INVITED COMMENTARY

Urinary proteomics: fancy gadgetry or a clinically useful diagnostic instrument? The end-user's perspective

George S. Reusz

1st Department of Pediatrics, Semmelweis University, Budapest, Hungary

Correspondence
George S. Reusz MD, PhD, 1st Department of Pediatrics, Semmelweis University, Bókay J. Street 53, 1083 Budapest, Hungary.
Tel.: +36-30-9869545;
fax: +36-1-3247795;
e-mail: reusz.gyorgy@med.semmelweis-univ.hu

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Late deterioration of kidney function is a major challenge in transplantation medicine, thus early detection of subclinical kidney damage is of outstanding importance [1]. Conventional laboratory parameters such as declining kidney function assessed by serum creatinine or creatinine clearance and proteinuria are late indicators of an already established damage [2,3].

While urine properties have long been the subject of medical investigations, classical parameters based on physico-chemical properties and microscopic analysis remain far from helpful for establishing a refined etiological diagnosis and generally do not indicate the stage of kidney disease.

Progress in the identification of urinary proteins by advanced enzymatic, electrophoretic and immunological methods has allowed identifying several marker molecules of acute and chronic kidney injury [4,5]. Despite the abundance of publications, they are still not part of daily clinical diagnostic practice.

The classical approach for the search of urinary protein markers of kidney diseases is hypothesis-based, where distinct urinary proteins known to be part of a pathway or related to cell injury are evaluated to assess their ability to distinguish individual kidney diseases. A combination of several markers may differentiate between different types of either acute, chronic, immune-mediated and/or other cell injury – depending on their behavior (i.e. increase or decrease) during disease progression [6].

The proteomic aspect of urine diagnostics is fundamentally different from the above approach, being more "pragmatic". A vast number (hundreds to thousands) of different peptides are "fished" or "mined" from the urine, identified and sorted using sophisticated hardware and software tools. The differences in urine proteins according to the various etiologies provided by conventional methods allows establishing a group of marker proteins and peptides exhibiting different patterns depending on the pathomechanism which may be characteristic (similarly to a fingerprint) and potentially be used for disease detection and diagnostics [7].

The analysis of the proteins and protein fragments involved may also provide new insight into the underlying biochemical and biological processes, since some of these components belong to matrix proteins, others to innate or adaptive immunity, to proliferation and/or fibrosis, etc. Their presence and quantity in a particular

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protein pattern may indicate the up- or downregulation of a given pathway during disease course [8].

Finally, if kidney biopsies are available, the proteomic results may be validated using tissue gene expression analysis [8–10]. However, disparities may still be present regarding gene RNA expression and protein abundance because of post-translational mechanisms. The proteomic approach from a "bird's eye view" of the different processes at play may paradoxically allow a deeper insight into the pathways involved and in establishing new connecting pathways based on the interconnection of the related proteins and mechanisms [8].

Chronic active antibody-mediated rejection (cABMR) plays a distinct role among the possible causes of kidney function loss. It is not only among the leading causes of long-term graft failure in adults, but becomes even more important in teenage and young adult owing to the high incidence of nonadherence at this stage of life [1,11,12]. Clinical diagnosis of cABMR relies on decreasing GFR in the presence of (de novo) donor-specific autoantibodies (DSA) in conjunction with specific histological signs in the graft biopsy [11]. However, serum creatinine and even cystatin C are poor indicators of kidney function loss, because of the large reserve capacity of the kidney. Thus, eGFR weakly and tardily reflects the decrease in the number of functioning glomeruli. This is especially true for children grafted with an adult kidney which by far overcomes the body's detoxification requirements. The presence of donor-specific HLA antibodies again is not a fully reliable indicator of cABMR, since antibodies not pathogenic and not involved in the disease processes may be detected and vice versa, and histopathological features of cABMR may be present without detectable antibodies [7,11,13,14]. Since the ultimate goal is early detection of rejection, some transplant centers perform protocol biopsies to evaluate the course of the allograft. However, graft biopsy is an invasive procedure, and continuous monitoring of the graft by way of biopsies during the entire post-transplant period is an unrealistic approach, hence the fervent search for noninvasive markers to identify cABMR.

In their work in the present issue of the journal, Kanzelmeyer et al. [15] used a urinary proteomic approach to identify the specific pattern of chronic antibody-mediated rejection.

In their case–control study, the authors compared the urinary proteomic profile of 24 pediatric renal transplant recipients with cABMR to that of 36 pediatric renal transplant recipients with stable kidney function without cABMR. From a set of 5616 peptides, 76 potential biomarkers were selected through a sophisticated statistical procedure to be part of a SVM (support vector machine)-classifier. This classifier was then tested for sensitivity and specificity on the independent test cohort. In addition, the performance of previously established classifiers of early CKD progression (CKD273 classifier), and an acute T-cell–mediated rejection (aTCMR) classifier were assessed in the test cohort [16,17]. In this setting, the new proteomic biomarker pattern allowed detecting cABMR with a reasonable specificity. The combination of the new classifier with the CKD273 classifier improved the detection of patients with cABMR with a misclassification of only 2/20 patients.

By assessing the origin of the proteins involved, most of the sequenced proteins were fragments of different collagen subtypes. The role of other marker peptides such as annexin, retinol binding protein 4 and IgG kappa chain C was partially discussed; however, the results of the study did not allow compiling a comprehensive pathomechanism.

From a practical standpoint, these results are promising; however, concerns still remain regarding the applicability of the proteomic approach in daily clinical routine. These questions mainly pertain to the true specificity, the ability of early detection as well as the reproducible, simple and fast technical feasibility of the proteomic-based test.

The potential of the proteomic approach to distinguish among transplant pathologies is contingent on the various entities included in the analysis. Other etiologies leading to slow and progressive function loss after transplantation include, among others, chronic calcineurin inhibitor nephrotoxicity, interstitial fibrosis and tubular atrophy (IF/TA), BK virus nephropathy. Since no comparator groups with these diagnoses were included in the study, the performance of the cABMR classifier remains undetermined with regard to differentiating these various conditions. An indirect reference to this concern is the overlap of the new classifier with the CKD273 classifier. Thus, validation in this context should be performed in a larger prospective setting.

Chronic active antibody-mediated rejection is a long-lasting process with slowly evolving histological features. From a purely theoretical standpoint, the urinary protein pattern should also change during the course of the disease, including the presence of proteins and peptides that are more specific to early development and others to later development of cABMR. Thus, this presents a second aspect of the need of independent validation.

Finally, with regard to the introduction of proteomics into clinical routine, it is of crucial importance to find a simple, universal and broadly accessible diagnostic platform for rapid and early diagnosis.

In conclusion, the answer to the general question of whether urinary proteomics represents a fancy gadgetry or a clinically useful diagnostic instrument is indubitably getting closer, although still pending.

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Conflicts of interest

The author has declared no conflicts of interest.

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