Biomarkers in diabetic kidney disease

Measures to assess kidney function and injury in the diabetic population are currently either inadequate or manifest late in the progressive phase, leading to extensive irreversible damage. Recent efforts to identify novel biomarkers in the diabetic population have been evolving either to detect early kidney injury or repair that ultimately lead to progression of diabetic kidney disease. Emerging roles of urinary biomarkers have proven beneficial due to the ability to standardize the various markers to creatinine or peptides already present in the urine. The role of neutrophil gelatinase-associated lipoprotein, kidney injury molecule-1 and podocin derivatives have gained enormous significance in the process of early identification of kidney injury in the diabetic population. The recent identification of various novel genomic and proteomic applications in investigations of acute kidney injury and chronic kidney diseases has garnered much attention. This article will discuss the evolution of novel biomarkers of diabetes early in the disease process and focus on the new applications being utilized for better understanding of the disease process and early detection.

KEYWORDS: ADMA ■ asymmetrical dimethylarginine ■ kidney injury molecule-1 ■ KIM-1 ■ MMP-9 ■ neutrophil gelatinase-associated lipocalcin ■ NGAL ■ TGF-β

Jaya P Buddineni[1], Kunal Chaudhary[2,3] & Adam Whaley-Connell[†2,3]

[1]University of Missouri-Columbia School of Medicine, Department of General Internal Medicine, Columbia, MO 65212, USA
[2]Harry S Truman VA Medical Center, 800 Hospital Drive, Columbia, MO 65211, USA
[3]University of Missouri-Columbia School of Medicine, Department of Internal Medicine, Division of Nephrology & Hypertension, Columbia, MO 65212, USA
[†]Author for correspondence:
Tel.: +1 573 882 7992
Fax: +1 573 884 4820
whaleyconnella@health.missouri.edu

Diabetes has become a global public health dilemma and will continue to pose a major challenge throughout the 21st century for healthcare providers. The prevalence of diabetes (both diagnosed and undiagnosed) is approximately 14% of the adult population in the USA according to a 2007 census, and this number is estimated to increase to 21–33% by the year 2050 [101]. All-cause mortality attributable to diabetes in the adult population in North America and the Caribbean region is higher than the general population for cardiovascular disease and cancer [1]. Past studies have shown that the onset and progression of Type 2 diabetes mellitus (T2DM) in high-risk individuals could be prevented and/or postponed with lifestyle modification and medications [2,3]. Recognizing individuals with a high risk of developing diabetes has therefore become an essential process for targeting preventive measures. Even though numerous risk equation scores based on genetic factors [4], clinical indicators [5] and lifestyle modifiers [6] have been published, none of them have been established as a universal practical tool for prediction of complications related to diabetes. Thus, the emergence of biomarkers has gained significant interest both to improve clinical predictions of diabetes and also to understand the pathogenesis of the disease.

The most commonly used measures for detection of kidney function in the diabetic population by healthcare providers, such as serum creatinine, calculation of glomerular filtration rate (GFR) and proteinuria, largely identify an underlying pathologic process that is well established [7]. Eventually, this has led to an increased effort to identify new biomarkers that reflect kidney function and injury that can ultimately predict the progression or regression of kidney damage early in the disease process. Newer technologies, such as genomic analysis involving RNA subtraction, DNA microarrays and proteomic approaches, to identify novel proteins that reflect renal injury are rapidly emerging. Urine itself is being used as a diagnostic medium for noninvasive detection of biomarkers. Although these biomarkers are not used in clinical settings at present, there is potential for clinical utility of new biomarkers in diabetic kidney disease. They may assist in the early detection of kidney disease in the preproteinuric phase as well as monitor the progression of kidney disease and help in differentiating diabetic kidney disease from other causes of proteinuria, thus enabling diagnosis and management of the disease process.

Albuminuria & renal injury

Albumin is the most abundant water soluble protein in plasma, originating from the liver. Under normal physiological conditions, most of the urinary albumin filtered through the glomerulus is later reabsorbed by proximal tubular cells. In diabetics, alterations in glomerular

part of future medicine fsg

filtration or proximal tubule reabsorption contribute to increased excretion of urinary albumin. Albuminuria (proteinuria) in diabetic patients is an independent risk factor for development of both renal and cardiovascular complications in diabetic and general populations [8,9]. Importantly, some investigators believe albumin *per se* may play a direct role in the progression of renal disease [9]. The most commonly used biomarker in the diabetic population is microabluminuria. Microalbuminuria is defined as excretion of 30–300 mg/day of albumin in the urine [10]. In the T2DM population, 20–40% of subjects develop microabluminuria within 10–15 years of diagnosis [11]. Therefore, all the hemodynamic changes in the glomeruli causing glomerular hyperfiltration and altered vascular endothelial tone must have occurred before the onset of microabluminuria, suggesting significant structural injury [12].

The mechanisms and pathogenesis of diabetic nephropathy are complex and multifactorial. The primary glomerular changes in diabetic nephropathy are diffuse and nodular glomerusclerosis with involvement of the glomerular basement membrane, leading to a five- to tenfold thickening of the glomerular basement membrane. Other glomerular changes include mesangial widening and hyaline deposits in the glomerular arterioles, particularly the efferent arteriole. Immunofluorescent staining reveals linear deposits of IgG, due to nonspecific adsorption on the glomerular capillary wall, usually with concurrent albumin deposition [7]. The combination of hyperglycemia, glycated proteins and oxidative stress cause an undue strain on the hemodynamic pathways and activate metabolic pathways that induce a group of growth factors in the kidney. Oxidative stress plays an important role in pathogenesis and various candidate biomarkers of oxidative stress have been mentioned in the literature for the diabetic population, including hydrogen peroxide, malonyldialdehyde, thiobarbituric acid reacting substances, superoxide dismutase, glutathione peroxidase and carbon monoxide [13–15]. The use of breath microassays of volatile organic compounds, which are produced by oxidation of polyunsaturated fatty acids in membranes, have been studied in the past [16–19]. Oxidative age, a new breath biomarker of oxidative stress, was developed to compare the abundance of alkanes and monomethylated alkanes in an individual's breath methylated alkane contour to the expected abundance in normal subjects of the same age [20]. Numerous mechanisms and pathways have been suggested to contribute towards diabetes-induced oxidative stress. Hyperglycemia-induced mitochondrial reactive oxygen species production has been studied to be a key event in the development of diabetic complications [21,22]. Urinary 8-hydroxydeoxyguanosine, a breakdown product related to oxidative injury to mitochondrial DNA, was studied as a key sensitive biomarker of intracellular oxidative stress *in vivo*. In another study, urinary 8-hydroxydeoxyguanosine excretion was shown as a very significant clinical marker to predict the development of diabetic nephropathy [23].

Even though the etiology of diabetic kidney disease is primarily glomerular, the involvement of tubulointerstitial injury is quite commonly noted and increasingly recognized. The extent of tubulointerstitial damage is thought to be a better indicator of long-term prognosis than the severity of damage to glomeruli [24,25]. Tubulointerstitial fibrosis is also noted to be caused by renin-angiotensin system activation and the nonhemodynamic effects of Ang II mediated by Ang I and Ang II receptors located in the glomerulus. In addition, the production of various prothrombotic factors, such as TGF-β, CTGF, EGF and other chemokines, are thought to play a major role in renal injury [26,27]. Many of these various biomarkers seem to appear long before the onset of microalbuminuria in urine, making them a significant source for the emerging platform for future research studies.

Biomarkers

Up to 25% of newly diagnosed T2DM patients already show evidence of systemic inflammation at the time of diagnosis, suggesting the disease process has been ongoing for a period of time [28]. The development of various biomarkers has initially focused on early detection of acute kidney injury along with etiology (i.e., ischemia vs toxin related) and location of tubular injury (i.e., proximal vs distal). Therefore, understanding the disease process and identification of early-onset biomarkers reflecting renal injury would be beneficial.

Inflammatory biomarkers

Recently, neutrophil gelatinase-associated lipocalcin (NGAL) and IL-18 have derived most of the attention in relation to acute kidney injury. Matrix metalloproteinases (MMPs) are a class of zinc-dependent proteinases that act in the degradation and turnover of extracellular matrix proteins. Of the many mammalian MMPs currently discovered, MMP-9 was noted to be in higher

concentrations in persons with obesity, metabolic syndrome and T2DM [29–31]. Binding of NGAL with MMP-9 has been demonstrated to decrease the degradation of these complexes and enhance their proteolytic activity [32]. Hence, the detection of urine NGAL has been shown to be sensitive for renal damage in T2DM, and NGAL along with MMP-9 in urine has also been noted in increasing concentrations in Type 1 diabetes mellitus (T1DM) [32–35]. Another study demonstrated the use of urinary NGAL and IL-18 as early predictors of needing dialysis and 3-month recovery of graft function in kidney transplant patients [36]. Both urinary and serum NGAL predicted chronic kidney disease (CKD) progression independently of age and estimated GFR [37].

Studies have identified that increased mRNA expression of NGAL in diabetic/obese mice and obese human beings is closely associated with insulin resistance and hyperglycemia [38]. A recent study conducted by Yang *et al.* demonstrated trending down of serum NGAL concentrations from the normoalbuminuria group to the macroalbuminuria group [34]. Urinary NGAL concentrations were noted to be higher in the macroalbuminuric group compared with the normoalbuminuric group. As the disease progression continued, urinary concentrations of NGAL were elevated due to the decreased absorptive functions of nephric tubules and the increased secretion of urine. Moreover, urinary NGAL was negatively correlated with MDRD (modification of diet in renal disease [calculation of GFR]) GFR and positively correlated with cystatin C, serum creatinine and blood urea nitrogen, thus suggesting the role of NGAL as a biomarker in the diabetic population [34].

Increasing concentrations of MMP-9 (gelatinase B) have been observed in subjects with obesity, metabolic syndrome and T2DM [30,31,39]. A recent study carried out by Thrailkill *et al.* demonstrated the positive correlation of urinary NGAL and MMP-9 excretion with HbA_{1c} and fasting plasma glucose. Moreover, they observed that the urinary concentrations of NGAL or MMP-9 excretions were higher in T1DM subjects with a longer duration of action. They also reported a significant increase in urinary MMP-9 concentrations with elevated urinary albumin excretion for T1DM subjects [32].

Urinary peptidomes

Qualitative detection of various urinary proteins may aid our understanding of the mechanisms that lead to CKD and thus may serve as early biomarkers to diagnose kidney injury. A recent study carried out in diabetic patients by Merchant *et al.* demonstrated increased expression of urinary proteins, such as cadherin-like protein FAT suppressor 2, inositol pentakisphosphate 2-kinase and zona occludens-3, in patients with early renal function decline as compared with patients with stable renal function. Furthermore, it also emphasized the decreased urinary expression of peptide fragments of extracellular matrix proteins, such as (α-I(IV) collagen α-I(V) collagen and tenascin-X), in patients with early renal function decline compared with normal subjects [40].

Podocin mRNA

Detection of podocytes and their fragments in the urine of subjects with glomerular disease has been an area of increasing focus for the past decade. It has been observed that the loss of podocytes in urine is associated with progressive worsening of CKD [37,41,42]. The presence of mRNAs of three different podocytes (nephrin, podocin and synaptopodin) has been demonstrated in the urinary sediment of patients with proteinuria [43]. The investigators concluded that the urinary expression of nephrin and podocin was useful for detecting the rate of progression of CKD compared with expression of synaptopodin in urine. In a rat model of podocyte injury, a single injection of diphtheria toxin resulted in the initial elevation of proteinuria, podocin and nephrin followed by a second peak 1 week later, which was only podocin-positive and nephrin-negative [44]. A study of *in vivo* podocyte damage using continuing versus transient injury along with primary and secondary injury concluded that the expression of viable podocytes in urine correlated with the active ongoing phase of glomerular damage compared with proteinuria, which could not differentiate ongoing damage from persistent glomerular defects [45]. In a study performed in 29 patients with CKD and 10 healthy controls who underwent kidney biopsy, investigators concluded the expression of TGF-β mRNA to have a direct correlation with the degree of tubulointerstitial injury and glomerular filtration rate [46].

Genetic biomarkers

The genetic basis for identification of susceptibility foci in T2DM has been undergoing extensive evolution in the past decade [47]. A recent study carried out in a Chinese population suggests the presence of a susceptibility locus at

13q31.1 as a novel biomarker in subjects with T2DM [48]. Genome-wide association studies conducted in Europe to identify susceptibility loci for GFR, estimated serum creatinine, cystatin C and CKD reported significant SNP associations with CKD subjects at the *UMOD* locus. As reported in the study, *UMOD* encodes Tamm Horsfall protein, the most common protein in human urine. Rare mutations of the *UMOD* locus are associated with the mendelian form of kidney disease [49,50]. In another study, carried out in a Japanese population, the identification of two new loci at *UBE2E2* on chromosome 3 and in *C2CD4A-C2CD4B* on chromosome 15 was associated with increased susceptibility towards development of T2DM in the Japanese population; this finding was also replicated in other east Asian populations [51]. Further research exploring genome-wide association studies would be beneficial to better conceptualize the biomarkers in the diabetes population.

Other biomarkers

Numerous other biomarkers have been a focus of interest and are being exclusively reviewed to predict the onset and progression of kidney injury. Plasma levels of asymmetrical dimethylarginine (ADMA) have been found to be elevated in patients with CKD and a strong predictor of future cardiovascular events in CKD populations [52]. Investigators have demonstrated the correlation between ADMA and progression of CKD [53]. ADMA concentrations have been shown to be elevated in T1DM patients who do not suffer from any diabetic vascular complications. Further studies are necessary in order to establish the role of ADMA in early detection or progression of diabetic compliations [54,55].

An evaluation of 31 novel biomarkers as predictors for clinically incident diabetes using a large population-based cohort followed-up for 10 years has been performed. After accounting for classic risk factors, it was concluded that the presence of adiponectin, apoB, CRP, IL-1ra and ferritin were the strongest risk factors for incident diabetes [56]. Other investigators have demonstrated the correlation of increasing levels of urinary liver fatty acid-binding protein (L-FABP) in transgenic cisplatinum-induced mouse models and the extent of histological injury along with glomerular filtration rates [57]. Another study demonstrated the increasing levels of urinary kidney injury molecule-1 (KIM-1) in patients with ischemic acute tubular necrosis as compared with other forms of CKD and acute renal failure [58]. Recent studies reported increased levels of urinary KIM-1 and *N*-acetyl-β-D-glucosaminidase (NAG) in patients with T1DM associated with microalbuminuria in comparison with diabetics associated with normoalbuminuria and nondiabetic healthy controls. As reported in the study, low urinary levels of KIM-1 and NAG were associated with regression of microalbuminuria during a 2-year follow-up [59].

It was also noted that urinary levels of KIM-1 and NAG were significantly elevated in T1DM subjects associated with microalbuminuria. The fact that proximal and interstitial cells respond to high concentrations of glucose with production of profibrotic mediators supports the theory that tubulointerstitial disease may be primary rather than secondary in diabetes mellitus [60,61].

Conclusion

Several novel biomarkers of kidney injury, as highlighted in this article, are under the scope of evolving research. Most of these biomarkers still need to be validated with larger prospective randomized cohorts before their routine use in diabetic populations. The advancements in the field of proteomic techniques and other biomarker panels would make their use more accessible and reliable in the near future. These techniques will also provide insight into the pathogenesis of diabetes, and guide in prevention and treatment. The role of KIM-1 and NAG in the diagnosis and monitoring of the course of renal disease in the diabetic population looks very promising. Thus, in future some of these biomarkers may play a role in the early detection and progression of diabetes as compared with the currently available tools. Furthermore, ongoing genome-wide association studies will help to better understand the biomarkers of diabetes and could open new avenues for diagnosis and management of kidney disease.

Future perspective

More studies need to be conducted to analyze novel risk factors for diabetes risk prediction, prevention and early diagnosis of kidney disease. Robust and large-scale studies are required to evaluate the clinical role of biomarkers in diabetic nephropathy, thus leading to early diagnosis as well as a better understanding of its pathogenesis. Although most of the studies evaluating diabetic nephropathy are related either to a single biomarker or a group of related biomarkers, incorporating information from multiple biomarkers with

diverse pathophysiological pathways could be beneficial and might prove to be more consistent. Further research exploring genome-wide association studies would be beneficial to better conceptualize the genetic biomarkers in the diabetic population. With further evolving research in proteomic techniques and genetic studies, it might be possible for early detection of diabetic kidney disease even in the preproteinuric phase.

Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

Executive summary

- Although none of the biomarkers are currently used in clinical settings, there is potential clinical utility of new biomarkers in diabetic kidney disease. They may assist in early detection of kidney disease in the preproteinuric phase as well as monitoring the progression of kidney disease and help to differentiate diabetic kidney disease from other causes of proteinuria, thus aiding in diagnosis and management of the disease process.
- Utility of biomarkers, such as IL-18, neutrophil gelatinase-associated lipocalcin and kidney injury molecule-1, are evolving to distinguish the site and degree of impairment in chronic kidney disease (CKD) patients. More importantly, the elevation of urinary levels of kidney injury molecule-1 and *N*-acetyl-β-D-glucosaminidase are noted in the Type 1 diabetes mellitus population with microalbuminuria. Plasma levels of asymmetrical dimethylarginine have been observed to be elevated in patients with CKD and noted as a strong predictor of future cardiovascular events in CKD populations. Asymmetrical dimethylarginine concentrations are known to be elevated in the Type 1 diabetes mellitus population without any known vascular complications.
- Identification of two new loci at *UBE2E2* on chromosome 3 and in *C2CD4A-C2CD4B* on chromosome 15 are associated with susceptibility of CKD in Type 2 diabetes mellitus populations.
- Detection of urinary podocytes and their fragments has been shown to be useful in determining the rate of CKD worsening in proteinuric patients.
- Urinary 8-hydroxydeoxyguanosine excretion has been shown to be a very significant clinical marker to predict the development of diabetic nephropathy.

Bibliography

Papers of special note have been highlighted as:
- ▪ of interest
- ▪▪ of considerable interest

1 Roglic G, Unwin N: Mortality attributable to diabetes: estimates for the year 2010. *Diabetes Res. Clin. Pract.* 87(1), 15–19 (2010).

2 Tuomilehto J, Lindstrom J, Eriksson JG *et al.*: Prevention of Type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N. Engl. J. Med.* 344(18), 1343–1350 (2001).

3 Knowler WC, Barrett-Connor E, Fowler SE *et al.*: Reduction in the incidence of Type 2 diabetes with lifestyle intervention or metformin. *N. Engl. J. Med.* 346(6), 393–403 (2002).

4 Lyssenko V, Jonsson A, Almgren P *et al.*: Clinical risk factors, DNA variants, and the development of Type 2 diabetes. *N. Engl. J. Med.* 359(21), 2220–2232 (2008).

5 Wilson PW, Meigs JB, Sullivan L, Fox CS, Nathan DM, D'Agostino RB Sr: Prediction of incident diabetes mellitus in middle-aged adults: the Framingham Offspring Study. *Arch. Intern. Med.* 167(10), 1068–1074 (2007).

6 Lindstrom J, Tuomilehto J: The diabetes risk score: a practical tool to predict Type 2 diabetes risk. *Diabetes Care* 26(3), 725–731 (2003).

7 Adler S: Diabetic nephropathy: linking histology, cell biology, and genetics. *Kidney Int.* 66(5), 2095–2106 (2004).

8 Ruggenenti P, Remuzzi G: Time to abandon microalbuminuria? *Kidney Int.* 70(7), 1214–1222 (2006).

9 Birn H, Christensen EI: Renal albumin absorption in physiology and pathology. *Kidney Int.* 69(3), 440–449 (2006).

10 Toto RD: Microalbuminuria: definition, detection, and clinical significance. *J. Clin. Hypertens.* 6(11 Suppl. 3), 2–7 (2004).

11 Remuzzi G, Schieppati A, Ruggenenti P: Clinical practice. Nephropathy in patients with Type 2 diabetes. *N. Engl. J. Med.* 346(15), 1145–1151 (2002).

12 Hayden MR, Whaley-Connell A, Sowers JR: Renal redox stress and remodeling in metabolic syndrome, Type 2 diabetes mellitus, and diabetic nephropathy: paying homage to the podocyte. *Am. J. Nephrol.* 25(6), 553–569 (2005).

13 Paredi P, Biernacki W, Invernizzi G, Kharitonov SA, Barnes PJ: Exhaled carbon monoxide levels elevated in diabetes and correlated with glucose concentration in blood: a new test for monitoring the disease? *Chest.* 116(4), 1007–1011 (1999).

14 Wierusz-Wysocka B, Wysocki H, Byks H, Zozulinska D, Wykretowicz A, Kazmierczak M: Metabolic control quality and free radical activity in diabetic patients. *Diabetes Res. Clin. Pract.* 27(3), 193–197 (1995).

15 Hartnett ME, Stratton RD, Browne RW, Rosner BA, Lanham RJ, Armstrong D: Serum markers of oxidative stress and severity of diabetic retinopathy. *Diabetes Care* 23(2), 234–240 (2000).

16 Kneepkens CM, Ferreira C, Lepage G, RoyCC: The hydrocarbon breath test in the study of lipid peroxidation: principles and practice. *Clin. Invest. Med.* 15, 163–186 (1992).

17 Kneepkens CM, Lepage G, Roy CC: The potential of the hydrocarbon breath test as a measure of lipid peroxidation. *Free Radic. Biol. Med.* 17, 127–160 (1994).

18 Phillips M: Method for the collection and assay of volatile organic compounds in breath. *Anal. Biochem.* 247, 272–278 (1997).

19 Phillips M, Herrera J, Krishnan S, Zain M, Greenberg J, Cataneo RN: Variation in volatile organic compounds in the breath of normal humans. *J. Chromatogr. B Biomed. Sci. Appl.* 729, 75–88 (1999).

20 Phillips M, Cataneo RN, Cheema T, Greenberg J: Increased breath biomarkers of oxidative stress in diabetes mellitus. *Clin. Chim. Acta.* 344(1–2), 189–194 (2004).

21 Nishikawa T, Edelstein D, Du XL *et al.*: Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 404(6779), 787–790 (2000).

22 Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 414(6865), 813–820 (2001).

23 Hinokio Y, Suzuki S, Hirai M, Suzuki C, Suzuki M, Toyota T: Urinary excretion of 8-oxo-7, 8-dihydro-2′-deoxyguanosine as a predictor of the development of diabetic nephropathy. *Diabetologia* 45(6), 877–882 (2002).

24 Ong AC, Fine LG: Loss of glomerular function and tubulointerstitial fibrosis: cause or effect? *Kidney Int.* 45(2), 345–351 (1994).

25 Nath KA: Tubulointerstitial changes as a major determinant in the progression of renal damage. *Am. J. Kidney Dis.* 20(1), 1–17 (1992).

26 Eardley KS, Zehnder D, Quinkler M *et al.*: The relationship between albuminuria, MCP-1/CCL2, and interstitial macrophages in chronic kidney disease. *Kidney Int.* 69(7), 1189–1197 (2006).

27 Aros C, Remuzzi G: The renin-angiotensin system in progression, remission and regression of chronic nephropathies. *J. Hypertens. Suppl.* 20(3), S45–S53 (2002).

28 Zhang Y, Dall TM, Mann SE *et al.*: The economic costs of undiagnosed diabetes. *Popul. Health Manag.* 12(2), 95–101 (2009).

29 Derosa G, D'Angelo A, Tinelli C *et al.*: Evaluation of metalloproteinase 2 and 9 levels and their inhibitors in diabetic and healthy subjects. *Diabetes Metab.* 33(2), 129–134 (2007).

30 Derosa G, Ferrari I, D'Angelo A *et al.*: Matrix metalloproteinase-2 and -9 levels in obese patients. *Endothelium* 15(4), 219–224 (2008).

31 Glowinska-Olszewska B, Urban M: Elevated matrix metalloproteinase 9 and tissue inhibitor of metalloproteinase 1 in obese children and adolescents. *Metabolism* 56(6), 799–805 (2007).

32 Thrailkill KM, Moreau CS, Cockrell GE *et al.*: Disease and gender-specific dysregulation of NGAL and MMP-9 in Type 1 diabetes mellitus. *Endocrine* 37(2), 336–343 (2010).

33 Devarajan P: Neutrophil gelatinase-associated lipocalin (NGAL), a new marker of kidney disease. *Scand. J. Clin. Lab. Invest. Suppl.* 241, 89–94 (2008).

34 Yang YH, He XJ, Chen SR, Wang L, Li EM, Xu LY: Changes of serum and urine neutrophil gelatinase-associated lipocalin in Type-2 diabetic patients with nephropathy: one year observational follow-up study. *Endocrine* 36(1), 45–51 (2009).

▪▪ **Role of urinary and serum neutrophil gelatinase-associated lipocalin in predicting the progression of diabetic nephropathy.**

35 Bolignano D, Lacquaniti A, Coppolino G *et al.*: Neutrophil gelatinase-associated lipocalin as an early biomarker of nephropathy in diabetic patients. *Kidney Blood Press. Res.* 32(2), 91–98 (2009).

36 Hall IE, Yarlagadda SG, Coca SG *et al.*: IL-18 and urinary NGAL predict dialysis and graft recovery after kidney transplantation. *J. Am. Soc. Nephrol.* 21(1), 189–197 (2010).

37 Bolignano D, Lacquaniti A, Coppolino G *et al.*: Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. *Clin. J. Am. Soc. Nephrol.* 4(2), 337–344 (2009).

38 Wang Y, Lam KS, Kraegen EW *et al.*: Lipocalin-2 is an inflammatory marker closely associated with obesity, insulin resistance, and hyperglycemia in humans. *Clin. Chem.* 53(1), 34–41 (2007).

39 Goncalves FM, Jacob-Ferreira AL, Gomes VA *et al.*: Increased circulating levels of matrix metalloproteinase (MMP)-8, MMP-9, and pro-inflammatory markers in patients with metabolic syndrome. *Clin. Chim. Acta.* 403(1–2), 173–177 (2009).

40 Merchant ML, Perkins BA, Boratyn GM *et al.*: Urinary peptidome may predict renal function decline in Type 1 diabetes and microalbuminuria. *J. Am. Soc. Nephrol.* 20(9), 2065–2074 (2009).

▪▪ **Role of urinary peptide fragments as potential mediators of diabetic nephropathy.**

41 Malyszko J, Malyszko JS, Bachorzewska-Gajewska H, Poniatowski B, Dobrzycki S, Mysliwiec M: Neutrophil gelatinase-associated lipocalin is a new and sensitive marker of kidney function in chronic kidney disease patients and renal allograft recipients. *Transplant Proc.* 41(1), 158–161 (2009).

42 Kriz W: Podocyte is the major culprit accounting for the progression of chronic renal disease. *Microsc. Res. Tech.* 57(4), 189–195 (2002).

43 Szeto CC, Lai KB, Chow KM *et al.*: Messenger RNA expression of glomerular podocyte markers in the urinary sediment of acquired proteinuric diseases. *Clin. Chim. Acta.* 361(1–2), 182–190 (2005).

44 Sato Y, Wharram BL, Lee SK *et al.*: Urine podocyte mRNAs mark progression of renal disease. *J. Am. Soc. Nephrol.* 20(5), 1041–1052 (2009).

45 Yu D, Petermann A, Kunter U, Rong S, Shankland SJ, Floege J: Urinary podocyte loss is a more specific marker of ongoing glomerular damage than proteinuria. *J. Am. Soc. Nephrol.* 16(6), 1733–1741 (2005).

46 Szeto CC, Chan RW, Lai KB *et al.*: Messenger RNA expression of target genes in the urinary sediment of patients with chronic kidney diseases. *Nephrol. Dial. Transplant.* 20(1), 105–113 (2005).

47 Qi L, Cornelis MC, Kraft P *et al.*: Genetic variants at 2q24 are associated with susceptibility to Type 2 diabetes. *Hum. Mol. Genet.* 19(13), 2706–2715 (2010).

48 Shu XO, Long J, Cai Q *et al.*: Identification of new genetic risk variants for Type 2 diabetes. *PLoS Genet.* 6(9), e1001127 (2010).

49 Kottgen A, Glazer NL, Dehghan A *et al.*: Multiple loci associated with indices of renal function and chronic kidney disease. *Nat. Genet.* 41(6), 712–717 (2009).

50 Kottgen A, Pattaro C, Boger CA *et al.*: New loci associated with kidney function and chronic kidney disease. *Nat. Genet.* 42(5), 376–384 (2010).

51 Yamauchi T, Hara K, Maeda S *et al.*: A genome-wide association study in the Japanese population identifies susceptibility loci for Type 2 diabetes at *UBE2E2* and *C2CD4A-C2CD4B*. *Nat. Genet.* 42(10), 864–868 (2010).

52 Zoccali C, Bode-Boger S, Mallamaci F *et al.*: Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: a prospective study. *Lancet* 358(9299), 2113–2117 (2001).

53 Fliser D, Kronenberg F, Kielstein JT *et al.*: Asymmetric dimethylarginine and progression of chronic kidney disease: the mild to moderate kidney disease study. *J. Am. Soc. Nephrol.* 16(8), 2456–2461 (2005).

54 Vallance P, Leone A, Calver A, Collier J, Moncada S: Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 339(8793), 572–575 (1992).

55 Altinova AE, Arslan M, Sepici-Dincel A, Akturk M, Altan N, Toruner FB: Uncomplicated Type 1 diabetes is associated with increased asymmetric dimethylarginine concentrations. *J. Clin. Endocrinol. Metab.* 92(5), 1881–1885 (2007).

▪ **Increased asymmetrical dimethylarginine concentrations in a Type 1 diabetic patient population.**

56 Salomaa V, Havulinna A, Saarela O *et al.*: Thirty-one novel biomarkers as predictors for clinically incident diabetes. *PLoS One* 5(4), e10100 (2010).

57 Negishi K, Noiri E, Doi K *et al.*: Monitoring of urinary L-type fatty acid-binding protein predicts histological severity of acute kidney injury. *Am. J. Pathol.* 174(4), 1154–1159 (2009).

58 Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV: Kidney injury molecule-1 (KIM-1), a novel biomarker for human renal proximal tubule injury. *Kidney Int.* 62(1), 237–244 (2002).

59 Vaidya VS, Niewczas MA, Ficociello LH *et al.*: Regression of microalbuminuria in Type 1 diabetes is associated with lower levels of urinary tubular injury biomarkers, kidney injury molecule-1, and *N*-acetyl-β-D-glucosaminidase. *Kidney Int.* 79(4), 464–470 (2010).

▪ **Urinary levels of kidney injury molecule-1 and *N*-acetyl-β-D-glucosaminidase are associated with regression of microalbuminuria in Type 1 diabetes.**

60 Ziyadeh FN, Snipes ER, Watanabe M, Alvarez RJ, Goldfarb S, Haverty TP: High glucose induces cell hypertrophy and stimulates collagen gene transcription in proximal tubule. *Am. J. Physiol.* 259(4 Pt 2), F704–F714 (1990).

61 Polhill TS, Saad S, Poronnik P, Fulcher GR, Pollock CA: Short-term peaks in glucose promote renal fibrogenesis independently of total glucose exposure. *Am. J. Physiol. Renal Physiol.* 287(2), F268–F273 (2004).

Website

101 Number of Americans with diabetes projected to double or triple by 2050. Center for Disease Control and Prevention Website. Published October 22, 2010 www.cdc.gov/media/pressrel/2010/r101022.html (Accessed 9 November 2010)