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# Commentary

## Serum proteomics for the diagnosis of nephrotic syndrome: Is there a ray of hope?

The term proteomics came into use only about 15 years ago[1]. Proteomics is the systematic analysis of proteins and peptides in a biological sample. It has been the natural offshoot of the Human Genome Project. A proteome is a list of all expressed proteins in the sample of interest. While genomics links gene activity with disease, proteomics investigates gene expression *i.e.* the proteins which are the ultimate effector molecules; and hence more relevant to identification of biomarkers. It is the simultaneous study of multiple proteins rather than one protein at a time as in traditional biochemistry[2]. Proteomics is capable of characterizing several thousand proteins in a single analysis. It has been used for better understanding of renal physiology and pathophysiology of renal diseases[1]. In clinical nephrology proteomics is a tool to identify biomarkers. The development of a biomarker essentially has three steps: biomarker discovery, validation and ultimately implementation[3]. Biomarkers are useful for early non-invasive diagnosis of disease, monitoring disease progression and prediction of drug efficacy[4]. A major advantage of the proteomic approach is its unbiased nature[3], and the major disadvantage is that biomarker studies are always underpowered since the number of samples required should exceed the number of observations[3]. The basic steps of proteomics include *(i)* albumin removal; *(ii)* proteins digestion; *(iii)* protein separation step; and *(iv)* analysis by mass spectrometry[2,5-7].

The methods commonly used currently for proteomic analysis are[6]: *(i)* two-dimensional gel electrophoresis followed by mass spectrometry (2DE-MS); *(ii)* liquid chromatography followed by mass spectrometry (LC-MS). It has high sensitivity but is time consuming; *(iii)* surface-enhanced laser desorption/ionization time of flight mass spectrometry (SELDI-TOF-MS); and *(iv)* capillary electrophoresis coupled to MS (CE-MS). It has a fast separation, but cannot detect proteins >20 kDa.

The biological samples used for renal disease include, urine, plasma/serum and kidney biopsy tissue. Urine is easily accessible non-invasively in large quantities and is relatively stable. The midstream sample of the second morning sample is recommended for proteomic analysis. Urine is a mixture of plasma and kidney proteins. The normal urinary proteome map was first established by Thongboonkerd and colleagues almost a decade back[8]. The urine has several hundred peptides mostly derived from albumin, β-2 microglobulin, uromodulin and collagen[9]. Weissinger *et al*[10] used urinary proteomics to classify patients with nephrotic syndrome into minimal change disease, membranous nephropathy and focal segmental glomerulosclerosis (FSGS). In another study, specific fragments of albumin and α1-antitrypsin were found in patients with nephrotic syndrome[11]. Subsequently, Varghese *et al*[12] used 2DE and MALDI-TOF-MS in 32 patients with membranous nephropathy, FSGS, lupus nephritis and diabetic nephropathy. The first 16 patients were used to create a prediction algorithm and the remaining 16 patients were used as the external validation set to test the accuracy of the algorithm. The model predicted the presence of the diseases with sensitivities between 75 and 86 per cent, and specificities from 67 to 92 per cent. Biomarkers in the urine have also been identified for IgA nephropathy[13], allograft rejection and acute kidney injury, besides urological malignancies[5].

The plasma proteome contains more than 3000 individual proteins and peptides with a wide range in molecular weights from pictograms to milligrams. Majority (99%) of the mass is constituted by a small number of proteins (albumin, immunoglobulins, *etc.*), the remaining 1 per cent may be useful for evaluation of

biomarkers. Blood may contain specific biomarkers for most diseases[14]. However, it is analytically challenging. The major amount of proteins needs to be removed before analysis. This can result in simultaneous depletion of many other factors. Another problem is activation of proteases leading to variability[9]. As compared to urine, there are very few studies using plasma. Very low molecular mass fragments of albumin have been found in patients with genetic forms of FSGS as compared to idiopathic FSGS[15]. Kaneshiro *et al*[16] found that the profile of serum short peptides was useful to discriminate IgA nephropathy and healthy adults.

In this issue Sui *et al*[17] have tried to identify patterns of proteins in the serum to characterize nephrotic syndrome. They have used magnetic beads based chromatography for fractionation followed by MALDI-TOF-MS. They identified groups of peptides specific for mesangioproliferative glomerulonephritis (MsPGN), minimal change disease (MCNS), FSGS and membranous nephropathy (MN). The authors feel that this may give an early idea of the pathology of nephrotic syndrome. The reproducibility of these results is a matter of concern. This study has other limitations also. Prior to enrolment, the study patients were having nephrotic syndrome for 1-3 years and presumably already receiving corticosteroids and/or other immunosuppressives. Whether the same proteomic profile would be seen in fresh cases before start of therapy (which is the purpose of this study) remains a matter of conjecture. Besides, the patients in the study had a wide range of proteinuria - from near normal to almost 10 g/day. Whether the degree of proteinuria affects the serum proteomics is also not clear. There is no external validation set in the study to test the algorithm they have developed. The authors have only used an in-built software programme. So, sensitivity and specificity cannot be calculated accurately. As mentioned by the authors, the sample size is small. Nephrotic syndrome may be caused by other conditions like membranoproliferative glomerulonephritis or primary amyloidosis. The study is silent on these conditions. Even if the diagnosis of MsPGN and FSGS by proteomics is correct, this diagnosis remains incomplete. Both MsPGN and FSGS are actually heterogeneous entities. FSGS is of five types, which can be diagnosed by histopathological examination. MsPGN requires immunofluorescence study to detect IgA nephropathy, C1q nephropathy or IF negative. Membranous nephropathy has five stages, which can only be differentiated by electron microscopy. These conditions differ in their prognosis, and hence complete characterization of these conditions is a must for appropriate management. The same authors have earlier studied the serum proteomic profile of uremic patients[18].

Nevertheless, this preliminary study provides a new insight into the proteomics of nephrotic syndrome. Renal disease is still awaiting biomarkers akin to troponin in cardiology and research in this field is welcome. It would be useful to know if the serum proteome of nephrotic syndrome has certain proteins common to all nephrotics irrespective of its histological type; and whether the primary form of nephrotic syndrome has certain differentiating features from the secondary form. Further, since the disease nephrotic syndrome itself causes loss of proteins in the urine, whether a combination of urine and serum proteomic analysis is more useful for the diagnosis of nephrotic syndrome. Biomarkers to predict those who are unlikely to respond to therapy or relapse subsequently would also be very useful. Further large studies are required to resolve these issues. Until then, clinicians would continue to perform kidney biopsy for the diagnosis of nephrotic syndrome.

**Dipankar Bhowmik* & Sanjay K. Agarwal**
Department of Nephrology
All India Institute of Medical Sciences
New Delhi 110 029, India
**For correspondence*:
dmbhowmik@yahoo.co.in

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