INTRODUCTION

Type 2 diabetes (T2DM) is the major cause of end-stage renal disease. It is estimated that T2DM is the primary cause leading to kidney disease in 20-40% of people starting dialysis. During the present decade, 30% of the predicted $1.1 trillion medical costs of dialysis worldwide will result from diabetic nephropathy. There is an unmet need for highly sensitive biomarkers for the detection of diabetic nephropathy. Currently this disease is not recognized early enough because of inadequate diagnostic methods, which increases the chances that early nephropathy and microalbuminuria will progress toward end-stage renal disease. Novel, more sensitive biomarkers that may be used to detect diabetic nephropathy at an early stage, and possibly also detect disease progression or regression after drug therapy, are discussed in this paper.

Diabetic nephropathy is normally established by the severity of urine albumin excretion. Research indicates that normal excretion is less than 7 mg/d (1, 2) although conventional cut-off for abnormal excretion (onset of microalbuminuria) is 30 mg/d. Excretion of 30–300 mg/d is generally termed microalbuminuria. Albumin excretion rates above 300 mg/d are generally accepted by experts as indicative of diabetic nephropathy (3).

Treatment of T2DM patients can delay the development of microalbuminuria. For example, the ACE inhibitor, trandolapril (4) and the angiotensin-receptor blocker, olmesartan (5) have both shown to delay the T2DM-related microalbuminuria.

The progressive nature of albuminuria is usually considered the result of escalating dysfunction in the capillary endothelium present in the glomerular tuft. The basement membrane and underlying epithelial cells also play important roles (6). Even in the healthy kidney, large amounts of albumin may traverse the glomerular endothelium and associated structures, but tubular rescue or degradation, two separate functions, may be compromised independently in micro- and macroalbuminuria (7). The degradation function may be the dysfunctional pathway in microalbuminuria (8).

As suggested above, excretion of albumin in quantities well below 30 mg/d are likely to be associated with marginally compromised renal function. However, it appears that biomarkers that are even more sensitive and whose presence in urine is associated with injury at specific sites in the nephron could help identify injury and localize it earlier.

Several novel biomarkers of kidney injury have been shown to increase in the urine of individuals with diabetes. These changes appear to occur even before microalbuminuria is established. Evidence is currently available indicating that the following biomarkers have may be useful in assessing early nephron injury in patients with diabetes.

NEUTROPHIL GELATINASE-ASSOCIATED LIPOCALIN (NGAL)

NGAL has been evaluated in several studies of diabetic subjects. In one study (9), NGAL was 5–10-fold higher in normo- or microalbuminuric patients as compared to controls. This indicates that tubular injury (proximal and distal) occurs early and perhaps before albumin excretion in patients with emerging diabetic nephropathy. NGAL levels increased 2–4 fold further when the injury progressed to diabetic nephropathy. Notably, serum NGAL showed an almost identical relationship with progression of diabetic nephropathy. These results were confirmed in another study of T2DM patients with normo-, micro- and macro-albuminuria where urine NGAL was positively correlated with cystatin-C, BUN, serum creatinine, as well as albuminuria, and negatively with GFR (10). Similar studies undertaken in Type 1 diabetic patients have found similar correlations between urine NGAL and progression of albuminuria from absent to severe (11, 12).
**N-acetylglucosaminidase (NAG)**

NAG is a biomarker of proximal tubule injury that has long been used in research and diagnostic settings (13). Recently, Nauta, et al (14), showed that urine NAG increases a surprising 9-fold in normoalbuminuric patients with diabetes compared to controls. It increases further with development and progress of microalbuminuria. Moreover, in another recent study of type 1 diabetic patients (T1DM), regression of microalbuminuria was associated with a highly significant reduction in urine NAG excretion (15).

**Kidney Injury Marker-1 (KIM-1)**

KIM-1 is another biomarker that is associated with proximal tubule injury. In the same study of T1DM patients where urine NAG was found to be correlated with regression of microalbuminuria, KIM-1 was also found to be associated with regression (15). No data were provided dealing with the KIM-1 levels in patients with progressing albuminuria.

Interestingly, in studies of patients with T2DM, KIM-1 was not found to be associated with progression of microalbuminuria (14,16). On the contrary, other biomarkers of proximal tubule injury were associated, including NGAL (14) and NAG (14,15). Studies on whether KIM-1 levels maximize early in kidney injury and, therefore, can not respond to further injury have not been reported.

**π-glutathione-s-transferase (π-GST)**

In a study of more than 450 diabetic patients, π-GST was found to increase across the spectrum of normo-, micro-, and macro-albuminuria (17). A study of children with T1DM also suggested this biomarker may be sensitive to changes in renal function that precede classic microalbuminuria (19). Thus, use of π-GST in assessing kidney injury in diabetic patients may allow distal tubular injury to be distinguished from that occurring in the proximal tubule.

**Cystatin C, β2-Microglobulin, and Type IV Collagen**

Beyond albumin, cystatin C and β2-microglobulin are well established urine biomarkers for monitoring the progression of glomerular injury. In diabetic patients, cystatin C increases with increasing excretion of albumin (14). β2-Microglobulin shows a similar relationship with urinary albumin in such patients (18). It must be noted that urinary levels of these two biomarkers also reflects proximal tubule dysfunction. Because it is specific for injury to the glomerulus, urinary type IV collagen appears to be useful in distinguishing diabetic glomerular injury from proximal tubule damage (17).

**Emerging Markers**

Other markers that may be helpful in following tubular injury in diabetic nephropathy include α-GST and retinol-binding protein 4 (18).

Of the few biomarkers shown to reflect injury to the Loop of Henle only osteopontin has evidence of association with diabetic nephropathy. Animal studies have shown the renal osteopontin expression increases in diabetic nephropathy (20), and serum levels of the biomarker are associated with its progression in humans (21). Studies of urine osteopontin in patients with diabetic nephropathy are currently lacking.

Collecting duct injury has been associated with calbindin D28. Thus, proteomic analysis of diabetic mouse kidney found dramatically upregulated expression of calbindin D28 (22). The biomarker was associated with the proximal and distal tubules, as well as the collecting duct, and it is not yet clear if elevated levels of this biomarker are found in the urine of diabetic patients.

All of these biomarkers are available or under development at Pacific Biomarkers. Please see Table for specifics.

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**Table: Biomarkers for Investigating Progression / Regression of Diabetic Nephropathy**

<table>
<thead>
<tr>
<th>BIOMARKER</th>
<th>SITE OF INJURY</th>
<th>BEST AVAILABLE METHODS</th>
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<td>Albumin</td>
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<td>Proximal Tubule, Distal Tubule</td>
<td>EIA; Automated Clinical Analyzers</td>
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<td>π-GST</td>
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<td>Cystatin C</td>
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<td>β2-Microglobulin</td>
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<td>Type IV Collagen</td>
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<td>Retinol-binding Protein-4</td>
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<tr>
<td>Osteopontin</td>
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