New Markers for Kidney Disease

There is ominous growth in chronic kidney disease among the US population, primarily related to type 2 diabetes (1). The current number of patients on dialysis (~300,000) is growing steadily as the prevalence of end-stage kidney disease grows geometrically with a doubling time of 10 years. The epidemic growth in diabetes, the leading cause of chronic kidney disease, portends continuation of this trend (2). Most patients with end-stage kidney disease will end up on dialysis because the supply of kidneys for transplants is limited to ~13,000 per year. A further warning sign from the National Health and Nutrition Examination survey is that an estimated 8 million Americans have a ≥50% reduction of their glomerular filtration rate (GFR) (3). This group with impaired kidney function is at risk for progression to end-stage kidney disease as well as for development of cardiovascular disease.

One source of hope for stemming the dire trends in kidney disease is progress in the treatment of impaired kidney function, if it is detected and treated early (1, 2). A recent initiative, termed the National Kidney Disease Education Program, seeks to increase public awareness for preventive therapy. Increased awareness will create new demands on laboratories for early and more sensitive detection of kidney disease and for identification of new laboratory markers, such as one evaluated in this issue of the Journal (4). With the recent development of new genomic and proteomic approaches, there is also the potential for identification of many new markers requiring evaluation.

The laboratory marker that has long served as the mainstay for detecting impaired kidney function is serum creatinine. Unfortunately, serum creatinine is an insensitive marker of kidney injury. Two factors underlie this insensitivity. The first factor is that the reference interval is relatively wide because it must encompass a large range of creatinine production, which is related to muscle mass. The second factor is that the inverse relationship between GFR and serum creatinine predicts that large reductions in GFR from normal produce only small absolute increases in serum creatinine. Indeed, for small or elderly people with small muscle mass, serum creatinine will remain within the usual reference limits despite substantial kidney damage.

Direct measurements of creatinine clearance are not an ideal solution to the shortcomings of serum creatinine because these results often are made inaccurate by tubular creatinine secretion and by errors in specimen collection (5). Measurement of creatinine clearance can be useful in patients on a vegetarian diet or with low muscle mass attributable to amputation or severe malnutrition. Measurement of the renal clearance of infused tracers such as inulin, iothalamate, or iohexol can provide accurate measurements of GFR, but they are too costly and cumbersome for routine clinical use.

The Kidney Disease Outcome Quality Initiative recently proposed that diagnosis and monitoring of chronic kidney disease should be based on calculated estimates of GFR (5). Several formulas have been developed for calculating GFR based on serum creatinine; one of the best-validated examples accounts for influences of age, gender, and race:

\[
\text{GFR} = 186 \times (\text{creatinine})^{-1.154} \times (\text{age})^{-0.203} \\
\times (0.742 \text{ if female}) \times (1.212 \text{ if African American})
\]

A different equation should be used for children (5). It is recommended that clinical laboratories report both the measured serum creatinine concentration and the calculated GFR value. Although calculation of GFR offers some improvement over serum creatinine, there still is a recognized need for better markers of renal dysfunction.

Decreases in GFR occur relatively late in many forms of chronic kidney disease, especially type 2 diabetes, which accounts for ~42% of all patients starting dialysis. The first sign of glomerular disease is increased urinary excretion of albumin, initially in the microalbumin range 30–300 mg/day, which gradually progresses over 15–20 years to proteinuria detectable by routine dipsticks. Annual testing of diabetic individuals for microalbumin is a recommended practice to provide early recognition of nephropathy and institution of therapies aimed at preventing progression of kidney disease (6). The National Kidney Foundation also recommends that laboratory evaluation of kidney function should include measurement of serum creatinine and estimation of GFR at least yearly (5).

There has been an ongoing search for improved markers for impaired renal function or injury. One promising candidate has been cystatin C, a small plasma protein of ~13 kDa that inhibits cysteine proteases (7, 8). It is produced by all nucleated cells and is small enough to be freely filtered at the glomerulus. The serum cystatin C concentration correlates inversely with GFR, and assays for cystatin C are commercially available. The production rate of cystatin C was initially claimed to be constant, although recent studies have detected decreased cystatin C production in transplant patients with low GFR (9, 10). Although this hinders interpretation of cystatin C concentrations in some patients with advanced renal failure, it may not limit the utility of cystatin C measurements to detect early renal dysfunction. Other low-molecular-weight proteins [reviewed in Ref. (7)] and a glycoconjugate of tryptophan (11) also are markers of renal function, but have not been investigated as thoroughly.

Many laboratories are searching for new markers by screening for messenger RNAs or proteins that are differentially expressed during kidney disease. These efforts apply new technologies such as RNA subtraction or DNA microarrays (12, 13) and the proteomic approaches of two-dimensional gel electrophoresis and mass spectrometry (14). Whereas it is easy to find messenger RNAs that are differentially expressed in the kidney, few of the
proteins they encode will be detectable in serum or urine, and even fewer will be regulated by injury. Although creatinine, other filtration markers, and proteinuria mainly measure glomerular injury, RNA methods are likely to identify markers of renal tubular injury. This is an area of extreme clinical interest because for many years studies have suggested that the interstitial changes correlate better with renal function than does the degree of glomerular disease (15, 16). Proteomic approaches have the advantage of detecting changes in the concentrations of proteins in relevant serum or urine specimens; however, current techniques may be unable to detect or quantify proteins of low abundance. Immunoassay techniques may be required to measure many potential markers that are of low abundance.

Although current investigative work is likely to identify many potential markers for renal disease in serum or urine, translating assays into forms suitable for routine clinical use and validating the appropriate clinical interpretation and application of the new markers will be challenging tasks. The article by Oda et al. (4), which describes the development and evaluation of a quantitative assay for lipocalin-type urinary prostaglandin D synthase (L-PGDS), is an instructive example of the efforts required to translate a new marker into a practical laboratory assay and to evaluate the utility of the assay. L-PGDS is a 26-kDa secretory glycoprotein that was termed β-trace when it was initially identified as a major component in cerebrospinal fluid. Concentrations of this protein in serum (17) and urine [reviewed in Ref. (4)] are increased in individuals with kidney disease.

Oda et al. (4) developed a practical assay to measure β-trace protein in urine. They developed monoclonal antibodies to L-PGDS, characterized the target epitopes and affinities of the antibodies to select suitable reagents for sandwich-type assays, optimized the characteristics of the immunoassays, and documented the assay results in a variety of kidney disorders. A wide spectrum of kidney diseases with diverse pathogenesis and inflammatory components were tested, as a particular marker may work better for detecting or differentiating specific kidney diseases. The authors correlated urinary L-PGDS with serum creatinine; however, it would be interesting to determine the correlation with 1/(estimated GFR). Perhaps some of the scatter is caused by inaccuracies in estimation of renal function by creatinine. The authors suggest that urinary L-PGDS, like many low-molecular-weight proteins, is filtered by the glomerulus and appears in the urine because it is not reabsorbed by damaged tubules. Because L-PGDS is also expressed in the kidney, an alternative explanation is that L-PGDS escapes from injured kidney cells. In either event, L-PGDS may serve as a marker for tubular injury.

Careful clinical classification will be an essential component of the study of any potential marker for kidney disease. Additional clinical data will be required to establish whether L-PGDS is a useful laboratory tool for the diagnosis and monitoring of kidney disease. However, the work in this report is a necessary foundation to allow further studies to be done and, if assay of L-PGDS is confirmed to have clinical value, to enable routine use of this marker in clinical laboratories. Similar efforts will be required for the translation of any other potential new marker into clinical practice.

References


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