Proteomic biomarkers in diabetic nephropathy—reality or future promise?

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In this issue, Ameur et al. [1] present a review on ‘proteomics in diabetic nephropathy’, a very timely subject, given the substantial burden of diabetic nephropathy (DN) and the expected increase in healthcare demand if the current situation does not undergo radical change. The article lists the recent work published in this area and gives a good overview of the field. The main question that is being extensively addressed is: what can proteomics deliver today that may help improving the situation with DN being a global challenge and the leading cause of end-stage renal disease in the Western world, and make a true change in the management of patients?

Detection of DN using urinary proteomics is a sensible first goal as a proof-of-principle. Urine proteomics has delivered biomarkers for DN, but the moderate consistency observed between biomarkers reported in different studies is puzzling. This inconsistency is in part attributed to different technologies used that resolve different parts of the proteome: 2D electrophoresis enables assessing the higher molecular weight proteome (>10 kDa), while capillary electrophoresis-coupled mass spectrometry (CE-MS) allows assessing the low-molecular-weight proteome/peptidome [2]. Furthermore, the lack of statistical power resulting in the reporting of artefacts may be another major contributing factor.

When combining the current knowledge on urinary proteomic biomarkers for DN, a significant increase in plasma proteins, including their degradation products, is observed in urine. This likely reflects significant alterations in the filtration barrier, at least in part owed to structural damages of glomeruli and tubuli, and to altered protease activity in DN. A further hallmark is the substantial decrease of extracellular matrix degradation products in urine. While the increase in urinary plasma proteins (or degradation products) may reflect later stages of DN, the changes in extracellular matrix components may indicate early stages and onset of fibrosis [3].

The identification of consistent proteomic changes clearly demonstrates that urine proteomics can deliver ‘biologically significant’ information. From the clinical perspective though, the question remains: can urine proteomics deliver ‘clinically useful’ information that will have a positive (and tangible) impact on patient management? The current clinical challenges in DN that may be approached employing urinary proteomics are: (i) detection of high-risk patients,
(ii) early detection of disease, (iii) prediction of prognosis and (iv) prediction of response to treatment.

Several manuscripts have reported a substantial amount of urinary proteomic biomarkers of DN, but most did not address the issue of prognosis. This is not surprising since estimation of prognostic value requires knowledge on the outcome, which may take 5–10 years. The use of surrogate markers for outcome (e.g. development of microalbuminuria) appears not advisable as these are still under debate, in part due to substantial variability and lack of accuracy [4–6]. While association of biomarkers with albuminuria is to be expected, the accuracy of a prognostic biomarker should be assessed using clinically accepted hard end points [7]. As a consequence, and in order not to spend several years of observation, retrospective assessment of prospectively collected samples seems to be best suited to address the question: which of the described biomarkers may in fact be of prognostic value?

The first promising approach for detection of DN prognostic biomarkers was reported by Ohu et al. [8]. This study raised a high hope in the field, but no further data validating the findings were published. We could recently demonstrate that biomarkers for DN serve as predictors of developing diabetic nephropathy in microalbuminuric patients [9]. These biomarker panels and biomarkers for chronic kidney disease described (Good et al. submitted) could be validated in independent cohorts [10,11].

At the ASN meeting in San Diego, CA, USA in November 2009, the SysKID (www.syskid.org) meeting in Vienna and the EuroKUP (www.eurokup.org) meeting in Rotterdam, both in March 2010, the first data that were presented demonstrated the ability of proteomics to alter therapeutic strategies. In brief, investigation of samples from normoalbuminuric subjects collected in a longitudinal study over a period >10 years revealed that the urinary biomarker patterns enable prediction of clinically relevant disease (diabetic nephropathy) with very high accuracy (area under the ROC curve >0.9), on an average 5 years prior to disease, at a point in time where patients are still normoalbuminuric. Interestingly, these prognostic biomarker patterns share a common denominator: reduction of urinary collagen fragments. In a recent independent study, Merchant et al. reported similar findings: reduction of urinary collagen fragments being associated with development of DN [12].

The ultimate proof of the benefit of urinary proteomics in management of DN will be a clinical trial that aims at identification of patients at risk of developing DN at an early stage (before microalbuminuria) based on urinary proteomics, followed by a targeted intervention. Such a trial would prove that proteomics enables early and accurate detection of patients that will progress (= prediction) and, we hope, that intervention, targeted at only those patients that actually are at risk (at the very early stage of disease), will result in a very favourable outcome. The outline of such a trial that we and others currently seek to initiate is presented in Figure 1.

These developments present new opportunities with respect to management of patients with DN. While it is yet unknown if the proteomics-based early detection of disease and consequently early treatment will result in improved outcome, such questions may in part be answered by retrospectively assessing samples from some of the re-
cent large trials, like, e.g. the DIRECT trial [13], Ontarget [14] or Roadmap [15], where ACE inhibitors (ramipril) and ARB (candesartan, telmisartan or olmesartan) were compared with combinations or placebo. Urinary proteomics should enable identification of the subgroup of patients that is positive for early-stage DN at the beginning of the trial, and the result of drug treatment in only this group of patients could be investigated and compared with the outcome in placebo-treated high- and low-risk patients. A putative positive effect of treatment may have been hidden by the large number of patients included in the study that did not develop DN, irrespective of treatment. With the ability to identify patients at the early stage of disease, potentially benefitting from treatment, the number of patients required for future studies of new interventions can be reduced.

Another application for urinary proteomic profiling is monitoring of treatment efficacy. While lowering urinary albuminuria with antihypertensive agents is associated with an improved prognosis, the validity of albuminuria as a surrogate biomarker for therapeutic benefit is still under debate [4–6]. The antihypertensive treatment with ARB (candesartan or irbesartan) is associated with significant changes of specific urinary proteomic biomarkers in microalbuminuric type 2 diabetic patients, from ‘disease’ to ‘normal’ (Zurbig et al., submitted, and [16]). These biomarkers might represent tools to assess and follow up treatment efficacy and pending confirmation.

The data on urinary proteomic biomarkers available today will increase the knowledge on pathophysiology and enable a more detailed understanding of the molecular mechanisms responsible for disease onset and progression. As outlined in two recent articles [17,18], combining the knowledge on urinary biomarkers with data from animal models and histology in a systems biology approach may enable pinpointing the relevant molecular mechanisms, consequently targeted therapy. It is to be expected that the increasing knowledge on the early molecular events in DN will spur the development of new, alternative drugs, which may be better suited to target these events, and hence may prevent onset of disease entirely.

In addition, though, there is already evidence that urinary proteomic biomarkers enable detection of patients at a very early stage of DN with a very high accuracy. Given the current strain on health economics, it will be essential to bring cost-effective screening procedures and a targeted treatment to patients, and prevent extremely cost-intensive therapy [19] or over-treatment. This appears even more important in the light of discussions about cost efficiency [20]. Intervention with RAS blocking agents in diabetic macroalbuminuric or microalbuminuric patients has been shown to be cost-effective, and an earlier treatment results in better outcome [21]. However, there is a large unmet need as a significant risk for progression remains despite the effect of blockade of RAS in these stages. It is likely that intervention in normoalbuminuric patients at an early stage of disease, possibly with other classes of compounds targeted at early stages of disease, may be even more beneficial for outcome and cost efficacy.

References


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