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Urinary biomarkers—silver bullets to faster drug development and nephron protection

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Topic and summary of key findings

Drug-induced, iatrogenic renal damage is among the five most common causes of severe acute kidney injury (AKI) in humans [1]. When drugs are developed and therapeutic regimens are tested, the 'do no harm' commandment rehearsed in the Hippocratic oath must be followed first line. Thus, testing of drugs for their nephrotoxic potential is a mainstay in preclinical studies. Results indicating drug-related tubular or glomerular injury may thereafter prompt termination of clinical studies and/or help focusing on promising drug pipelines.

However, such preclinical development is delayed due to the dependence on traditional markers of renal injury, such as serum creatinine (sCrea) and blood urea nitrogen (BUN). These lack the sensitivity and/or specificity to detect early on and adequately nephrotoxicity before considerable loss of renal function is established. Histopathology as gold standard to assess and quantify nephrotoxicity requires an invasive, time-consuming and cost-intensive biopsy procedure and in some cases does not adequately reflect functional renal impairments.

In the May issue of *Nature Biotechnology*, a series of papers has been published [2–5] that all have in common the goal to assess the utility of urinary renal biomarkers to detect kidney injury in murine experimental models [6–8]. The work has been carried out by the 'Predictive Safety Testing Consortium', an initiative with participation of pharmaceutical companies and academic institutions, as well as one regulatory agency for drug development.

The primary approach in the studies was to systematically perform large-scale animal studies, that is the application of eight nephrotoxins such as antibiotics and anti-cancer medications, with a focus on time- and dosage-dependent kidney alterations. Controls included animals that were exposed to hepatotoxins or without application of toxins at all. The diagnostic value of seven urinary and one serum biomarkers was evaluated and correlated to histopathological changes, indicating kidney injury. Findings were compared with that of sCrea and BUN.

As a result, the Consortium demonstrates what is well known among nephrologists: current 'functional markers' sCrea and BUN to assess nephrotoxicity are only suitable for more advanced histopathological changes. Irrespective of the underlying mechanism of kidney injury and independent of the extent of histopathological alterations, urinary KIM-1, albumin and clusterin levels increase as well as trefoil factor 3 concentrations fall and outperform the predictive value of sCrea and BUN with regard to proximal tubular injury (Figure 1A; [2–4]). Glomerular injury with subsequent impairment of tubular reabsorption was best detected by urinary cystatin C, total protein and beta2-microglobulin levels, again outperforming the traditional marker proteins (Figure 1B, [2]). A panel of these urinary biomarkers correlated with early changes and indicated reversibility of acute renal injury following withdrawal of nephrotoxins [5].

Discussion

Urinary biomarkers are the most prominent clinically applicable breakthrough technologies in the early detection of AKI, and the implementation of non-invasive, urinary

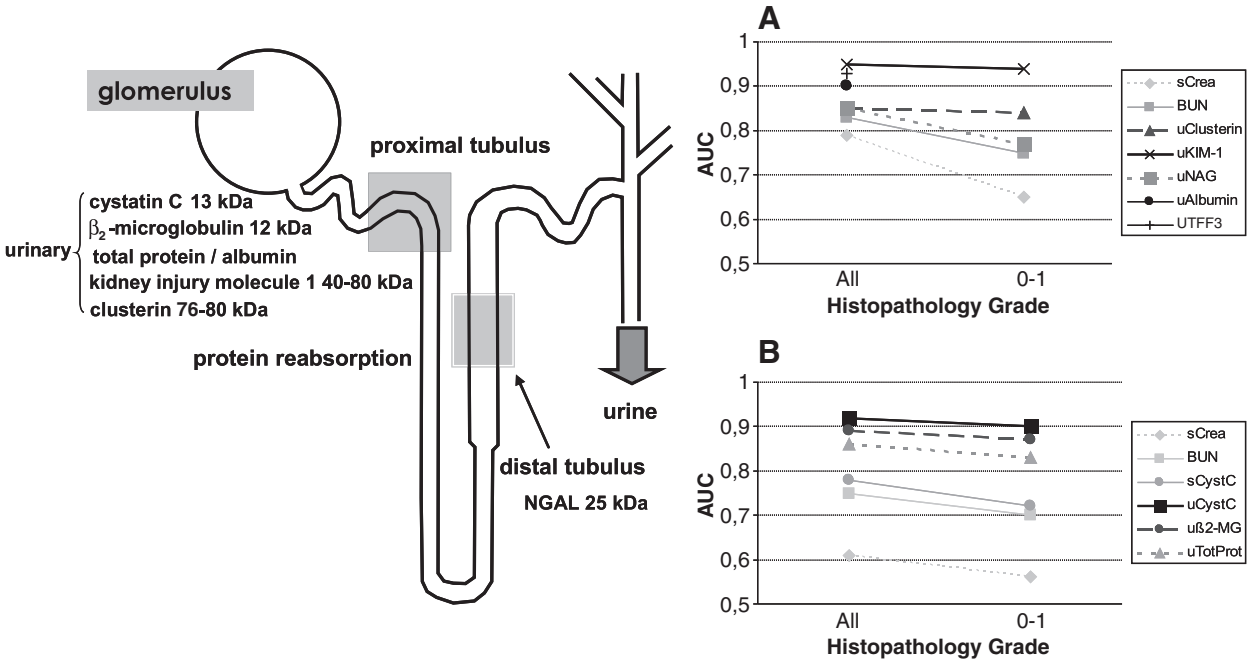

Fig. 1. A. Area under the curve (AUC) of urinary biomarkers for 'tubular' injury compared with the 'gold standard' histopathology (for all and for minor histopathology grade); summary of [2–4]. **B.** AUC of urinary biomarkers for 'glomerular' injury compared with the 'gold standard' histopathology (for all and for minor histopathology grade); summary of [2]. **A**, **B.** sCrea, serum creatinine; BUN, blood urea nitrogen; KIM-1, kidney injury molecule 1; NAG, *N*-acetyl-glucosaminidase; TFF 3, trefoil factor 3; CystC, cystatin C; β2-MG, β2-microglobulin; TotProt, total protein; u, urinary; s, serum. The diagnostic performances of the biomarkers BUN and sCrea were summarized by receiver operating characteristic (ROC) curves, which are plots of the true-positive rate (sensitivity) against the false-positive rate (1 − specificity) for a continuous variable (biomarker) against a specific reference standard (histopathology). The corresponding AUC is a measure of the diagnostic performance of the corresponding biomarker, whereby a perfect biomarker corresponds to an AUC of 1, and a biomarker not better than a random guessing corresponds to an AUC of 0.5.

structural damage markers might represent a substantial progress in the preclinical assessment of nephrotoxicity [2–5]. All tested biomarkers, none of which was newly discovered, underwent an evaluation process by the Food and Drug Administration (FDA) and the European Medicines Agency (EMEA). Both institutions regard these markers as acceptable in the context of non-clinical drug development for detection of acute, drug-induced renal toxicity. They provide additional and complementary information. The immediate intent of the collaborative effort was to apply the patterns of renal injury discerned using these biomarkers. The derived knowledge base eventually permits preclinical testing to assess and predict renal injury in a (pre) clinical setting, before overt nephrotoxicity and nephron loss become apparent. However, there is no sufficient evidence to completely replace histological assessment in preclinical models. Biomarker measurements may accelerate the approval process for newly developed drugs in the future. Furthermore, the covered studies may help to spare animal lives when novel drugs are tested and may spark the discussion on the clinical use of 'novel' biomarkers for early diagnosis of AKI. However, biomarker 'negativity' in preclinical studies may not always preclude drug toxicity in humans where surveillance needs to be continued.

There is an exciting development in the field to establish and assess biomarkers of renal injury. In the long run, it will modify the rules in preclinical drug development.

Measuring the levels of sCrea and/or BUN and/or urinary output has not fulfilled the needs in the clinical setting of early predictors of tubular damage and compromised renal function. As evidences on the predictive value and usefulness of novel biomarkers in humans accumulate, the chapter of AKI diagnosis and early intervention may be rewritten in the near future.

Besides the biomarkers assessed in animal experiments in the presented paper series, so far, neutrophil gelatinase-associated lipocalin (NGAL) is one of the most promising biomarkers for early clinical AKI detection [9,10]. It was discovered using unbiased transcriptomic approaches [11,12], is rapidly induced and released from the injured distal nephron in experimental models and human diseases [12–14], and appears to play a key role in early regeneration and repair in AKI [12,14]. A closer look at the determined properties of this marker reveals important aspects on what an ideal marker should look alike. Its urinary and plasma concentrations increase proportionally to severity and duration of renal injury [12,15,16]. On the other hand, its concentration rapidly decreases with attenuation of renal injury [17]. Quantification is reproducibly possible in plasma [15] and urine samples [16]. In animal experiments, an increase in urine NGAL occurred within 3 hours of cisplatin administration in a dose- and duration-dependent manner and notably preceded increases in serum creatinine or urinary N-acetyl-glucosaminidase [18]. This was confirmed in patients with various types of cancer receiving cisplatin, where the NGAL increase preceded AKI by 4.5 days [19].

Overall, the clinical implications of novel renal biomarkers are wide. They may be used in patients at risk of AKI or those after renal transplantation to monitor for rejection

episodes. Early kidney-damaging side effects of newly commenced drug therapies may be monitored for early nephrotoxicity. In case of biomarker 'positivity', change of treatment regimens (doses and schedules) or termination and, if necessary, replacement of the suspected drug may be considered. Finally, the potential nephroprotective role of substances such as bicarbonate deserves and may gain further attention, especially when initiated early on due to timely diagnosis of AKI [17].

AKI is a multifactorial syndrome with sometimes unpredictable outcome. It may progress from one compartment of the kidney to another, an observation which establishes the need for site- and type-of-injury-specific biomarkers. Advanced transcriptomic and metabolomic techniques certainly accelerate the *de novo* discovery of high-quality renal biomarkers for AKI detection and specification.

Take-home message

Complementation of classical renal evaluation methods, including serum creatinine, blood urea nitrogen and histopathology, with (novel) urinary biomarkers will allow a faster preclinical drug development, potentially sparing animal lives and reducing drug toxicity and nephron loss. It therefore strengthens our armamentarium to detect AKI and allow for a timely intervention.

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