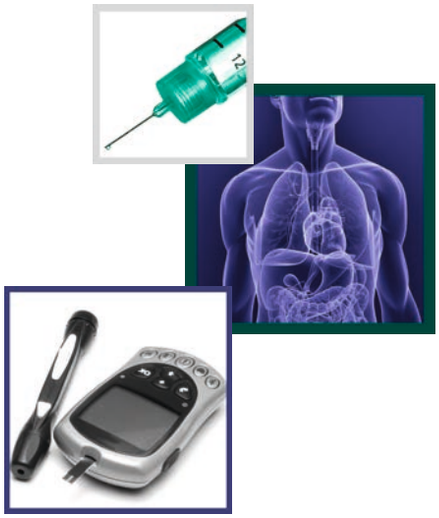
REVIEW

Biomarkers for evaluating renal function decline in diabetes: where are we now?



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Practice Points

- Diabetic nephropathy (DN) is a major cause of increased mortality in diabetes.
- Hypertension and hyperglycemia are well-recognized risk factors.
- Not all of the risk of DN is explained by known risk factors.
- Renal function decline can begin prior to the development of proteinuria.
- A variety of methods exist for measuring estimated glomerular filtration rate (GFR).
- Novel biomarkers such as cystatin C may perform marginally better than serum creatinine for estimating GFR.
- Emerging risk factors for renal function decline include advanced glycation end products, inflammation and insulin resistance.
- Genome-wide association studies may be useful to identify genetic markers associated with renal function decline.

SUMMARY Diabetes causes increased mortality and healthcare costs, and prevalence is increasing worldwide. Diabetic nephropathy is the leading cause of end-stage renal disease, and is closely tied to cardiovascular disease, the main cause of death in people with diabetes. Renal function decline as measured by estimated glomerular filtration rate has emerged as a more reliable marker of kidney damage than urinary albumin, and markers for renal function decline are discussed in this review. Renal damage appears to be related to a series of effects of the hyperglycemia and insulin resistance that accompanies both Type 1 and Type 2 diabetes, including insulin resistance, accumulation of advanced glycation end products, uric acid and oxidative stress, often mediated by genetic susceptibility.

Diabetes and hyperglycemia are major determinants of morbidity and mortality, and the global burden of these disorders is increasing worldwide [1]. Type 2 diabetes (T2D) is the most common form, accounting for 90–95% of all cases of diabetes. T2D has an estimated prevalence of 9.8% in men and 9.2% in women, affecting approximately 25 million individuals in the USA, and nearly 350 million people globally [1]. Increasing obesity levels have contributed to a rising incidence of T2D, particularly in developing nations, and have fueled fears of a

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global epidemic of diabetes. Prediabetes, defined as increased glucose and/or hemoglobin A_{1c} (HbA_{1c}) but not yet clinical diabetes, affects a reported 35% of US adults aged 20 years or older. Type 1 diabetes (T1D) affects approximately 1.4 million people in the USA and 30 million globally [2], and the incidence of the disease has been increasing by 3–5% per year [3]. While not as common as T2D, T1D is usually diagnosed earlier in life, leading to a high burden of complications by middle age. T1D contributes substantially to cardiovascular disease (CVD) and premature death among younger individuals [4,5].

Diabetes is the leading cause of end-stage renal disease (ESRD), responsible for nearly half of all new cases annually, and is the leading cause of severe visual impairment, CVD and premature death in the general population [6]. Healthcare costs for people with diabetes are over twice those for the general population, and diabetes costs the USA at least US$174 billion annually in medical costs and lost income [7,8].

Diabetic nephropathy (DN) has historically been the leading cause of death among people with diabetes. While the incidence of DN has decreased significantly over the past 50 years [9], up to 20–30% of T1D and T2D patients still develop DN [10,11]. Patients with T1D are particularly at risk for progression of DN, as a large proportion of T1D patients who have persistent microalbuminuria and who are not treated continue to have increased excretion of protein in the urine and develop overt proteinuria within 10–15 years [12]. Half of people with T1D who have overt proteinuria progress onto kidney failure within 10 years, and within 20 years this percentage jumps to 75% if they are not treated [12]. While patients with T2D are less likely to progress to ESRD, only approximately 20% of those with overt proteinuria progress to kidney failure after 20 years [12], many patients with T2D already have significant levels of microalbuminuria and even overt proteinuria at diagnosis, due to the substantial burden of undiagnosed T2D with a reported 4–7-year lag time between development of T2D and diagnosis [13]. Improvements in treatment have resulted in reduced progression of macroalbuminuria, such that the majority of these patients do not progress, and pancreatic transplantation in patients with T1D has been shown to improve kidney function, even among proteinuric patients [14,15]. Finally, ESRD rates and mortality in T1D patients with DN remain high and have not improved since 1991 [16]. As a result, prevention of DN and treatment of proteinuric patients is critical to reducing the burden of ESRD and related mortality.

Furthermore, recent studies have suggested that mortality among T1D subjects who are free from DN may not differ from that in the general population [17,18], suggesting that DN is responsible for much, if not all, of the increased risk for mortality in people with T1D. The likely explanation is that DN accelerates CVD – the main cause of death in all patients with diabetes today [19,20]. Therefore, understanding protective and predictive factors for DN is a major public health goal.

Risk factors for DN

Hyperglycemia and hypertension are major risk factors for DN, but they do not explain all of the risk. The Diabetes Control and Complications Trial (DCCT) in T1D [21] and the United Kingdom Prospective Diabetes Study (UKPDS) in T2D [22] have established that reducing hyperglycemia has significant and sustained effects on reduced microvascular complications, including retinopathy, neuropathy and DN [21]. Hypertension is often present in people with T2D by the time they are diagnosed [23], often secondary to the presence of obesity [24], insulin resistance and the metabolic syndrome [12]. In patients with T1D, on the other hand, hypertension often develops as a result of microalbuminuria. Regardless of the etiology of the hypertension, both systolic and diastolic hypertension accelerate the course of renal function decline, and antihypertensive treatment is the standard of care to reduce the progression of diabetic kidney disease, with an associated decrease in mortality from 94 to 45%, and over a 40% decrease in the number of patients requiring kidney dialysis or transplantation over 16 years [12]. However, substantial improvements in both HbA_{1c} levels and blood pressure control have reduced but not removed the risk of DN in people with diabetes [25,26], demonstrating that there are additional risk factors and potential areas for further intervention.

While current glycemic control certainly plays a role in DN, there is also evidence of a 'metabolic memory' from glycemic control in prior years, and this is hypothesized to be related to the accumulation of advanced glycation end products (AGEs) [27]. Inflammation, insulin

resistance and elevated uric acid have also been suggested to play a role in the development of DN, and underlying genetic susceptibility has been investigated among patients with T1D utilizing large-scale genome-wide association studies (GWAS).

In this review, we will discuss the epidemiology of renal function decline in diabetes, and the current level of evidence for these biomarkers of DN risk. Articles were accessed via PubMed, and searches included keywords of 'diabetes', 'type 1 diabetes', 'type 2 diabetes', 'diabetic nephropathy' and 'biomarkers'.

Development of DN & renal function decline

The paradigm for the natural history of diabetic nephropathy used to be that microalbuminuria is an early marker of renal damage, which may then lead to overt proteinuria and eventual end-stage renal disease (ESRD). Treatment can reverse microalbuminuria, and it was presumed that a return to normal urinary albumin levels indicated a reversal in the disease process. However, biopsy studies have revealed that lesions are already present by the time microalbuminuria is detected [28], and these lesions and the disease process can progress even when treatment is able to reverse microalbuminuria [29]. In a Pima Indian population with Type 2 diabetes who were followed longitudinally for renal function decline, 32% of those with normal levels of urinary albumin at baseline experienced renal function decline over 4 years, while 42% of those with microalbuminuria and 74% of patients with macroalbuminuria had renal function decline. Therefore, microalbuminuria only resulted in a slight increase in the progression of renal function decline [30]. Decline in renal function was associated with a nearly fivefold increased risk for ESRD over 10 years in this population.

Studies of the natural history of diabetic nephropathy have resulted in a shift in the paradigm from a focus on microalbuminuria as the earliest sign of kidney damage, and a recognition of the need for novel biomarkers that can detect changes in renal function prior to the development of the lesions that occur by the time patients demonstrate microalbuminuria. Furthermore, it has been demonstrated that renal function as measured by glomerular filtration rate (GFR) often declines significantly prior to the development of microalbuminuria [31,32], perhaps pointing to earlier clinical manifestations of diabetic kidney disease.

Definition of renal function decline

ESRD is defined as a GFR of less than 15 ml/min/1.73 m^2, requiring renal replacement therapy (renal maintenance dialysis or renal transplantation), whereas normal renal function is defined as the absence of renal damage (including normoalbuminuria) and a GFR ≥90 ml/min/1.73 m^2. The process through which renal function declines from normal levels to ESRD is extremely variable among individuals with DN, and was long thought to begin only once overt proteinuria was present. However, recent studies have demonstrated significant declines in renal function much earlier in the disease process, when only microalbuminuria is present. In a study of 79 patients with Type 1 diabetes and incident microalbuminuria who were followed for 12 years, progression to advanced chronic kidney disease stages (CKD 3–5) occurred without overt proteinuria preceding the development of advanced CKD [33]. In fact, among the 23 patients who progressed to advanced CKD, 11 had either persistent microalbuminuria or reverted to normoalbuminuria, while the 12 who had proteinuria developed CKD and proteinuria at the same time.

As a result, it is now better understood that the process of renal damage in diabetes begins before the development of overt proteinuria, and primary prevention efforts must occur before the development of microalbuminuria. Furthermore, not all patients with microalbuminuria progress to further stages of diabetic kidney disease, and a significant proportion regress to normoalbuminuria, particularly when hypertension and hyperglycemia are appropriately treated. In a cohort of patients with T2D followed over 8 years in Japan, 21% of microalbuminuric patients regressed, while only 17% progressed to macroalbuminuria [34].

The detection of early renal function decline through serial examinations of estimated GFR can provide the opportunity to detect evidence of renal damage prior to the development of microalbuminuria [35], or in the setting of regression of microalbuminuria, when damage to the kidney may continue to occur despite the return to normoalbuminuria. Furthermore, the regression of microalbuminuria does not always indicate that renal function has returned to normal, as renal damage on biopsy has been shown to be

present even before microalbuminuria [36], and renal function can continue to decline despite a return to normoalbuminuria.

The decline in renal function is often examined as the change in estimated or measured GFR per year, and varies among individuals with DN from 2 to 20 ml/min/year [12]. Of note, once DN is present, the fall in GFR is similar between people with T1D and T2D.

Measuring renal function

The direct measurement of GFR can be accomplished using tracer clearance testing with iothalamate, inulin or other tracers, but this is not practical for large epidemiologic studies. A number of methods for estimating GFR have therefore been developed in order to track renal function decline and response to therapies within populations. Traditionally, calculated GFR has been based on serum levels of creatinine. However, serum creatinine levels are affected by factors such as muscle mass, gender, race and age, which are usually accounted for in calculations of GFR based on serum creatinine. However, there is concern that a level of inaccuracy could still be introduced by the effect of factors independent of kidney function that affect serum creatinine levels. More recently, cystatin C has emerged as a novel marker of kidney function, perhaps less susceptible to factors outside of kidney function [37]. In patients with T1D, serial measurements of cystatin C have been shown to be superior to serum creatinine-based GFR estimates for detecting a decline in renal function [38]. In a study comparing GFR measured directly using inulin with estimated GFR using cystatin C or serum creatinine via the 4-variable Modified Diet in Renal Disease (MDRD) equation, cystatin C was able to accurately detect increases in GFR during hyperglycemic clamp studies, and performed better than the serum creatinine-based estimated GFR [39]. Similarly, in a group of 210 subects with T2D, GFR was measured using tracer clearance, and estimated GFR using serum creatinine-based equations and cystatin C were compared [40]. Cystatin C was more accurate and less biased than the serum creatinine-based equations, and performed particularly well in patients with very poor control (HbA_{1c} >10.8%).

It remains unclear whether the use of cystatin C-based estimated GFR improves clinical decision-making, and the assay cost is higher than serum creatinine, and so serum cystatin C has not been widely used clinically. A number of methods for estimating GFR have therefore been developed; some based on serum creatinine, some based on cystatin C and some using both markers to estimate GFR.

Risk factors for renal function decline

■ AGE–RAGE axis

Poor glycemic control is closely tied to DN [41]. A sustained effect of hyperglycemia on long-term risk for complications (a 'metabolic memory') has been demonstrated by the Epidemiology of Diabetes Interventions and Complications (EDIC) follow-up study of the DCCT [21]. Despite similar levels of HbA_{1c} in the intensive and standard treatment groups following the end of the DCCT, the intensively treated study subjects were less likely to develop DN over the ensuing 12 years.

The 'metabolic memory' is speculated to be at least partly explained by the accumulation of AGEs [42]. AGEs develop as a result of hyperglycemia, inflammation and increased reactive oxygen species, and AGEs bind to the cellular receptor (RAGE) to form cross-linked proteins that are not reversible. As shown in **Figure 1**, bidirectional relationships between the inflammation and hyperglycemia that accompany diabetes contribute to the AGE–RAGE complexes, and may contribute to both microvascular and macrovascular complications [43].

A RAGE knock-out mouse model has demonstrated that the deletion of RAGE protects from glomerular basement membrane thickening, podocyte damage, mesangial sclerosis and the development of albuminuria [44]. Soluble RAGE (sRAGE) can bind AGEs and prevent the formation of AGE–RAGE complexes; higher levels of sRAGE may protect from diabetes complications [45,46]. On the other hand, associations of higher sRAGE with mortality have been reported [47], and little is known regarding AGE–RAGE activation and the risk of DN in diabetes. These inconsistent results may reflect imprecise measurement of tissue AGEs.

The EDIC Study reported higher levels of skin collagen AGEs (N[ε]-[carboxymethyl] lysine [CML] and pentosidine) in T1D diabetic patients with microvascular complications than in those without, adjusting for HbA_{1c} [48]. However, the levels of the AGEs did not differ between previous DCCT intensive and standard treatment groups, suggesting a mechanism independent of recent glycemic control.

Inflammation

Diabetes and diabetic nephropathy appear to be associated with a chronic, low-grade inflammation [49,50]. Increased inflammatory markers including TNF-α, soluble receptors I and II, IL-1, IL-6, IL-8 and MCP-1 are associated with decreasing renal function in both T1D [51–53] and T2D [54]. Activation of innate immune system Toll-like receptors (TLR) by hyperglycemia is thought to stimulate release of pro-inflammatory cytokines (IL-6, TNF, IL-1 and MCP-1), and this process appears to be mediated by the formation of AGE–RAGE complexes leading to the release of reactive oxygen species (ROS) [55,56].

In a study at the Joslin Diabetes Center (MA, USA), Wolkow and colleagues reported an association between a panel of inflammatory markers and renal function decline in the First Joslin Study of the Natural History of Microalbuminuria in Type 1 Diabetes, showing that levels of all five urinary inflammatory markers (IL-6, IL-8, MCP-1, IFN-γ-inducible protein and macrophage inflammatory protein-1β) were increased among patients with progressive renal function decline [53].

In a cohort of 410 subjects with T2D, elevated levels of TNF receptors 1 and 2 were associated with the risk for developing ESRD over 12 years of follow-up, which occurred in 17 per 1000 patient-years [54].

While improved glycemic control may reduce inflammation, the inflammatory cytokine (TNF-α and IL-6) and Toll-like receptor-mediated pathways have both been linked to DN [54,55], and could be ameliorated by specific anti-inflammatory agents, potentiating the effect of optimal glycemic control.

In animal models of diabetes, higher levels of IL-6 and TNF-α were associated with higher urinary albumin excretion and were reduced by antihypertensive treatment [57,58], and urinary TNF-α levels were positively associated with markers of both glomerular and tubulointerstitial damage [59]. In humans, treatment with irbesartan in the IRMA2 trial of patients with T2D resulted in reduced inflammatory cytokines, including highly sensitive c-reactive protein and fibrinogen, and slowed the increase in IL-6 over 2 years [60]. Furthermore, the changes in IL-6 was correlated with changes in albumin excretion. There are no human data demonstrating that treatment of inflammation decreases lesions in the kidney, however, and so while it is clear that inflammation is increased in diabetes and among people with diabetic nephropathy, this association may not be causal.

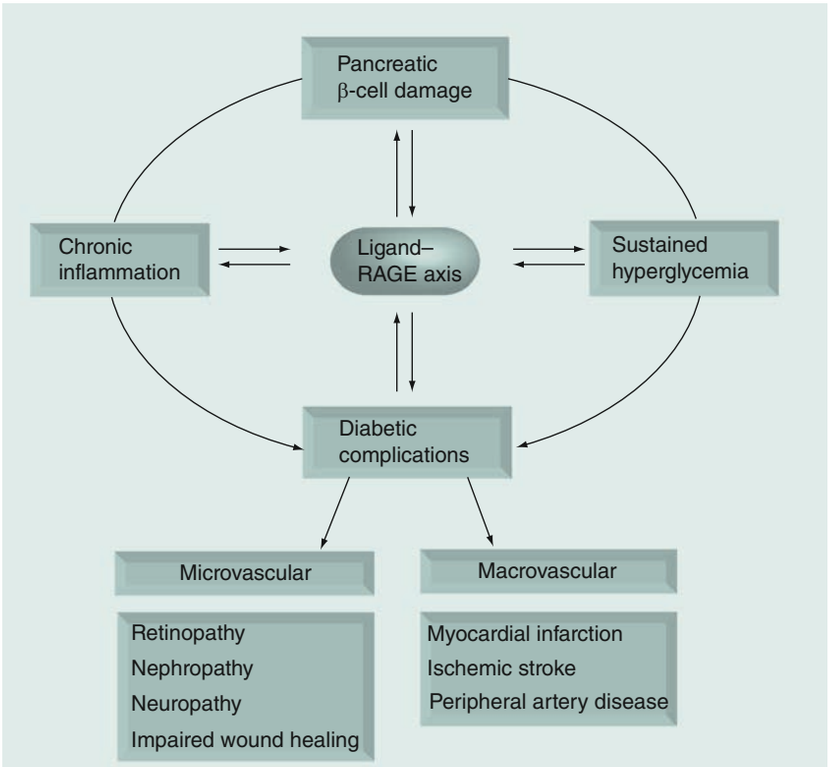

Figure 1. The relationship between the AGE–RAGE axis and chronic inflammation and hyperglycemia in diabetes, leading to both microvascular and macrovascular complications.
Adapted with permission from [43].

Insulin resistance

Insulin resistance is a major risk factor for T2D, and usually precedes the diagnosis of diabetes in these individuals. Obesity increases insulin resistance, and this is one of the mechanisms through which obesity can lead to T2D. It is therefore well established that insulin resistance is a prominent feature of T2D, but less known is the role of insulin resistance in T1D. Adults with T1D have approximately half the insulin sensitivity of adults without diabetes in both skeletal muscle and adipose tissue, even when the level of obesity is similar [61]. The causes and clinical correlates of insulin resistance in T1D patients [61–63] are complex and vastly different from those in T2D or obesity, although rates of obesity are increasing in T1D [64]. Insulin resistance in T1D is primarily driven by iatrogenic peripheral hyperinsulinemia and can already be observed in adolescents [65].

Regardless of the cause of insulin resistance, this appears to be an important risk factor for diabetes complications. Increased insulin resistance is associated with a more atherogenic lipid profile [66] and a more atherogenic lipoprotein cholesterol distribution [67]. As shown in **Figure 2**, insulin resistance can also lead to renal injury through a number of different pathways, including increased inflammation, oxidant stress, decreased nitric oxide generation and direct effects on renal tissues [68]. Insulin resistance can be measured directly with a hyperinsulinemic–euglycemic clamp, but owing to its invasive nature, cost and length, the clamp procedure is not practical for epidemiological or clinical practice. Owing to these challenges, relatively scant data exist on the association between insulin resistance and DN. However, an insulin resistance score developed from clinical factors (estimated glucose disposal rate [eGDR]) [63] strongly predicted overt nephropathy [69].

■ Uric acid

Both observational and mechanistic studies demonstrate that elevated serum uric acid increases the risk of developing DN in people with T1D [70]. Data from the Joslin Diabetes Center, Steno Diabetes Center (Gentofte, Denmark) and the Barbara Davis Center (CO, USA) have consistently demonstrated hyperuricemia to be associated with worsening of renal function in T1D, including early GFR loss [71], transition from normoalbuminuria to microalbuminuria or worse [72], progression of subclinical atherosclerosis [73], and microalbuminuria 18 years later [74].

In a cohort of patients with T1D free from urinary albuminuria (n = 324) and who were followed for 6 years for the development of micro- or macro-albuminuria, higher levels of baseline uric acid predicted progression to albuminuria **(Figure 3)** [72].

In T2D, uric acid levels have been shown to correlate with microalbuminuria as well as with subclinical atherosclerosis, as measured by carotid intima-media thickness [75], and lowering of uric acid decreased renal complications in db/db mice [76]. In clinical trials in humans, there is some evidence that lower uric acid using allopurinol may decrease proteinuria in patients

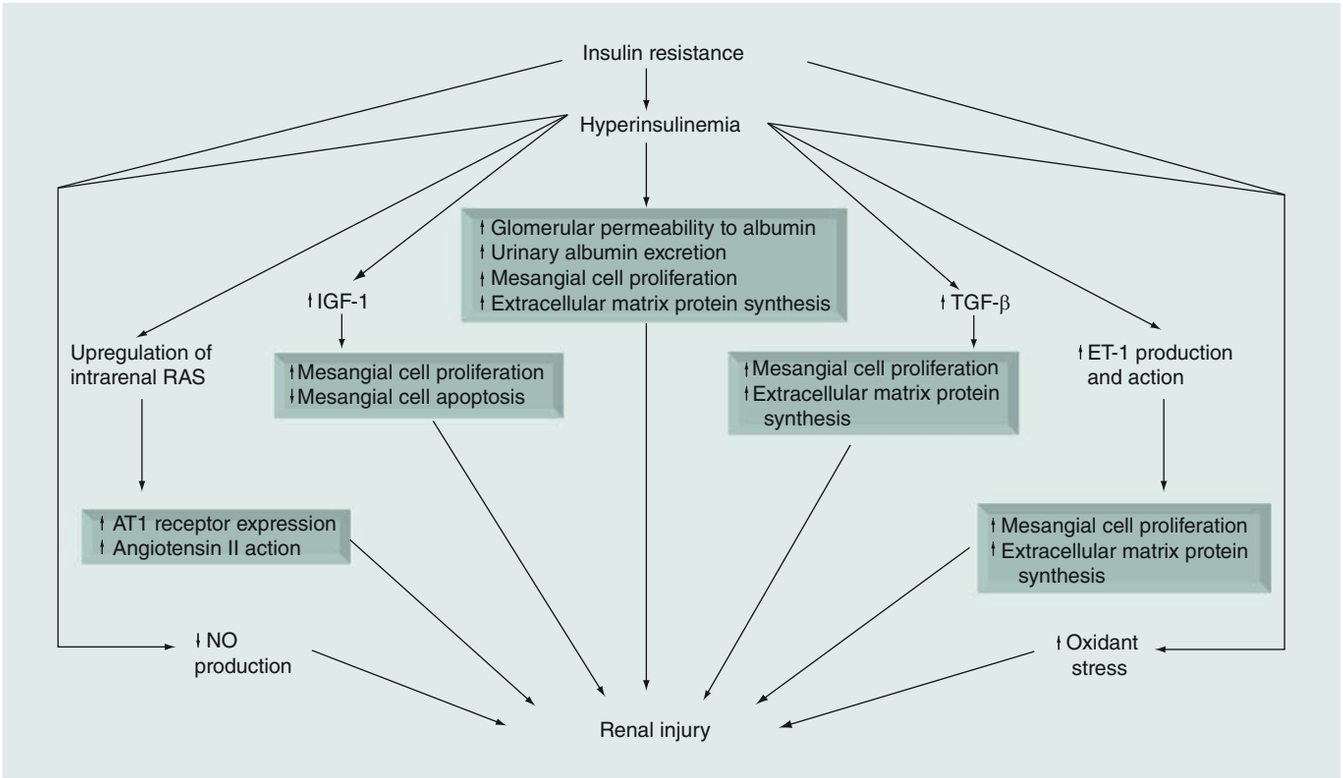

Figure 2. Pathways through which insulin resistance leads to renal injury, including the generation of oxidant stress, inflammatory cytokines and decreased nitric oxide production.
Adapted with permission from S. Karger AG, Basel, Switzerland [68].

with T2D, and that treatment with losartan may exert some of its renal protective effects through decreasing uric acid levels [77].

Mechanistic explanations for uric acid's association with renal function decline include alterations in nitric oxide pathways [78,79] and production of inflammatory cytokines [80,81], and xanthine oxidase generation resulting in increased oxidative stress [82,83].

■ Novel markers of kidney disease: the role of proteomics

Urine proteomics, a rapidly developing research area, holds promise to improve prediction of DN and monitoring of the effect of interventions. Recently, a panel of biomarkers (THP, α-1-acid glycoprotein, clusterin and progranulin) generated from proteomics discovery work was shown to predict the development of microalbuminuria and renal function decline [84]. Urinary markers have also been shown to differ between T1D, T2D and nondiabetic control individuals, shedding light on potential differences in the mechanisms of kidney damage in each type of diabetes [85,86].

The predictive value of novel markers can only be evaluated prospectively using well-characterized cohorts of diabetes patients followed for development of DN using traditional and novel outcomes [87,88]. Collaborative efforts to validate novel methods for predicting DN have proven useful. An example is a recent manuscript describing the development of an equation to predict the development of microalbuminuria, which resulted from the development of the prediction equation in the EURODIAB study, and subsequent validation of this equation in collaboration with the FinnDiane study, the Epidemiology of Diabetes Complications study, and the Coronary Artery Calcification in Type 1 Diabetes Study [88].

■ Genetic markers

Underlying genetic susceptibility to kidney disease and genes associated with diabetic nephropathy has been an area of increasing study, with the advent of large-scale genetic studies such as GWAS, making it possible to identify putative genetic markers predicting CKD, microalbuminuria and renal function decline.

Several GWAS have been conducted with the aim to identify genetic loci associated with CKD and DN. The heritability of kidney disease in patients with T2D has been estimated to be 0.75 for GFR and 0.46 for albumin/creatinine ratio [89], and the heritability of albumin/creatinine was similar in diabetic and nondiabetic family members in a study of 63 families [90], suggesting that the same genetic susceptibility factors are operating in individuals with and without diabetes. Therefore, genetic susceptibility in the setting of hyperglycemia and diabetes may lead to DN.

The idea that diabetes and other chronic conditions increase the effect of underlying genetic susceptibility is supported by a recent GWAS study that found that variants at the uromodulin gene (*UMOD*) were associated with both CKD, and this association was increased among people with diabetes [91]. The *UMOD* gene was also found to be the strongest predictor of both CKD and estimated GFR in a GWAS study conducted in four large population-based studies [92].

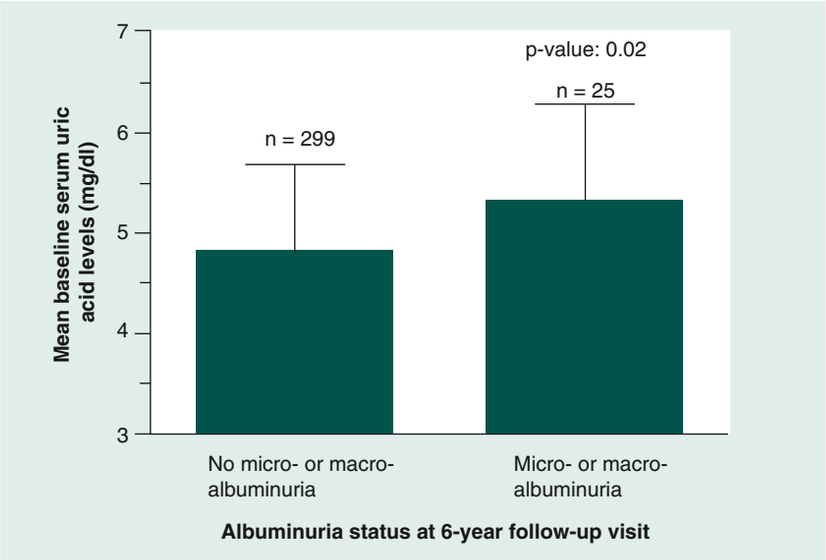

Figure 3. Mean baseline serum uric acid level predicts the development of urinary albuminuria over 6 years of follow-up in adults with Type 1 diabetes. Adapted with permission from [72].

Conclusion & future perspective

While advances in the care of individuals with diabetes have led to improved glycemic control and reduced hypertension, there remains an increased risk of kidney complications in people with both T1D and T2D. Furthermore, reliance on microalbuminuria as a risk marker for progression of diabetic kidney disease has been recognized to have significant limitations, while renal function decline has emerged as a

potential outcome for monitoring the effectiveness of therapies and for determining the risk for both DN and cardiovascular complications.

Novel biomarkers for renal function decline may both help to identify patients at risk for progression to DN who should be treated more aggressively, and may also allow for the monitoring of response to therapy. As discussed in this review, progress has been made towards identifying novel biomarkers for renal function decline, and a greater understanding of the associations between insulin resistance, advanced glycation end products, inflammation and oxidant stress should be the focus of future research.

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