Urinary Biomarkers of Tubular Injury in Chronic Kidney Disease

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Abstract: It has been suggested that urinary biomarkers of tubular injury might help predict progression to end-stage renal disease. In this issue Hsu et al. report, that in those with established CKD, this information doesn't add to what we know by quantifying creatinine and albuminuria. Here we discuss the evidence for urinary tubular injury markers in predicting renal outcomes in CKD and the areas where measurement of these molecules might be useful in the future.

Since chronic dialysis became widely available there has been substantial effort focused on understanding which of our patients will go on to require dialysis and/or kidney transplantation. Our duty as clinicians is to provide care founded on shared and informed decision making. This requires patients with chronic kidney disease (CKD) and their doctors to be able to access precise information about the risks of progression so as to plan for the future. This process also translates into hard clinical outcomes; for example, those who receive an adequate period of pre-dialysis preparation are more likely to start dialysis with definitive access and consequently

are at lower risk of early complications and death. To this end biomarkers such as creatinine and albuminuria are established tools in helping determine who will go on to need dialysis but as a renal community, we should always be asking: Can we do this better?

The search for predictive biomarkers has also been a focus of the Acute Kidney Injury (AKI) field where the failure of the serum creatinine to rise within a clinical useful window for intervention has made this a priority. Investigators have searched for and established the utility of markers of direct renal tubular damage. Kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), N-acetyl-β-D-glucosamininidase (NAG) and liver fatty acid-binding protein (L-FABP) are all proteins that are released into the urine following acute injury to the renal tubule (Figure 1). A comprehensive review of the biology of these molecules is beyond this commentary but is available elsewhere.[1] Given the increasing recognition of the interplay between CKD and AKI[2] and the fundamental role of the tubular-interstitial compartment in progressive CKD it is intuitive that these urinary biomarkers might also provide important predictive information in chronic disease.

A number of moderately sized studies, in cohorts without CKD at baseline but at high risk of developing renal impairment, have demonstrated useful additional power using tubular biomarkers (specifically NGAL) to predict outcomes such as incident stage 3 CKD.[3] However, this doesn't answer the question as to whether these novel urinary markers have predictive value in patients with established CKD. To date, studies have reported conflicting results on the utility of NGAL when added to traditional predictors of progressive renal disease such as baseline eGFR and albuminuria.[4,5] Against this background the report by Hsu and colleagues in this issue of Kidney International provides additional clarification[6]. The authors report data from the Chronic Renal Insufficiency Cohort (CRIC) study on the associations between KIM1, NGAL, NAG and L-FABP and the combined outcome of end-stage renal disease (ESRD) or halving of eGFR, a conservative but specific and FDA approved end-point. Although all of the urinary biomarkers were associated with the outcome, none of them provide additional discrimination over and above baseline eGFR and urinary albumin excretion.

These findings seem robust: This is the largest cohort in which such an analysis has been performed. The patients included were recruited from multiple centres and with a broad range of underlying kidney diseases. Importantly methodological considerations surrounding possible biomarker degradation were controlled for. Finally the authors demonstrate that the outcomes of their analyses were not dependent on adjustment, or not, of biomarker levels for urinary creatinine concentration. This has been a controversial issue as although it is intuitive to use creatinine to control for differences urinary concentration, urinary creatinine levels will depend not only on overall urinary concentration but also creatinine production, something that may systematically differ between study participants with stable CKD and those with progressive disease.

So what are the implications of these findings? Firstly, it is important to acknowledge that publication of what are fundamentally negative findings in high profile journal plays a crucial role in pursuit of scientific advancement. One has to wonder how many moderately sized but negative studies examining these associations never even made it to a first draft as the lead investigator considers the findings not worthy of the effort (and expense) needed to submit a manuscript. Following publication of this paper we can now say with some degree of certainty that tubular injury markers (at least KIM1, NGAL, NAG and L-FABP) aren't likely to be helpful in predicting renal outcomes in a typical clinic population with CKD.

Of course we should also ask what help from biomarkers are we are looking for? As the authors allude to when faced with a patient with established CKD in clinic we are already have pretty good tools to identify those who will progress to the need for dialysis. We routinely quantify eGFR and albuminuria which, unlike risk factors for atherosclerotic cardiac events such as hyperlipidaemia that predict occurrence of new disease, are markers of established pathology and/or physiological compensation and therefore provide a window onto the disease itself. Furthermore, if we add change in eGFR over time to our decision-making (something which the authors of this report did not do) we can increase our ability to determine who will progress to dialysis even further.[7] Of course there remains a degree of uncertainty, and given the interplay between AKI and CKD one wonders whether this is explained

by episodes of the former. The implication being that much of this uncertainty as to renal prognosis might not be possible to capture using biomarkers. After all, future information on the events that lead to AKI, e.g. the prescription of a nephrotoxic medication or the timing of the acquiring a bacterial pneumonia are unlikely to be obtainable from urine.

So is this the end for urinary biomarker based research in CKD? We would argue no, but it means thinking more carefully about what we are trying to predict, why and in whom. Although the (change in) eGFR and albuminuria perform well in relatively advanced disease (i.e. eGFR<60mL/min) across all forms of CKD, perhaps research should focus on CKD where these pathological processes are not already established or alternatively on aetiologically distinct kidney condition(s) where a single disease process is at work.

Early CKD, where the eGFR sits above the threshold of 60mL/min/m^2, prior to the development of established glomerulosclerosis, might be a clinical scenario where urinary biomarkers might play a useful role. Indeed when Hsu and colleagues performed a post-hoc subgroup analysis in those at intermediate risk of progression (still a rather heterogeneous group) the inclusion of KIM-1 did marginally improve the prediction of renal outcomes.

Furthermore there are subsets of patients with CKD who remain relatively free from albuminuria despite progression; tubular biomarkers may be useful in this population, and subgroup analyses of some cohorts support this approach.[8] For example, young patients with Autosomal Dominant Polycystic Kidney disease may want to know their long-term prognosis to plan their professional and private life, yet it is incredibly difficult to predict at age 20 at what age an individual with this disease will reach ESRD. Similar issues pertain for patients with chronic allograft injury though the time frames are shorter. Thinking globally, a disease of interest might be CKD of undetermined cause (CKDu) where young agricultural workers from low and middle-income countries develop ESRD in the absence of known risk factors or albuminuria.[9] Could identifying those with pre-clinical CKDu allow us to get a better insight in to the causes of this epidemic?

Finally, biomarkers of progression are likely to become more important in the age of personalized medicine. If we are going to develop therapies to retard renal scarring we need to be able to stratify our patients who fall under the umbrella term CKD, not only by underlying aetiology but also by the activity of mediators of disease progression. Targeting specific (pro-inflammatory or pro-fibrotic pathways) is more attractive if the individuals who would benefit form these interventions can be identified and the effects of the intervention monitored over time. Given the shortage of effective treatments for progressive CKD it is perhaps with this last aim in mind that researchers should prioritize efforts in the search for novel biomarkers.

Overall then, Hsu and colleagues work confirms what many suspect, not that we can't identify novel markers of progressive kidney dysfunction in those with established CKD stage 3-5, but the tools we have to quantify risk of progression are already pretty accurate. The future of urinary biomarkers in CKD is likely to be focused on either individual diseases, personalizing therapy targeted at renal scaring or in identifying those with preserved kidney function who might go on to develop some form of kidney impairment going forward.

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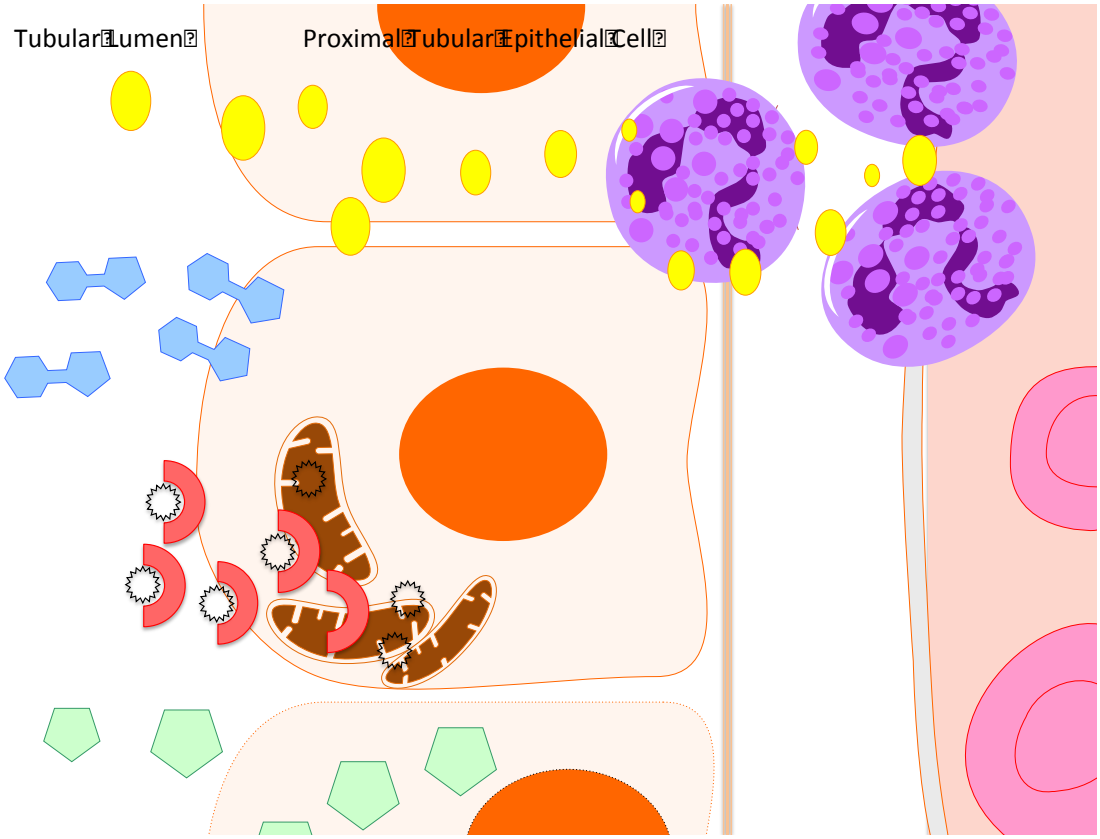
Figure 1: Urinary biomarkers of tubular injury.

Origin of the different markers measured in the report by Hsu et al. Kidney injury molecule-1 (blue) expression is upregulated on the surface of proximal tubular cells in response to injury; neutrophil gelatinase-associated lipocalin (yellow) is predominantly produced by inflammatory cells and released into blood and urine; N-acetyl-β-D-glucosamininidase (green) is a constitutively expressed proximal tubular enzyme released following cell damage; liver fatty acid-binding protein (red) is a

mitochondrial carrier protein which binds reactive oxygen species (black stars) and is released into the urine in response to cellular stress. Adapted from reference 1.


Tubular Lumen
Proximal Tubular Epithelial Cell