Serum NGAL and Cystatin C Comparison With Urinary Albumin-to-Creatinine Ratio and Inflammatory Biomarkers as Early Predictors of Renal Dysfunction in Patients With Type 2 Diabetes

Marcelo R. Bacci1, Ethel Z. Chehter1, Ligia A. Azzalis2, Beatriz Costa de Aguiar Alves1 and Fernando L.A. Fonseca1,3

1Department of General Practice, Faculdade de Medicina do ABC, Santo André, Brazil; 2Earth Science’s Department of UNIFESP, São Paulo, Brazil; and 3Clinical Analysis Laboratory of Faculdade de Medicina do ABC, Santo André, Brazil

Introduction: Diabetic nephropathy is associated with specific histological changes. Early detection of poor glomerular and tubular function can be achieved with biomarkers of diabetes. The aim of this study was to evaluate the accuracy of kidney dysfunction biomarkers in type 2 diabetes (T2D).

Methods: Patients with T2D were grouped according to their glycated hemoglobin level. Patients’ urine and blood samples were taken to measure cystatin C (CysC), neutrophil gelatinase-associated lipocalin, beta-trace protein levels, and the first morning void albumin-to-creatinine ratio. Patients in the end stage of renal disease or receiving dialysis were not included. Receiver operating characteristic curves were generated, and the areas under the curve were compared with the performance of the biomarkers used to evaluate kidney dysfunction in T2D.

Results: Ninety patients with T2D were chosen. CysC was positively correlated with creatinine ($P < 0.001$), estimated glomerular filtration rate ($P < 0.001$), and urinary beta-trace protein ($P = 0.01$). The area under the curve was 0.635 for CysC, 0.621 for serum neutrophil gelatinase-associated lipocalin, and 0.660 for the albumin-to-creatinine ratio. A crude logistic regression model showed a positive association between serum CysC ($P = 0.01$) and serum neutrophil gelatinase-associated lipocalin ($P < 0.001$). A linear regression model showed a positive association between serum CysC, creatinine, and estimated glomerular filtration rate ($P < 0.001$) but did not show a positive association with glycated hemoglobin ($P = 0.892$).

Discussion: Neutrophil gelatinase-associated lipocalin and serum CysC were positively associated with the presence of renal dysfunction and had better performance on receiver operating characteristic analysis than the other markers evaluated in patients with T2D without kidney dysfunction.

Kidney Int Rep (2017) 2, 152–158; http://dx.doi.org/10.1016/j.ekir.2016.10.001

KEYWORDS: albuminuria; biomarkers; cystatin C; diabetic kidney disease; NGAL

© 2016 International Society of Nephrology. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
glomerular basement membrane thickening and mesangial expansion.\textsuperscript{3}

In an animal model using mice, an increase in kidney size above hyperfiltration was attributed to high net reabsorption in the proximal convoluted tubule, causing reduced tubular hydrostatic pressure and inhibiting fluid secretion at the end portion of the segment.\textsuperscript{2}

An ideal renal function marker must have constant production, rapid diffusion in the extracellular space, free clearance, an absence of resorption and/or tubular secretion, an absence of deletion or extrarenal degradation, and the existence of accurate and reproducible tests without interference from other components.\textsuperscript{5}

Many markers have been used to predict early-stage renal dysfunction in diabetic patients; however, the reference levels have not been established for these markers. The presence of microalbuminuria is a sign of the presence of diabetic kidney disease and marks the beginning of more intense therapy.

Thus, the primary objective of this study was to evaluate the accuracy of neutrophil gelatinase-associated lipocalin (NGAL) and serum cystatin C (CysC) as markers of renal dysfunction in T2D. The secondary objectives were to evaluate the performance of other urinary and inflammatory markers and to understand their interaction in patients with T2D.

**METHODS**

**Study Population**

The study population consisted of patients who had T2D for at least 5 years according to their medical records and were older than 21 years. The study was approved by the ABC Medical School ethics committee, and all individuals gave signed informed consent before inclusion.

Ineligible patients were those with chronic kidney disease with a cause other than T2D, those who were receiving dialysis, those who had undergone hospitalization for any reason within 30 days before sample collection, those who had cancer and were being treated, those who had AIDS and were taking immunosuppressive drugs, and those who had undergone kidney or renal and pancreatic transplants.

**Laboratory Measurements**

**GFR Valuation**

The Modification of Diet in Renal Disease simplified equation was used to calculate the estimated GFR (eGFR) in ml/min per 1.73 m\textsuperscript{2}.\textsuperscript{2,6} Renal dysfunction was defined as eGFR < 60 ml/min per 1.73 m\textsuperscript{2} according to the Kidney Disease: Improving Global Outcomes chronic kidney disease definition.

Blood samples were taken to determine serum creatinine, NGAL, and CysC levels as glomerular markers of kidney function.

Serum creatinine was measured using the colorimetric method. The NGAL concentrations and CysC were determined using an automated enzyme-linked immunosorbent assay (IBL International, Hombrechtikon, Switzerland) with the aid of Labotech (Adaltis, Rome, Italy) enzyme-linked immunosorbent assay equipment.

**Tubular Function Evaluation**

To evaluate the presence of tubular dysfunction, the following urinary markers were measured: urinary NGAL, CysC, urinary gene expression of beta-trace protein (BTP), and urinary albumin-to-creatinine ratio (ACR). The cutoff values used in determining the normality of NGAL and CysC were defined according to each calibration reagent used. The ACR was considered to be normal if its value was less than 30 mg/g; microalbuminuria was indicated by levels between 30 and 300 mg/g, and macroalbuminuria was indicated by levels above 300 mg/g.

**Glycemic Control Evaluation**

Glycemic control assessment was performed using the values of fasting glucose and glycated hemoglobin (HbA1c). Values above 140 mg/dl for glucose and above 7% for HbA1c were considered abnormal.

**Inflammatory Profile Evaluation**

The inflammatory profile of each patient was obtained by measuring the serum levels of the following markers: ultrasensitive C-reactive protein, interleukin-6, tumor necrosis factor-\textalpha, homocysteine, serum beta-2 microglobulin (B2M), and 25-OH vitamin D. Measurements were performed using the immunoenzymatic method with chemiluminescence values determined using Immulite 1000 equipment (Siemens, Erlange, Germany).\textsuperscript{7} For each marker, the cutoff value was defined according to the guidelines of the kit.

**Urinary Gene Expression of BTP**

The urinary gene expression of BTP was determined by plasma RNA isolation (initial amount, 1 \mu g). Synthesis of the cDNA was performed using SSIII first-strand quantitative polymerase chain reaction (qPCR) Supermix (Invitrogen, cat no. 11752050; Carlsbad, CA, USA).

The BTP urinary gene expression was evaluated by quantitative real-time reverse transcriptase-PCR. Specific primers for each selected gene were designed using Input Primer3 Blast program version 0.4.0.

Glyceraldehyde-3-phosphate dehydrogenase was used as a reference gene to normalize the relative expression of the target gene expression values. The initial standardization of quantitative real-time reverse transcriptase-PCR amplifications occurred in a thermocycler: Applied Biosystems 7500 real-time PCR.
the inclusion of 100 patients with T2D. Ten patients had kidney disease with a cause other than diabetes and were excluded, resulting in 90 patients in this sample.

Table 1 shows the demographics of the sample. Among the patients, the HbA1c was 6.21% for the group with better glycemic control and 9.2% for the group with worse glycemic control. Furthermore, the average eGFR for both groups was greater than 60 ml/min per 1.73 m², specifically 83.8 ml/min per 1.73 m² for the group with HbA1c less than 7% and 76.7 ml/min per 1.73 m² for the group with worse glycemic control.

**ROC Curves for the Various Biomarkers in Patients With T2D**

The performance of each tested marker was analyzed by considering the change in renal function endpoint, which is defined by the eGFR calculated by the simplified formula Modification of Diet in Renal Disease and the value of serum creatinine, which are shown in Table 2.

The area under the curve (AUC) of serum CysC was 0.635. The ACR showed the best performance in determining the presence of renal dysfunction in

**Table 1. Demographics and baseline characteristics of the whole study population**

| Variable                  | Group 1 (HbA1c < 7) | Group 2 (HbA1c ≥ 7) |
|---------------------------|---------------------|---------------------|
| Male (%)                  | 40.00               | 53.30               |
| Age (yr)                  | 61.50 (9.80)        | 61.20 (9.88)        |
| Ethnicity (%)             |                     |                     |
| Caucasian                 | 76.60               | 80.00               |
| Black                     | 33.40               | 20.00               |
| Hypertension (%)          | 90.00               | 85.00               |
| BMI (kg/m²)               | 32.00 (4.80)        | 29.60 (4.98)        |
| Urea (mg/dl)              | 41.00 (20.90)       | 48.40 (28.40)       |
| Creatinine (mg/dl)        | 1.07 (0.41)         | 1.22 (0.64)         |
| eGFR (ml/min per 1.73 m²) | 83.80 (35.80)       | 76.70 (36.12)       |
| sCysC (ng/ml)             | 3.42 (1.44)         | 3.94 (1.55)         |
| uCysC (ng/ml)             | 0.50 (0.10–0.50)    | 0.50 (0.07–0.50)    |
| sNGAL (ng/ml)             | 0.80 (0.37–1.07)    | 0.80 (0.40–1.08)    |
| uNGAL (ng/ml)             | 0.01 (0.01–0.37)    | 0.01 (0.01–0.37)    |
| uBTP (mg/l)               | 10.0 (10.0–3.13)    | 10.1 (10.0–3.04)    |
| ACR (mg/g)                | 36.10 (9.82–103.60) | 38.30 (10.10–104.00) |
| HbA1c (%)                 | 6.21 (2.02)         | 9.20 (4.24)         |
| TNF-α (pg/ml)             | 9.82 (4.83)         | 9.09 (4.84)         |
| IL6 (pg/ml)               | 2.00 (2.00–3.08)    | 2.00 (2.00–3.08)    |
| CRP (mg/l)                | 5.15 (1.83–11.70)   | 5.15 (1.82–11.02)   |
| Homocisteine (µmol/l)     | 11.58 (6.00)        | 12.30 (5.86)        |
| B2M (mg/ml)               | 2365.00 (1786.50–3028.50) | 2439.00 (1850.25–3049.00) |
| Vitamin D (ng/ml)         | 17.46 (8.11–22.16)  | 17.55 (8.11–21.71)  |

Data presented as mean ± SD and median with interquartile ranges.

ACR, albumin-to-creatinine ratio; B2M, beta 2 microglobulin; BMI, body mass index; BTP, beta-trace protein; CRP, ultrasensitive C-reactive protein; CysC, cystatin C; eGFR, estimated glomerular filtration rate; HbA1c: glycated hemoglobin; IL6, interleukin 6; NGAL, neutrophil gelatinase-associated lipocalin.

**RESULTS**

Patients at the outpatient diabetic kidney setting of ABC Medical School, Sao Paulo, Brazil, were included sequentially during the years 2013–2015. They were evaluated after giving their informed consent, resulting in
patients with T2D, with an AUC value of 0.660. The values of the other markers were not considered to be significant.

In this study, the urinary gene expression of BTP had an AUC of 0.567.

### Interaction Among the Biomarkers and Outcomes Tested

Table 3 shows the results of Spearman’s correlation test using creatinine values, GFR, and the urinary gene expression of BTP.

Serum CysC and ACR had significant P values with respect to the worst outcome of renal function in patients with T2D.

Urinary NGAL showed a positive correlation with the urinary gene expression of BTP (P = 0.02). We did not find a positive correlation between urinary BTP and renal outcome but did find a tendency for a positive correlation between urinary BTP and eGFR (P = 0.06).

The results of the univariate and multivariate regression models are shown in Table 4, with the variables as dichotomous outcomes for the presence of abnormal kidney function. Markers that showed a positive association in both models are also indicated in Table 4.

In the univariate model, a positive association was observed between the values of serum CysC (P = 0.01), serum NGAL (P < 0.001), increased age, and male gender.

All inflammatory markers in the univariate model showed positive associations with the outcome of abnormal kidney function (eGFR < 60 ml/min per 1.73 m²).

The multivariate model showed a positive association between the inflammatory markers. Serum 25-OH vitamin D levels showed a negative association with worse renal function, with an odds ratio of 0.91 and a P value of 0.002 in the univariate model and an odds ratio of 0.81 with P = 0.007 in the multivariate model. Male gender also showed a strong association with worse renal function in both models.

The multivariate logistic regression model built with a backward selection of variables (Table 5) confirmed the association of male gender, serum CysC, and age with the outcome of worse kidney function. However, vitamin D was associated with better kidney function.

### DISCUSSION

Patients with diabetes can develop macro- and microvascular complications. The generation of glucose degradation products and the endothelial damage triggered by hyperglycemia contribute to a permanent state of inflammation and encourage the persistence of oxidative stress. Thus, the possibility of jointly evaluating previously reported inflammatory markers—interleukin-6, tumor necrosis factor-α, C-reactive protein, homocysteine, and vitamin D—makes the results of this study even more interesting.

### Urinary Markers

BTP is widely found in body tissues and fluids, and its elevation has been studied as a marker of reduced GFR. However, BTP presents great variability in study outcomes due to the differences in measurement methods and the variations between individuals.

### Table 2. Receiver operating characteristics curve values for the biomarkers tested in patients with type 2 diabetes

| Variable | Sensitivity | Specificity | AUC (95% CI) |
|----------|-------------|-------------|--------------|
| uNGAL   | 0.15        | 0.96        | 0.526 (0.42-0.64) |
| sNGAL   | 0.75        | 0.53        | 0.621 (0.51-0.71) |
| sCysC   | 0.37        | 0.88        | 0.635 (0.52-0.73) |
| uCysC   | 0.70        | 0.46        | 0.569 (0.44-0.69) |
| ACR     | 0.70        | 0.62        | 0.660 (0.53-0.78) |
| uBTP    | 0.85        | 0.35        | 0.567 (0.46-0.67) |

ACR, albumin-to-creatinine ratio; AUC, area under the curve; BTP, beta-trace protein; CI, confidence interval; CysC, cystatin C; HbA1c, glycated hemoglobin; NGAL, neutrophil gelatinase-associated lipocalin.

### Table 3. Correlation of P values of urinary gene expression of BTP and eGFR with the biomarkers tested in patients with type 2 diabetes

| Biomarkers | Creatinine (mg/dl) | eGFR (ml/min per 1.73 m²) | uBTP | P value |
|------------|--------------------|--------------------------|------|---------|
| sNGAL (ng/ml) | 0.140 | 0.370 | 0.310 | |
| uNGAL (ng/ml) | 0.960 | 0.740 | 0.020 | |
| sCysC (ng/ml) | <0.001 | <0.001 | 0.010 | |
| uCysC (ng/ml) | 0.680 | 0.740 | 0.800 | |
| ACR (mg/g) | 0.020 | 0.030 | 0.250 | |
| uBTP | 0.090 | 0.080 | |
| HbA1c (%) | 0.980 | 0.700 | 0.280 | |

ACR, albumin-to-creatinine ratio; BTP, beta-trace protein; CysC, cystatin C; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; NGAL, neutrophil gelatinase-associated lipocalin; Spearman’s correlation test, P < 0.05.

### Table 4. Variables associated with worse kidney function in patients with type 2 diabetes. Univariate and multivariate regression models

| Variables | Univariate | | | Multivariate | |
|-----------|------------|------------------|------------------|------------------|------------------|
| Age       | 1.05       | 1.00–1.10        | 0.030            | 1.06             | 0.99–1.14        | 0.080            |
| Male      | 9.80       | 3.69–25.98       | <0.001           | 14.85            | 3.34–65.93       | <0.001           |
| sNGAL     | 3.39       | 1.36–8.42        | <0.001           | 3.53             | 0.80–15.46       | 0.090            |
| sCysC     | 1.48       | 1.08–2.04        | 0.010            | 1.48             | 0.84–2.60        | 0.170            |
| B2M       | 4.01       | 1.49–10.77       | 0.005            | 1.80             | 0.37–8.55        | 0.450            |
| IL6       | 2.70       | 1.06–8.85        | 0.030            | 1.93             | 0.40–9.24        | 0.400            |
| Homocysteine | 1.15   | 1.04–1.26        | 0.004            | 1.07             | 0.93–1.23        | 0.320            |
| Vitamin D | 0.91       | 0.87–0.96        | 0.002            | 0.88             | 0.81–0.96        | 0.007            |

B2M: beta 2 microglobulin; BMI, body mass index; CI, confidence interval; CysC, cystatin C; HbA1c, glycated hemoglobin; IL6, interleukin-6; NGAL, neutrophil gelatinase-associated lipocalin; OR, odds ratio.
Selvin et al.⁹ evaluated the coefficient of variability of various markers of the GFR, such as CysC, B2M, and BTP. Among all of the evaluated markers, BTP showed the highest intraindividual variability by mass analysis.⁹ The RNA extraction and gene amplification process was the most sensitive method, but the amount of free RNA was small. This problem resulted in an isolation issue, thereby requiring fast and optimal processing of the urinary samples.¹⁰

Compared with nondiabetic individuals, diabetic individuals had an association between BTP and renal dysfunction, with an AUC value of 0.567. This result differs from previous studies, such as that by Uehara et al.,¹¹ who found an AUC value of 0.84 when measuring urinary BTP by reaction to latex.

There is a direct association between the urinary values of NGAL and worse progression of renal function in T2D without the presence of albuminuria, as shown by Wu et al.¹² Thus, the presence of a larger amount of this protein before creatinine elevation is an early marker of tubular dysfunction. The AUC value of urinary NGAL in this experiment was 0.526, which was not significant; however, the increase in urinary NGAL value and the urinary gene expression of BTP were positively correlated, according to Spearman’s test.

Matys et al.¹³ found an AUC value of 0.59 for urinary NGAL in diabetic patients with stable coronary disease. The authors concluded that in addition to serum and urinary NGAL, CysC was also not higher than the eGFR in these patients.¹²,¹³

When the other urinary markers were evaluated, NGAL and CysC did not have the same result as the ACR but had AUC values of 0.526 and 0.569, respectively.

An elevated ACR is clinically relevant because it indicates a loss in glomerular selectivity and/or reduced tubular reabsorption. In an Indian cohort of Pima ethnicity, those with microalbuminuria had a 2.1 times higher risk of progression to terminal kidney disease and were 9.3 times more likely to develop diabetes than those with normoalbuminuria.¹⁴

In this sample, the average ACR was 86.6 mg/g in the group with HbA1c less than 7% and was 116.5 mg/g in the group with HbA1c exceeding 7%. Moreover, there was no division into groups based on ACR, unlike the approach used by most of our peers in their respective analyses. To assess the ACR and its interaction with the other markers, we categorized patients who showed no reduction in GFR by their glucose levels.

The observed AUC of ACR was 0.660, which was the best value among the evaluated urinary markers. This value was positively correlated with creatinine and the eGFR as expected, and the correlation was a linear relationship, as indicated by univariate linear regression.

### Serum Biomarkers

HbA1c was tested for its ability to predict renal injury in accordance with the combined outcome of creatinine and eGFR, which was estimated using the Modification of Diet in Renal Disease formula. The AUC value was found to be unsuitable, having a value of 0.568. However, this study used a cross-sectional analysis, and the best performance of HbA1c lies in the long-term monitoring of diabetes.

During a 4-year period, Lee et al.¹⁵ analyzed the effects of glycemic control as determined by HbA1c values in GFR in T2D. The subjects were divided according to HbA1c control; intermediate control was considered to be approximately 7% to 9%, and the worst control was considered to be above 9%. Lee et al. noted that a higher baseline HbA1c was associated with a greater decline in GFR over the studied period. The most significant outcome was shown in patients with poor glycemic control and an ACR higher than 300 mg/g.¹⁵

CysC and NGAL were positively correlated with worse eGFR, with AUC values of 0.635 and 0.621, respectively; in this analysis, these values are only lower than the value for ACR. In addition, the authors showed a positive relationship with the combined endpoint of renal injury assessment, with the univariate logistic regression model showing $P = 0.01$ and $<0.001$ for CysC and NGAL, respectively.

CysC showed a positive relationship with renal injury outcome when there was a choice of variables in the adjusted multivariate logistic regression model with $P = 0.005$. There were also linear relationships with the continuous values of creatinine and eGFR.

Assal et al.¹⁶ assessed the pattern of CysC and urinary NGAL, among other markers, in diabetic subjects with different levels of eGFR. Subjects were stratified according to their baseline ACR, and the CysC values were higher among those with macroalbuminuria and increased creatinine values. Nevertheless, the AUC value in this experiment was 0.727.¹⁶

NGAL indicates the elevation of early known problems in secondary acute kidney injury models—such as

---

**Table 5. Multivariate logistic regression model built with backward selection of variables with $P < 0.05$**

| Variables | OR   | 95% CI | $P$  |
|-----------|------|--------|------|
| Age (yr)  | 1.07 | 1.00–1.14 | 0.03 |
| sCysC (ng/ml) | 2.04 | 1.23–3.40 | 0.005 |
| Vitamin D (ng/ml) | 0.89 | 0.83–0.96 | 0.003 |
| Male      | 19.95 | 4.96–80.22 | $<0.001$ |

CI, confidence interval; CysC: cystatin C; OR, odds ratio.
ischemia, cardiopulmonary bypass, and vasoconstriction—as shown in vascular examinations. NGAL has a good accuracy in predicting early kidney damage in these situations, but its use in chronic renal injury models is still under debate.17,18

In a prospective analysis, Chou et al.19 followed a cohort of diabetic patients stratified according to GFR and albuminuria. They noted that those with greater reduction in GFR during the follow-up period, and therefore, a greater variation in GFR value, had a higher serum NGAL value, and NGAL had a positive correlation with eGFR.19

**Inflammatory Biomarkers**

In our sample, serum homocysteine was related to kidney dysfunction in the univariate analysis and linear model built using the continuous values of creatinine and eGFR. These findings are consistent with studies showing deterioration of GFR and elevated levels of homocysteine in both diabetic and nondiabetic patients.20,21

However, low vitamin D levels were associated with worse eGFR and a greater creatinine value in all of the analyses. Zoppini et al.21 conducted a retrospective analysis of the serum values of 715 patients with T2D and found vitamin D deficiency in 36.6% of these diabetic patients. Nevertheless, these patients had more microvascular complications than those with higher vitamin D levels.21

The other inflammatory markers, C-reactive protein, interleukin-6, tumor necrosis factor-α, and B2M, did not show the same behavior as homocysteine and vitamin D. B2M had a positive association in the univariate logistic regression model using the outcome of kidney damage. This study has some limitations. First, this is a cross-sectional analysis. In a longitudinal follow-up, perhaps the effect of HbA1c variation in kidney function could be better evaluated. Another important point to consider is the variability in urine sample analysis, particularly with regard to the extraction of RNA and its amplification in the process of gene expression analysis of urinary BTP. However, our results confirm that it is possible to assess markers that compare gene expression in samples of urinary sediment and to understand the correlation with other markers. In addition, ACR was found to be a reliable marker in diabetic patients with poor glycemic control, in the absence of detected renal dysfunction.

In conclusion, NGAL and serum CysC were positively associated with the presence of renal dysfunction and had better performance in terms of ROC analysis than that of the other markers evaluated in patients with T2D without kidney dysfunction. NGAL had an AUC of 0.621, with a sensitivity of 75% and a specificity of 53.06%. CysC had an AUC of 0.635, with a sensitivity of 37.5% and a specificity of 88%. The urinary concentrations of these markers did not show the same performance.

Homocysteine and vitamin D were associated with the presence of renal dysfunction in all the models tested. Furthermore, B2M and interleukin-6 showed a positive correlation with renal dysfunction in the multivariate logistic regression model.

**DISCLOSURE**

All the authors declared no competing interests.

**ACKNOWLEDGMENTS**

This study received funding from Fundação de Amparo à Pesquisa do Estado de São Paulo under the registry 2014/04596-8 and from Nucleo de Assistência à Pesquisa Clínica under the registry 01/2014. The results presented in this paper have not been published previously in whole or part except in abstract format.

**REFERENCES**

1. Parving HH, Mauer M, Fioretto P, et al. Diabetic nephropathy. In: Taal MW, Brenner, BM, Rector FC, eds. Brenner and Rector’s The Kidney. 9th ed. Philadelphia, PA: Elsevier/Saunders; 2012:1411–1454.
2. Castellano I, Covarsí A, Novillo R, et al. Renal histological lesions in patients with type II diabetes mellitus. Nefrologia. 2002;22:162–169.
3. Caramori ML, Kim Y, Huang C, et al. Cellular basis of diabetic nephropathy: 1. Study design and renal structural-functional relationships in patients with long-standing type 1 diabetes. Diabetes. 2002;51:506–513.
4. Bak M, Thomsen K, Christiansen T, et al. Renal enlargement precedes renal hyperfiltration in early experimental diabetes in rats. J Am Soc Nephrol. 2000;11:1287–1292.
5. Peres LA, Cunha Júnior AD, Schäfer AJ, et al. Biomarkers of acute kidney injury. J Bras Nefrol. 2013;35:229–236.
6. Levey AS, Bosch JP, Lewis JB, et al. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med. 1999;130:461–470.
7. Gascón TM, Schindler F, Oliveira CG, et al. Evaluation of chemiluminescence method for the analysis of plasma homocysteine and comparison with HPLC method in children samples. Einstein (Sao Paulo). 2010;8:187–191.
8. Bacci MR, Cavallari MR, de Rozier-Alves RM, et al. The impact of lipocalin-type-prostaglandin-D-synthase as a predictor of kidney disease in patients with type 2 diabetes. Drug Des Devel Ther. 2015;9:3179–3182.
9. Selvin E, Juraschek SP, Eckfeldt J, et al. Within-person variability in kidney measures. Am J Kidney Dis. 2013;61:716–722.
10. Cheng L, Sun X, Scicluna BJ, et al. Characterization and deep sequencing analysis of exosomal and non-exosomal miRNA in human urine. Kidney Int. 2014;86:433–444.
11. Uehara Y, Makino H, Seiki K, et al. Urinary excretions of lipocalin-type prostaglandin D synthase predict renal injury in type-2 diabetes: a cross-sectional and prospective multi-centre study. *Nephrol Dial Transplant*. 2009;24:475–482.

12. Wu J, Ding Y, Zhu C, et al. Urinary TNF-α and NGAL are correlated with the progression of nephropathy in patients with type 2 diabetes. *Exp Ther Med*. 2013;6:1482–1488.

13. Matys U, Bachorzewska-Gajewska H, Malyszko J, Dobrzycki S. Assessment of kidney function in diabetic patients. Is there a role for new biomarkers NGAL, cystatin C and KIM-1? *Adv Med Sci*. 2013;58:353–361.

14. Woo KS, Choi JL, Kim BR, et al. Urinary neutrophil gelatinase-associated lipocalin levels in comparison with glomerular filtration rate for evaluation of renal function in patients with diabetic chronic kidney disease. *Diabetes Metab J*. 2012;36:307–313.

15. Lee CL, Li TC, Lin SY, et al. Dynamic and dual effects of glycated hemoglobin on estimated glomerular filtration rate in type 2 diabetic outpatients. *Am J Nephrol*. 2013;38:19–26.

16. Assal HS, Tawfeek S, Rasheed EA, et al. Serum cystatin C and tubular urinary enzymes as biomarkers of renal dysfunction in type 2 diabetes mellitus. *Clin Med Insights Endocrinol Diabetes*. 2013;6:7–13.

17. Isetti V, Bonventre JV. Biomarkers in acute and chronic kidney diseases. Acute kidney injury. In: Taal MW, Brenner, BM, Rector FC, eds. *Brenner and Rector’s The Kidney*. 9th ed. Philadelphia, PA: Elsevier/Saunders; 2012:1016–1043.

18. Bolignano D, Lacquaniti A, Coppolino G, et al. Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. *Clin J Am Soc Nephrol*. 2009;4:337–344.

19. Chou KM, Lee CC, Chen CH, et al. Clinical value of NGAL, L-FABP and albuminuria in predicting GFR decline in type 2 diabetes mellitus patients. *PLoS One*. 2013;8:e54863.

20. Li J, Shi M, Zhang H, et al. Relation of homocysteine to early nephropathy in patients with type 2 diabetes. *Clin Nephrol*. 2012;77:305–310.

21. Zoppini G, Galletti A, Targher G, et al. Lower levels of 25-hydroxyvitamin D3 are associated with a higher prevalence of microvascular complications in patients with type 2 diabetes. *BM J Open Diabetes Res Care*. 2015;3:e000058.