting MMP-9 from degradation [19]. Moreover, NGAL can activate the MMP-9 precursor directly, and counteract the inhibiting effect of TIMP-1 [20]. Thus, we presume that NGAL is activated to delay the proceeding of renal fibrosis in diabetic nephropathy, by preserving the enzymatic activity of MMP-9. Third, an expanding body of data now strongly suggests that inflammation contributes to diabetes mellitus and diabetic nephropathy [21–24]. The disorder of glycometabolism stimulates the expression of inflammatory factors, and the infiltration of the latter could not only ruin the kidney tissue directly but also provoke the secretion of type IV collagen, fibrin and so on, thereby, kidney sclerosis is accelerated. It is proposed that NGAL has immuno-modulatory activity by binding and clearing lipophilic inflammatory mediators [25], such as the neutrophil tripeptide chemoattractant. In addition, NGAL has been found to be able to induce cell apoptosis through an autocrine/paracrine pathway. For these reasons, we presume that NGAL may protect the diabetic kidney through restraining inflammation reaction and inducing apoptosis of neutrophil granulocytes in the nephric tubules and interstitium.

However, this is just a pilot study for NGAL and diabetic nephropathy. There are important limitations to this study. First, it is a single-center study with a relatively small sample size, which might underestimate the relationship between NGAL (especially for serum NGAL) and any other parameter. Second, the follow-up period is too short to show the exact changes of the disease. Serum NGAL also increased in the other two groups at follow-up level in our study, but we had no evidence to judge what direction the disease went in the two groups except the declining of serum creatinine in macro-albuminuria group, and further follow-up study for observation was necessary. Third, the exact role NGAL play in the disease is debatable. Whether NGAL is protective as fore cited or proximate to injury or even an innocent bystander remains unclear. It will therefore be critical in future multicenter prospective studies to confirm the role serum and urine NGAL play in the progression of patients with diabetic nephropathy. It is likely that not any one biomarker but, rather, a combination of strategically selected proteins will constitute the elusive "panel" for accurate monitoring and prediction of the disease. The present study suggests both serum and urine NGAL as strong candidates for inclusion in such a panel.

In one word, NGAL may participate in the genesis and progression of diabetic nephropathy. Both serum and urine NGAL are sensitive for predicting the progression of diabetic nephropathy but they may change in different directions. Serum NGAL may be more sensitive than urine NGAL since the former changed more sharply and earlier than the latter. The outcome is reliable since the result kept highly consistent at both baseline and follow-up levels. We expect for more and more studies about NGAL and diabetic nephropathy in the future.

Acknowledgments This study was supported by the funds from the Scientific and Technological Projects of Guangdong Province (2006B36005007), Guangdong Scientific Fund Key Items (No. 7118419), National Natural Science Foundation of China (No. 30672376) and Specialized Research Fund for the Doctoral Program of Higher Education of China (No. 20050560003).

References

1. E. Ritz, I. Rychlik, F. Locatelli, S. Halimi, Am. J. Kidney Dis. **34**, 795–808 (1999)
2. A.S. Levey, J. Coresh, E. Balk et al., Ann. Intern. Med. **139**, 137–147 (2003)
3. M. Mussap, M. Dalla Vestra, P. Fioretto et al., Kidney Int. **61**, 1453–1461 (2000)
4. M. Kanauchi, H. Nishioka, T. Hashimoto, K. Dohi, Nippon Jinzo Gakkai Shi **37**(11), 649–654 (1995)
5. L. Kjeldsen, A.H. Johnsen, H. Sengelov et al., J. Biol. Chem. **268**(14), 10425–10432 (1993)
6. J.B. Cowland, N. Borregaard, Genomics **45**, 17–23 (1997)
7. J. Yang, D. Goetz, J.-Y. Li et al., Mol. Cell **10**, 1045–1056 (2002)
8. K. Mori, H.T. Lee, D. Rapoport et al., J. Clin. Invest. **115**(3), 610–621 (2005)
9. Y. Wang, K.S.L. Lam, E.W. Kraegen et al., Clin. Chem. **53**(1), 34–41 (2007)
10. Y.C. Ma, L. Zuo, J.H. Chen et al., J. Am. Soc. Nephro. **17**, 2937–2944 (2006)
11. S. Supavekin, W. Zhang, R. Kucherlapati et al., Kidney Int. **63**, 1714–1724 (2003)
12. J. Mishra, K. Mori, Q. Ma et al., Am. J. Nephrol. **24**, 307–315 (2004)
13. M. Nguyen, G. Ross, C. Dent, Am. J. Nephrol. **25**, 318–326 (2005)
14. J. Mishra, C. Dent, R. Tarabishi, Lancet **365**, 1231–1238 (2005)
15. M.M. Mitsnefes, T.S. Kathman, J. Mishra et al., Pediatr. Nephrol. **22**(1), 101–108 (2007)
16. M. Coles, T. Diercks, B. Muehlenweg et al., J. Mol. Biol. **289**(1), 139–157 (1999)
17. S. Mclennan, D.K. Yue, J.R. Turtle, Nephron **79**(3), 293–298 (1998)
18. E. Ishimura, Y. Nishizawa, S. Shoji et al., Life Sci. **58**(16), l331–l1337 (1996)
19. L. Yan, N. Borregaard, L. Kjeldsen et al., J. Biol. Chem. **276**(40), 37258–37265 (2001)
20. C. Murray, D. Tammo, M. Bernd et al., J. Mol. Biol. **289**(40), 139–157 (1999)
21. F.P. Schena, L. Gesualdo, J. Am. Soc. Nephrol. **16**, 30–33 (2005)
22. E. Galkina, K. Ley, J. Am. Soc. Nephrol. **17**, 368–377 (2006)
23. C. Mora, J.F. Navarro, Curr. Diab. Rep. **5**(6), 399–401 (2005)
24. T.W. Meyer, Kidney Int. **63**, 377–378 (2003)
25. J.R. Bundgaard, H. Sengelov, N. Borregaard, L. Kjeldsen, Biochem. Biophys. Res. Commun. **202**(3), 1468–1475 (1994)