Over the last decade, the pursuit for drugs to prevent or treat acute kidney injury (AKI) has been replaced by a search for novel biomarkers of kidney damage. Prompted by expert opinion that lack of sensitive and specific biomarkers has thwarted progress in the field, several new biomarkers have emerged and are jockeying to become the ‘golden’ test for AKI. As clinical experience with these biomarkers accumulates, it is increasingly evident that no single biomarker will likely take the crown. There is a slow realization that the consistent emphasis on the weak and limitations of existing markers may be misguided. In fact, there may be several opportunities to reevaluate existing techniques in combination with newer biomarkers for managing patients with AKI.

Urinary microscopy has long been considered to be a window to the kidney; however, its clinical value was questioned after publications suggested that urinalysis was not helpful in discriminating between functional and intrinsic renal disorders, especially in sepsis [1–3]. Nevertheless, there has been a recent resurgence in interest in urine microscopy as a tool to characterize AKI spurred by data from two groups.

Chawla et al. [4] developed an AKI cast scoring index to standardize urine sediment analysis and were able to show good precision of the index to detect acute tubular necrosis (ATN). In that study, urine sediment also correlated with outcomes in patients with ATN. Renal recovery was worse in patients with a higher cast scoring index (2.55 ± 0.9 versus 1.7 ± 0.79; \( P = 0.04 \)), and the area under the receiver operating characteristic (ROC) curve of the cast scoring index for the prediction of non-renal recovery was 0.79. Perazella et al. [5] proposed a different scoring system for differentiating ATN from decreased kidney perfusion in AKI (pre-renal AKI). Using final AKI diagnosis at discharge as the gold standard, urinary microscopy on the day of nephrology consultation was highly predictive of ATN. The odds ratio for ATN incrementally increased with a higher score. In patients with an initial diagnosis of ATN, any granular casts or renal epithelial tubular cells increased in value of 100% and a negative predictive value of 44%. Lack of renal epithelial tubular cells or granular casts in patients with an initial diagnosis of decreased kidney perfusion (functional

Biomarkers for acute kidney injury: combining the new silver with the old gold

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AKI) had a sensitivity of 0.73 and a specificity of 0.75 for the final diagnosis of ATN. The same group developed a scoring point system of urinary microscopy findings to predict adverse outcomes [6]. They evaluated the correlation of urinary sediment score and the Acute Kidney Injury Network (AKIN) stage at nephrology consultation. The score of urinary microscopy was associated with higher risk of worsening AKI in a dose-dependent manner.

In this issue of the journal, Schinstock et al. reinforce the value of urinary microscopy as a predictor of AKI and AKI severity. They performed urine microscopy and measured Neutrophil Gelatinase-associated Lipocalin (NGAL) levels in urine samples collected in the emergency room (ER) from 363 patients who were subsequently admitted to the hospital. Patient charts were reviewed to determine whether they developed AKI using the AKIN creatinine criteria of a rise in creatinine of >0.3 mg/dL within the 48 h following admission. Normal ranges and thresholds for the NGAL ELISA were established in a cohort of 125 healthy volunteers. Seventy-six patients (21%) developed AKI with 65% in AKIN Stage 1. Finding a renal epithelial cell, renal epithelial cell cast or granular cast had separately a high specificity (93.0–98.6%) to discriminate AKI from non-AKI patients. The presence of these elements as a group showed a higher sensitivity (from 6 to 22%) and good specificity (91%), determining a low negative but high positive predictive value (81.6%). The discriminative performance was even better when comparing more severe AKIN Stage 2 or 3 from AKIN Stage 0 or 1: sensitivity 30%, specificity 90% and positive predictive value 94%. In contrast, urine NGAL at ER admission had a sensitivity of 64.5% and a specificity of 64.5% to predict the development of AKI, which was significantly lower than the admission serum creatinine (sensitivity of 84.2% and specificity of 77.7%). Urine NGAL levels also predicted AKIN stage; however, urine microscopy additionally predicted death.

Nickolas et al. [7], in a single-center study of ER patients, showed sensitivities and specificities of urinary NGAL at a cutoff of 130 µg/G creatinine of 0.9 and 0.995 in predicting AKI. However, in a subsequent multicenter study of ER patients, the same authors reported that urinary NGAL had an 81% specificity and 68% sensitivity at a 104 ng/mL cut-off for intrinsic AKI in comparison to serum creatinine which had a 82% specificity and 81% sensitivity for values >1.4 mg/dL [8]. In both these studies, urine samples were collected in the ER; however, details of the timing of collection are not provided and a urinalysis was not performed. The Schinstock study design was somewhat different as it assayed NGAL in urine that was collected and stored for varying periods of time, at room temperature, prior to assay. One of the difficulties in implementing any biomarker-based strategy for AKI is standardizing the sample collection, preparation and assay measurements [9]. In the Schinstock study, urine samples were assayed for NGAL in the waste urine that was discarded after a standard automated urinalysis had been run through a central lab. No attempts were made to standardize the time to assay from collection or the timing of NGAL measurements after the urine sample had been processed by the laboratory. Additionally, no special handling of the samples was done with respect to storage prior to assay, and it is assumed that they were maintained at room temperature. These conditions replicate real-life scenarios where urine samples may sit at room temperature for several hours prior to assay. It is thus reassuring that NGAL levels in healthy controls were not affected by the storage conditions after centrifugation within the first 7 days after collection, regardless of the storage temperature of −4, −20 and −70°C. This is in contrast to earlier studies that have emphasized strict sample collection protocols for fear that longer storage times result in degradation of urinary biomarkers, particularly casts. Standardizing biomarker sample processing at room temperature eliminates the need for specialized handling and would facilitate the implementation of the use of biomarker measurement in conjunction with urinalysis.

Schinstock et al. also point out the fundamental issues that need to be considered before the widespread clinical use of NGAL as a biomarker for AKI. They verified the influence of urine white blood cells in NGAL assessment and the need for a systematic approach for sample handling and the assays used to improve reproducibility. It has been recently shown that the monomeric form of NGAL is the predominant form secreted by tubular epithelial cells, and the dimeric form is the predominant form secreted by neutrophils [10]. However, currently used ELISA methods mostly detect both forms of NGAL and cannot make this distinction. Nickolas et al. [8] have shown an excellent correlation of the ARCHITECT platform (Abbott Laboratories, Abbott Park, IL) to the measurement of monomeric forms of NGAL using an immunoblot technique, thereby confirming the specificity of these assays for AKI. As already demonstrated by other studies [11, 12], they confirmed the influence of gender and established different reference ranges for men and women. It is interesting that the authors picked a threshold for NGAL levels of ≥42.7 ng/mL from an ROC curve analysis that was within the 95th percentile reference value of ≤65.0 ng/mL in females and twice that of ≤23.4 ng/mL in males. This highlights that thresholds for each biomarker need to be representative of the population at risk and normalized for the assay platform and sampling conditions. It is clear that a consensus guideline on sample handling for urinalysis and biomarker preparation is urgently needed to enhance application of these techniques.

The first clinical studies of the new biomarkers of kidney injury showed promising results, with high diagnostic and predicting ability [13, 14]. In more heterogeneous populations, in which time of renal injury is poorly defined, performance of some biomarkers to detect AKI earlier was equivalent to clinical evaluation and standard laboratory measurements [15–17]. Ideally, the performance of the biomarkers should be tested in addition to clinical and laboratory evaluation available in the current clinical practice. There is consensus that most of the emerging kidney injury biomarkers may be able to detect injury earlier than serum creatinine demonstrates functional loss. However, the pattern of elevation in relationship to renal injury is not completely understood, except in homogeneous populations in which time of injury is known, for example, following cardiopulmonary bypass or exposure to nephrotoxins such as iodinated contrast. In addition, biomarker performance is measured by assessing diagnostic or predictive performance based on the actual standard, serum creatinine [8, 18–20].
However, two main issues are associated with the use of serum creatinine as the standard measurement. In the critically ill, serum creatinine changes are poorly related to function, and the baseline serum creatinine, which is needed to compare the subsequent values, is often unknown [21, 22]. Additional factors other than age and muscle mass influence serum creatinine values. In the ER, the dilutional effect of positive fluid balance can also be responsible for its lack of accuracy. With positive fluid balance, the volume of distribution of serum creatinine increases and could be associated with a delay for the diagnosis and estimation of severity of AKI [23]. In their study, Schinstock et al. did not describe the timing of peak serum creatinine in relation with fluid balance, diuretic use or fluid resuscitation. The absence of a known baseline renal function and use of a value after days in hospital and possible loss of muscle mass could both increase the inaccuracy [24]. In comparison with a known baseline serum creatinine, the use of these surrogates can falsely increase the incidence of AKI and thus interfere with the prediction ability of the biomarkers.

Since all the parameters used to predict and diagnose AKI have limits and biomarkers have varying performance, combining information from urine microscopy, biochemistry and biomarkers could help the clinician to predict AKI and its severity. In the Schinstock study, the association of a functional parameter with a biomarker increased sensitivity and specificity. As patients with functional AKI also reach aKIN criteria, the authors stratified patients based on the FeNa and showed that NGAL discriminative ability was better in those with an FeNa higher than 1% compared to those with FeNa less than 1%. They showed that urinary NGAL might be especially useful among patients without evidence of a reversible transient AKI evaluated by the FeNa. This finding demonstrates the value of combining functional and injury markers with varying sensitivity and specificity to provide better predictive performance for the clinician. Both urinalysis and NGAL could thus be combined to detect AKI or rule it out with more certainty than either test alone.

It seems clear that AKI biomarkers will not replace clinical evaluation and expertise will be required for meaningful interpretation [16, 25–27]. The ability of urinary biomarkers to diagnosis AKI or stratify patients at higher risk for AKI beyond clinical criteria will require the knowledge of the pattern of the biomarker in relationship to the type of injury etiology, duration and severity of AKI. The correlation of the underlying renal function and comorbidities with baseline levels of these biomarkers still requires validation in heterogeneous populations [16, 25, 26]. Standardized approaches for handling urine for urine microscopy and biomarker measurement will increase the ability of performing both tests. This requires a clear delineation of the collection procedure, including preservatives, spinning process, storage characteristics and the time since collection.

We believe that we are in an unprecedented era for equipping clinicians with tools to evaluate patients with AKI. The traditional measures of urinalysis and serum creatinine are being complemented with more specific biomarkers of kidney injury. As we expand the armamentarium for tackling AKI, we will undoubtedly create new standards for utilizing these methods [26]. Combining existing with newer techniques could inform appropriate triage, guide therapeutic interventions and provide information about the course of AKI and recovery of kidney function. As we learn more from the emerging field of AKI biomarkers, we should pay attention to the adage ‘make new friends but keep the old ones; new is silver and the other’s gold’.

### CONFLICT OF INTEREST STATEMENT

None declared.

(See related article by Schinstock et al. Urinalysis is more specific and urinary neutrophil gelatinase-associated lipocalin is more sensitive for early detection of acute kidney injury. *Nephrol Dial Transplant* 2013; 28: 1175–1185.)

### REFERENCES

In this issue of Nephrology Dialysis Transplantation, Heaf et al. [1] describe a novel haemodialysis approach, the so-called multipass system, which differs from the traditional dialysis set-up in a way that spent dialysate is returned to its container, where it is mechanically mixed with fresh dialysate before being recirculated into the dialysis circuit. This allows passing the same dialysate several times through the dialyser and reducing the volume of water needed; however, at the expense of a decreased removal capacity and thus dialysis adequacy per time unit. This negative aspect is then compensated by longer