Serum creatinine and the search for new biomarkers of acute kidney injury (AKI): the story continues

Davide Bolignano

AKI: the problem

The term ‘acute kidney injury’ (AKI) currently identifies a wide spectrum of clinical conditions ranging from a minimal elevation in serum creatinine levels to the requirement for renal replacement therapy. Therefore AKI includes both slight injury and/or a severe impairment in renal function, a condition formerly known as acute renal failure (ARF). AKI is a global problem which occurs less frequently in the community than in the hospital, where is commonly found on pediatric, oncology and surgical wards, with the highest frequency in the intensive care unit (ICU). It has been estimated that severe AKI requiring admission to an ICU occurs in about 10 patients per 100,000 per year, whereas AKI affects up to 30% of all ICU admissions usually as a manifestation of a multi-organ failure syndrome (1). Patients with chronic kidney disease (CKD) are particularly susceptible to AKI and AKI in turn may act as a cause of new-onset CKD or a promoter of underlying CKD progression. A rough estimate of the yearly incidence of end stage renal disease (ESRD) due to AKI is about 0.3 per 100,000 (2), and CKD can be detected in an average of 30% of AKI survivors (3). Irrespective of its nature, AKI affects both short-term and long-term cardiovascular outcomes and the in-hospital mortality currently remains dramatic (4). The early identification and correct diagnosis is therefore crucial to establish appropriate therapeutic measures in a timely manner, saving costs and improving patients’ outcomes and quality of life.

Why we need to look beyond serum creatinine?

The Acute Dialysis Quality Initiative Working Group has recently tried to standardize the definition of AKI in adults. The consensus classification RIFLE (risk, injury, failure, loss, and end-stage renal disease) defines three grades of severity [risk (class R), injury (class I) and failure (class F)], and two classes of clinical outcome (loss and ESRD) based on the entity of serum creatinine increase from baseline and/or the reduction of urine output over time (5, 6). In 2007, a modified version of the RIFLE criteria was proposed by the AKI Network (the AKIN criteria) (6). In this refinement version, the categories of Risk, Injury, and Failure were replaced with Stages 1, 2 and 3 and the outcome categories Loss and ESRD were eliminated. However, irrespective of the classification used, increasing evidence indicates that serum creatinine is far from being a decisive marker for diagnosing and staging AKI (7) due to several limitations that cannot be underestimated. First of all, the increase in serum creatinine is often not specific for organic AKI, requiring at least a differentiation from other extra-renal causes of azotemia. Second, serum creatinine is usually considered as a passive marker of renal function, both in health and disease. As a consequence, serum creatinine concentrations are not specific for renal tubular lesions (pathogenetically related to AKI), but rather reflect the loss of glomerular filtration function which follows the development of AKI. The increase in serum creatinine is therefore often detected later than the actual GFR changes due to the fact that creatinine accumulates over time. Under normal conditions (assuming tubular secretion and extra-renal elimination to be zero), the filtered load of creatinine equals its generation, leading to a stable creatinine balance and circulating levels. After an acute decline of GFR the generation of creatinine is unchanged but its filtration and excretion are reduced, resulting in retention of the marker (a rising positive balance) and a rising serum level (non-steady state). During this time the estimated GFR (eGFR: using the commonest creatinine-based formulas such as Cockcroft-Gault, Modification of Diet in Renal Disease (MDRD) or Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)) is higher than the real, measured GFR (mGFR). Although GFR remains reduced, the rise in serum level leads to an increase in filtered load (the product of GFR times the serum level) until filtration equals generation. At that time, the cumulative balance and the circulating levels finally reach a new steady state where eGFR approximates mGFR. However, after normalization of GFR, serum creatinine decreases to its previous levels. While serum creatinine decreases, eGFR underestimates mGFR. Again, when the new steady state is attained, eGFR will approximate once more mGFR (Figure 1).

It is also important consider that circulating creatinine reflects not only the GFR but also other physiologic processes including creatinine generation by muscle catabolism and diet, renal tubular secretion of creatinine, extra-renal elimination by the gut, and the distribution volume of creatinine. The normal range of this protein is also influenced by some demographic characteristics such as age, sex and race. Giving an example, to have the same GFR the serum
creatinine of a young black male will nearly twice than that of an elderly white female. Given that the serum creatinine definition of AKI is based at least on two consecutive values, another problem is when two creatinine measurements are obtained by different laboratories. While the coefficient of variation of serum creatinine is usually modest by various clinical testing methods (i.e., <5%), the variation (bias) from one laboratory to another may be considerably higher (8, 9). Furthermore, daily variation in creatinine due to differences in diet, physical activity and hydration status may be as great as 10%. To this extent, the hydration status is another crucial point because both dehydration (e.g., as consequence of diuretic abuse, strenuous physical exercise or gastro-intestinal loss) and hyperhydration (as consequence of water load or exaggerate fluid replacement) (10) can induce pseudo-alterations of creatinine levels which can mask or mislead the correct estimation of renal function, a concept recently remarked by the Program to Improve Care in Acute Renal Disease (PICARD) study investigators (11).

New biomarkers to predict AKI: what lies ahead?

Several different biomarkers have been proposed as potential ‘holy grails’ for the early and correct diagnosis of AKI over the last decade (5). The promising results attained in experimental and small pilot studies were, however, not always confirmed in wider and heterogeneous populations. In some cases, we suffer from a lack of targeted randomized trials to assess, in a definitive manner, to what extent the introduction of a given biomarker in the clinical practice can offer in terms of outcomes improvement and cost saving, compared to the traditional (and limited) clinical models currently in use. From a general point of view, the ideal biomarker for the diagnosis of AKI should be sensible, as much as possible related to kidney diseases (even better if almost immeasurable under health conditions), early, non-invasive, reliable, quick to perform, accurate and inexpensive. From a specific point of view, it should allow the differentiation between intra- and extra-renal causes of AKI (also distinguish it from acute renal glomerular injury) as well as to recognize its etiology; it should eventually correlate with the histological findings of kidney biopsies; be site-specific to detect early injury and identify pathological changes in various segments of renal tubules; reflect the degree of tubular injury (detecting even minor changes) or the onset of more severe damage. Finally, such a marker should be detectable throughout the entire course of AKI with defined threshold values to assess progression and prognosis of renal injury. Despite intense research, no single ideal biomarker with such features has yet been found and, probably, the use of a multipanel including different biomarkers represents the best solution to improve the diagnostic power in strictly defined scenarios (12).

Figure 1  Timeline of generation, filtration, excretion, balance and circulating levels of creatinine after an acute decline in renal function.
Is neutrophil gelatinase-associated lipocalin (NGAL) the biomarker we were looking for?

In this issue of Clinical Chemistry and Laboratory Medicine (CCLM) the articles of Bakker and Syperda (13), Lippi et al. (14, 15), Clerico et al. (16) and respective co-workers suggest new potential applications and raise new concerns about the clinical usefulness of one the most promising biomarkers of AKI, the neutrophil gelatinase-associated lipocalin (NGAL). NGAL is a 25-kDa protein initially found in the gelatinase of human neutrophils which is also expressed in very low concentrations in numerous human tissues. In the kidney, the NGAL gene is strongly induced after various kinds of injury like ischemia, sepsis or nephrotoxicity and the NGAL protein is accumulated mainly in the proliferating cells of the proximal tubule where it induces tubular re-epithelialization by increasing the internalization, transport and storage of iron particles (17). In a seminal paper (18), Mishra et al. measured NGAL in 71 children undergoing cardiopulmonary bypass, a treatment usually associated with a very high risk of AKI. The authors performed serial urine and blood NGAL measurement by Western blots and ELISA, defining AKI as a 50% increase in serum creatinine from baseline. The diagnosis of AKI using serum creatinine was only possible 24–72 h after cardiopulmonary bypass. Conversely, 2 h after the end of the procedure, NGAL increased over 100-fold and about 20-fold in urine and serum, respectively, AUC for urinary NGAL at 2 h was 0.99, with a sensitivity of 1.00 and a specificity of 0.98 with a best cut-off value of 50 μg/L. Finally, by multivariate analysis, the amount of NGAL in urine at 2 h after cardiopulmonary bypass was the most powerful independent predictor of AKI. Unfortunately, further studies analyzing NGAL in adult population at high risk of AKI failed to confirm at least in part these astonishing findings, and revealed an extreme variability in the diagnostic profile of this biomarker. A recent, systematic review (19) has collected and meta-analyzed data about the predictive power of NGAL obtained from 19 studies and eight countries involving 2538 patients, of whom 487 (19.2%) developed AKI. The overall diagnostic-odd ratio (DOR)/area under the curve (AUC)-ROC of NGAL to predict AKI was 18.6/0.815 (95% CI 0.732–0.892). When the results were stratified according to the clinical setting, the DOR/AUC-ROC of NGAL was 13.1/0.775 (95% CI 0.669–0.867), in cardiac surgery patients, 10.0/0.728 (95% CI 0.615–0.834) in critically ill patients and 92.0/0.894 (95% CI 0.826–0.950) after contrast infusion. The diagnostic accuracy of plasma/serum [17.9/0.775 (95% CI 0.679–0.869)] appeared rather similar to that of urine NGAL [18.6/0.837 (95% CI 0.762–0.906)], while age seemed to have a strong impact on the diagnostic profile of NGAL with a better predictive ability in children [25.4/0.930 (95% CI 0.883–0.968)] than in adults [10.6/0.782 (95% CI 0.689–0.872)]. This last aspect is probably one of the most important limitations of NGAL as a reliable AKI biomarker. Several studies have revealed that NGAL is far from being a true ‘kidney-specific’ protein, because even urine levels can be influenced by several co-morbidities such as diabetes (20), cardiovascular diseases (21, 22), neoplasias (23), inflammation (24, 25), multiple trauma (26), underlining CKD (27) as well as acute leukocytosis (28), most of which are usually present in adults rather than in children. The heterogeneity of the study populations therefore may hinder the ability of this biomarker to improve the predictive power of the clinical models currently available. In their study, Siew et al. (29) prospectively analyzed urine NGAL in a heterogeneous population of 451 critically ill adults. The AUCs for NGAL to predict the occurrence of AKI within 24 and 48 h were 0.71 and 0.64, respectively, whereas the AUC of a clinical model including serum creatinine, age, disease severity, sepsis and intensive care unit (ICU) location was 0.81. Although NGAL remained independently associated with AKI even after adjustment for this clinical model, the addition of NGAL measurement only marginally improved the predictive performance of the model, slightly increasing the AUC from 0.81 to 0.82. Although urine NGAL independently predicted severe AKI during hospitalization [HR 2.60, 95% CI 1.55–4.35] even after adjustment for APACHE II score (an ICU illness severity score), a single measurement of NGAL revealed only a moderate predictive utility for the development and severity of AKI, with a very limited contribution to conventional clinical risk predictors in this heterogeneous ICU population.

Conclusions

Although interesting, NGAL does not seem to match all the requirements for a perfect AKI biomarker and thus remains a ‘promising one’. We still have to identify in which population (children, adults...), in which specimen (urine, serum, both...) (28, 30) and at which time point(s) its measurement can give the better diagnostic capacity, considering that the low kidney-specificity can hinder its predictive capacities when other co-morbidities are present. Furthermore, as for other promising biomarkers used in several highly prevalent disorders such as cancer (31), neurological diseases (32–36), liver fibrosis (37), cardiovascular and disease and thrombosis (38, 39), we are still waiting for targeted randomized trials which would allow to quantify the impact on hard outcomes of NGAL in daily practice, with respect to current available diagnostic weapons. Given the dimensions of the AKI problem and the already cited limitations of serum creatinine measurement, the search for the perfect biomarker thus continues, becoming day-by-day even more mandatory.

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Davide Bolignano, MD, CNR-IBIM
Clinical Epidemiology and Pathophysiology of Renal Diseases and Hypertension, Ospedali Riuniti, Via Vallone Petrara, 89100 Reggio Calabria, Italy
Phone: +39 0965397012, Fax: +39 0965397014,
E-mail: davide.bolignano@gmail.com