Unlocking the code: mining the urinary proteome after renal transplantation

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Diagnosis of transplant dysfunction usually requires kidney biopsy. Sidgel et al. compared urinary proteomics with matched kidney biopsies to develop a biomarker panel to differentiate acute rejection, BK viral nephropathy, and chronic allograft nephropathy. The results suggest that monitoring a panel of urinary peptides may ultimately facilitate noninvasive diagnosis and management of common transplant complications. Kidney International (2016) 89, 1183–1185; http://dx.doi.org/10.1016/j.kint.2016.03.008

Kidney transplantation represents optimal treatment for many, perhaps most patients with end-stage kidney disease. However, both acute and chronic injury characterize the trajectory of most transplanted kidneys from time of transplantation to eventual decline in function. Such progressive dysfunction and eventual allograft loss with return to dialysis is associated with increased mortality and morbidity. When renal function is initially poor or lost soon after transplantation, acute kidney injury, acute rejection, and calcineurin inhibitor toxicity are important differential diagnoses. BK viral nephropathy, antibody-mediated rejection and chronic allograft nephropathy ([CAN]; encompassing both immunologic and nonimmunologic injury) are critical differential diagnoses for later decreases in renal function. Unfortunately, confirmation of each diagnosis requires biopsy of the transplant kidney. Consequently, there is considerable interest in identifying biomarkers for the noninvasive assessment of renal transplant dysfunction.

Urine is the oldest noninvasively accessed source of human biomarkers. Assessed since the time of Galen (to see if the bodily humors—blood, phlegm, yellow and black bile—were in balance), the image of a doctor examining a round-bottomed urine-filled flask, the matula, became standard medical practice during the medieval period. Urine wheels depicting the color, smell, and later taste, became abundant after the invention of printing. However, most diagnoses (and prophecies) made from examination of the urine had little to do with the kidney and by the 15th century, the practice of uroscopy was falling into disrepute. Scientific rigor brought urine microscopy and eventually assays for proteinuria and then albuminuria, culminating in point-of-care dipstick analysis as well as more sophisticated assays. These techniques are now established practice in screening for renal-specific disease, with albuminuria and a reduced estimated glomerular filtration rate also having strong predictive value for cardiovascular-specific mortality (Figure 1).2

Recognition of the renal specificity afforded by measurement of urinary low-molecular weight protein biomarkers has seen an explosion of interest in these and application to diagnosis of acute and more recently to chronic renal disease.3 Such applications include prediction of delayed graft function after renal transplantation.4,5 Whereas novel markers of renal cell injury could be useful in early detection of transplant nephrotoxicity, the mechanistic reasons why these could help diagnose or predict rejection are less direct unless these processes involve inflammation or oxidative stress for which there are identified markers. A systematic approach to discovery of appropriate biomarkers for these transplant outcomes is offered by Omics strategies. These describe the simultaneous study of multiple cellular and system components within the genome, transcriptome, proteome, or metabolome. Such studies are typically hypothesis-free, systematic, and comprehensive. The hope is that such approaches will ultimately be more successful in identifying diagnostic and prognostic markers. This approach even allows refinement of mechanistic pathways. For example, new genes were identified following a comprehensive proteomic analysis of adult and fetal human tissues to create a draft map of the human proteome.6

Omics approaches have found their way into the search for urinary biomarkers, the “urinome.” The various omics strategies utilized to search for biomarkers identifying calcineurin inhibitor toxicity earlier than the predictably late changes identified by relying on serum creatinine were reviewed recently.7 A genomic approach to urinary biomarkers of transplant rejection identified a 3-gene signature of 18S ribosomal RNA–normalized measures of CD3ε mRNA and interferon-inducible protein 10 mRNA, and 18S ribosomal RNA itself in urinary cells (in prospectively acquired samples of urinary sediment); this signature became apparent weeks before renal biopsy showed acute rejection.8 The nontargeted approach utilized by proteomics enables simultaneous

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identification of multiple biomarkers, which can then be combined into high-dimensional classifiers. Importantly, such high-dimensional classifiers based on multiple, well-defined biomarkers generally outperform individual markers, because such classifiers account for disease complexity and molecular heterogeneity.9

In this issue, Sidgel et al.10 (2016) report a proteomic analysis of renal transplant injury, which continues important work in developing a proteomic analysis of renal transplant injury. From a bank of 2016 urines, part of a bank of meticulously collected urine and histology samples from 2000 to 2011, samples with matching renal transplant biopsy from 396 kidney transplant recipients were analyzed by isobaric Tags for Relative and Absolute Quantitation, or iTRAQ, reagent labelled and label-free liquid chromatography–mass spectrometry to quantitate the urinary proteins. Blinded histology data from paired graft biopsies were used to classify the urine samples into the renal injury phenotypes, namely acute rejection, CAN, BK virus nephropathy, and stable graft. From a group of over 900 proteins identified in transplant injury, 131 peptides were assessed for their significance in accurately segregating the cause of renal injury in an independent cohort of 151 urine samples. Finally, a 33-member biomarker panel was selected that comprised 11 urinary peptides for acute rejection, 12 urinary peptides for CAN, and 12 urinary peptides for BK virus nephropathy. This panel segregated acute rejection with an area under the curve of 93%, CAN with an area under the curve of 99%, and BK virus nephropathy with an area under the curve of 83%. If validated, the results suggest that urinary proteomics can identify urine protein panels for rapid differentiation of kidney transplant injury without the need for a renal biopsy.

Grouping chronic changes under the umbrella of CAN, which is known to be multifactorial in etiology, is a limitation of the study. Contributors to CAN include ongoing immune damage, drug toxicity, and patient factors such as hypertension. The fundamental concern is likely the nonspecific nature of histology in this chronic setting and the absence of convincing etiology-specific structural change. One solution could be to use histology-independent definitions, such as the response to therapeutic intervention, albeit a retrospective adjudication rather than a guide to clinical management.

The failure of early proteomics studies led to a series of recommendations for proteomic biomarker research.10 Although much of the methodology and data are only present in the supplementary data, the Sidgel et al.10 study conforms to many of these recommendations. Each urinary biomarker panel has a clear context for performance and aims to satisfy a clearly defined clinical problem (except for CAN). The use of sample pooling and bootstrapping in the validation may have reduced statistical power, although multiple pools have been used. Although the group has a precious, large and well-documented biobank, the panels will require validation in other large independent data sets. Cost limitations need careful evaluation before this approach can be rolled out for clinical use. Although a biomarker panel with multiple peptides or proteins seems an inherently expensive parameter to assess repeatedly, the investigators highlight that the wide availability of mass spectrometers in hospitals may enable clinicians to add screening urinary protein/peptide panels to other routine posttransplant monitoring parameters at a relatively low cost.

The difficulty inherent in identifying suitable biomarkers in the posttransplant context might be compared with a search for a needle in a haystack (composed of simultaneous events all injurious to the transplant kidney). However, studies such as that of Sidgel et al.10 reveal proteomics as a sophisticated way of assessing the whole haystack rather than searching through it. This is a significant step along the long road ahead.

DISCLOSURE
ZE has received honoraria as a member of the Novartis Transplant Advisory Board. MF declared no competing interests.

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Unraveling the mechanisms of progressive peritoneal membrane fibrosis

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Continuous glucose exposure contributes to severe ultrafiltration failure in peritoneal dialysis. In their study, Wang et al. describe a mechanistic pathway involving direct activation by glucose of mesothelial cell protein kinase C α that, when blocked, or absent in a mouse knockout model, prevents fibrosis and the associated reduction in ultrafiltration. Interestingly, this pathway involves the 3 main mechanisms of membrane injury (inflammation, neoangiogenesis, and fibrogenesis), offering a potential target for therapeutic intervention.

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