Biomarkers in kidney fibrosis: are they useful?

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With the escalating cost of monitoring and follow-up required in the care of patients with chronic kidney disease (CKD), biomarkers are increasingly being investigated for their utility in predicting patients most at risk of decline in renal function in order to rationalize and target care. Putative biomarkers have also emerged as treatment targets, with the potential to develop novel therapeutics. However, biomarker studies in CKD are largely derived from single-sample collections in observational or nested case-control studies that are suboptimal in study design, analyses, and end points relevant to confirm the utility of specific biomarkers. It has been demonstrated that biomarker expression may be modified by declining kidney function. Hence, their value in predicting future kidney dysfunction is limited. Therefore, understanding the nature, mechanism of action, and how specific biomarkers interact with the CKD disease process is a crucial step in defining the potential for biomarkers to predict outcome, or alternatively, develop as a therapeutic target. Unlike conventional risk factors that, albeit partly, enable us to distinguish an individual at risk of cardiovascular disease, biomarkers in patients with CKD may not be required to be modifiable either directly or indirectly in the disease process or by therapy. Reproducibility and prospective validation remain major challenges for the burgeoning number of purported biomarkers in patients with CKD. It is highly likely a combination of conventional and novel biomarkers will be needed to accurately predict the risk of end-stage kidney disease. This review will focus on recently identified biomarkers and their utility in predicting progressive kidney fibrosis.

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Chronic kidney disease (CKD) is recognized as the single biggest risk factor for cardiovascular disease, and the combination of diabetes and kidney disease confers a significant increase in the risk of death.[1] The cost of treating end-stage kidney disease (ESKD) poses a significant risk to the community.[1,2] Hence CKD, and in particular diabetic nephropathy as the most common cause of CKD, is a major international health and socioeconomic burden.

Strategies to mitigate the development of diabetic nephropathy are primarily limited to blood pressure optimization and maximizing renin–angiotensin–aldosterone system blockade, but a treatment gap still exists. Hence additional therapies are urgently needed, and the identification of accurate and early predictors to define those at high risk of declining renal function may assist in timely intervention with targeted therapy.

Currently, new biomarkers are largely being developed based on both mechanistic and 'data trawling' approaches. Validation of potential biomarkers in prospective studies as surrogate end points for hard clinical outcomes is often complicated by the long lag time to so-called 'hard' renal end points, as evidenced by ESKD. Hence, validated surrogate markers for progressive renal disease are required to facilitate clinical trial programs, potentially yielding novel diagnostics and therapies to aid in prognostication and treatment of patients. This review will focus on current concepts and application of biomarkers, in particular, those relating to the risk of kidney fibrosis and progressive CKD.

WHAT ARE BIOMARKERS?

The term 'biomarker' has been defined as a 'quantitative indicator of biologic or pathologic processes that vary continuously with progression of the process'.[3] This was further refined by the National Institutes of Health working group report in 2001 and included its role in measuring and monitoring pharmacologic responses to a particular therapeutic intervention.[4] Biomarkers used in nephrology can be measured from blood and urine specimens, and more recently from DNA and microRNA analyses, kidney biopsy specimens, and from imaging modalities.

Ideally, biomarker levels vary continuously with the activity or degree of progression of the disease process. Increasingly, biomarkers are measurable intermediary components of molecular or cellular pathways involved in the biologic process, which may be disease specific or relevant to

treatment toxicity. As such, single biomarker measurements represent a temporal slice through one of possibly many pathways that converge to define that process and its treatment. For instance, the estimated glomerular filtration rate (eGFR) and albuminuria are generally accepted as 'standard' biomarkers for the prediction of cardiovascular mortality and risk of ESKD.[5] However, the classification inherently implies that those with renal impairment are likely to progress. The challenge is to identify those at risk of complications and progressive renal disease earlier in its development.

WHY DO WE NEED BIOMARKERS IN CKD?

A biomarker is different from a risk factor or susceptibility marker. Most known risk factors for CKD can rarely distinguish patients who will develop CKD from those who will not. Conversely, surrogate end points can also be described as biomarkers that are strongly associated with a distant clinical event. They can reliably substitute for hard clinical end points in predicting clinical benefit, lack of benefit, or harm from a treatment. For that reason, albuminuria has been widely used as a surrogate marker in large-scale clinical trials for both cardiovascular and renal end points to improve clinical trial efficiency and decrease the need for lengthy studies of slowly progressive diseases such as CKD.[6] However, the use of albuminuria as a surrogate marker for such end points is controversial.[7]

Therefore, we need biomarkers that can:

a) accurately predict an individual's risk of developing CKD or hard renal end points such as ESKD or death,
b) identify additional risk factors other than known conventional risk factors for CKD,
c) identify and quantify a pathological process within the kidney, which may or may not be modifiable, and
d) act as an indicator of treatment response?

Emerging technologies in the field of genomics, metabolomics, and proteomics are providing new platforms for biomarker discovery. Although molecular genetic typing of kidney biopsy specimens may eventually replace classical renal pathology in diagnosis of various nephropathies, the vast majority of patients with the potential for development of CKD, or indeed existing CKD, will not undergo a renal biopsy. Hence peripheral blood biomarkers are still required. The role of epigenetic modification is increasingly recognized to be associated with disease phenotype and an area of active investigation, but its predictive association with progression of CKD is as yet unclear.

HOW DO WE IDENTIFY POTENTIAL BIOMARKERS?

Targeted pathophysiologic investigations, especially in animal models of disease, have been the predominant method of biomarker discovery in the past. The primary goal of these studies has been an understanding of disease mechanisms, and biomarker discovery was usually a secondary outcome. A 'fishing' approach involves exhaustive quantitative analyses of mRNA or proteins in patient samples (tissue, blood, urine) and the use of analytical methods to search for patterns in the resulting large data sets. The major drawback of this approach is data overload, with the potential for overfitting of models, and lack of immediate pathophysiological insight.[8] The risk of spurious associations is, therefore, evidently present. However, with the recent advances in urinary proteomic and gene expression arrays, this approach allows rapid screening of huge numbers of possible associations and the increased likelihood of serendipitous discovery.

LIMITATIONS IN THE IDENTIFICATION OF NOVEL BIOMARKERS

Once new target biomarkers are identified, or existing ones are refined, preclinical trials are used to validate them in individuals with or without disease. Subsequently, retrospective case-control studies are used to determine assay sensitivity and specificity. This is followed by prospective screening studies in large cohort studies to determine reproducibility. Finally, the biomarker is validated as a disease-predictive tool in alternative cohort studies or randomized controlled trials. Most biomarker studies are conducted within population-based studies, epidemiological cohorts, or in clinical trials. These investigations are generally designed as either a cohort study or a nested case-control study extracted from a larger cohort. Participants are selected based on criteria including the absence of CKD at baseline and the subsequent outcome based on defined end points of CKD during follow-up. The methodology is usually based on the availability of specimens (blood or urine, single or two time points), and the cost and feasibility of the assay to be performed, which generally is not factored into the cost of the primary study. In either case, multivariate analysis is typically used to control for confounding variables that may alter associations between the biomarkers and risk of progressive CKD.

Validation of the performance of a new biomarker is the most problematic aspect of biomarker development and is best achieved by prospective validation with a separate cohort of patients.

STATISTICAL METHODS FOR DETERMINATION OF BIOMARKER UTILITY

Relative risk indicators, such as odds ratio or risk ratios, are the most frequently used measures of association between a biomarker and outcomes. Owing to the nature of distribution of biomarker levels, which typically overlap in cases and controls, the risk ratios do not provide direct information as to whether a biomarker affects risk prediction.[9] Therefore, *c*-statistics are used to measure risk discrimination of the biomarkers. The *c*-statistic is more commonly known as the area under the receiver-operating characteristic curve. The *c*-statistic ranges from 0.5 (no better than random guessing) to 1 (perfect discrimination), and represents the trade-off between sensitivity and specificity of a candidate biomarker.[10]