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Novel renal biomarkers of acute kidney injury and their implications

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Acute kidney injury (AKI) is a common problem in the community, in hospitalised patients, and on a global scale.[1,2] It is independently associated with increased morbidity, mortality, and risk of subsequent chronic kidney disease (CKD).[3,4] The diagnosis of AKI and its consensus definition rest upon two criteria: the serum creatinine concentration and the urinary output. Such criteria have also been used to stage its severity as outlined by the Kidney Disease Improving Global Outcomes (KDIGO) classification.[5]

However, serum creatinine and urinary output have serious limitations in defining the presence and severity of AKI. Serum creatinine is insensitive and typically only starts to increase when approximately 50% of glomerular filtration rate (GFR) is lost.[6] Moreover, it is influenced by several confounders such as muscle mass, fluid therapy, muscle injury, and a variable degree of secretion into the tubular lumen.[6] Urinary output may be a more sensitive marker of decreased GFR, but lacks specificity.[7] Even more than creatinine, it is affected by confounders such as intravenous fluid therapy, diuretics, and haemodynamic status. In addition, both biomarkers mostly reflect GFR and kidney *function*, without providing information on tubular damage or tubular stress and/or dysfunction. Despite these shortcomings, they have dominated the clinical world of AKI for decades and continue to do so.

More recently, however, the increasingly sophisticated world of biochemical analysis and proteomics has led to the discovery of several protein biomarkers. These biomarkers can be measured in blood and, more usefully and easily, in urine and can predict the subsequent development of traditionally defined AKI. Their predictive performance has been measured by several methods including the area under the receiver operating characteristic curve (AUC ROC), the integrated discrimination improvement index, and the net reclassification index.[8]

Such measurements from multiple observational studies, as well as commercial development, have narrowed the current field of clinical use mostly to two major biomarkers: neutrophil gelatinase associated lipocalin (NGAL) and the combination of two key protein markers of tubular cell cycle arrest: insulin like growth factor binding protein-7 (IGFBP-7) and tissue inhibitor of metallo-proteinase 2 (TIMP-2).[9-19] The former has been studied in >1000 studies across multiple populations; the latter has been studied in more defined but fewer populations.[13-18] However, the investigations of the latter have been more structured, multicentre, and sophisticated in scope, size, and design. Accordingly, the combination of IGFBP-7 and TIMP-2 as measured in the urine has now been approved by the United States Federal Drug Administration (FDA) for the early diagnosis of AKI under the commercial name of Nephrocheck™.

What do novel AKI biomarkers represent?

The identification of these novel biomarkers has raised biological questions. These questions go beyond the predictive value of these biomarkers. For example, we now know that NGAL is involved in the control of free iron and is a complex molecule.[19] NGAL can be found in blood and urine in several forms: a homodimer of two combined NGAL molecules and/or a heterodimer of NGAL combined with lipocalin (typically of neutrophil original) and/or a monomer of NGAL alone (essentially of tubular origin).[19] NGAL is also complex in its origin because, even though it can be released into the tubular lumen or, perhaps into the blood stream from the tubules, it is also released by neutrophils under conditions of activation, and most of all by the liver in conditions of inflammation (an interleukin-6 dependent process)[20] and in a form that can be filtered by the glomerulus. Moreover, if filtered, its absorption is

regulated by the expression and availability of megalin, a binding protein that can easily be made less available by the presence of proteinuria.[21] Finally, the epitopes for commercially available assays are variable and cannot reliably distinguish likely tubular NGAL (monomer) from filtered neutrophil NGAL (dimers). Accordingly, beyond its predictive value for AKI, the biological meaning of an elevated NGAL level in relation to subsequent AKI remains unclear.

The meaning of the Nephrocheck™ biomarkers remains similarly unclear. Even though these proteins appear to contribute to cell cycle arrest (and presumably, thereby, decrease bio-energetic stress), [13, 17] they may not necessarily represent tubular cell damage but rather tubular cell stress or leakage. In animal (mice) models of AKI, the excretion of cell cycle arrest biomarkers in the urine was rapid (4 hours after injury) and associated with increased concentrations in the renal cortex, while mRNA levels remained normal. In addition, albuminuria strongly correlated with their levels in the urine, while immunohistochemistry showed progressive cell cycle arrest losses from proximal tubules. Crucially, inhibition of endocytic reabsorption in normal mice tripled urinary levels of cell cycle arrest biomarkers suggesting that saturation of this mechanism may contribute in great part to higher concentrations in the urine.[22]

Irrespective of whether they represent cell stress or cell damage or failed endocytosis, they have now been shown to predict loss of renal functional reserve after cardiac surgery, even in the absence of changes in serum creatinine.[23]

What is the role of novel biomarkers of AKI?

A major problem with the assessment of the role of these biomarkers is related to their performance as predictors of subsequent changes in serum creatinine. Their performance assessed by the AUC ROC method varies between 0.75 and 0.82 depending on the population studied and has been judged imperfect. However, such judgement is illogical because these biomarkers reflect tubular events and not GFR changes. Thus, using imperfect biomarkers of GFR (creatinine and/or urinary output) to judge the diagnostic value of biomarkers of tubular stress or dysfunction makes no sense at all. Accordingly, these novel biomarkers cannot be "validated" by a relevant gold standard and must stand alone.

If that is the case, as is logical, the biological evidence that they reflect a form of tubular pathophysiology (stress or damage or leakage or failed endocytosis as it may be) is strong. Thus, the key issue is not about the associated changes in GFR that may or may not be detected by serum creatinine or urinary output, but whether such biomarkers should now be included in the definition of AKI. The biological case for such inclusion is clear.[24,25] The clinical case for their use in daily practice is rapidly growing and, in the opinion of many experts, these measurements are ready for daily use. In particular, the increasing ability to measure these novel AKI biomarkers and the observation that they likely identify an earlier phase of tubular AKI has now opened the door to investigations of the use such biomarkers as triggers for therapeutic interventions aimed at preventing AKI diagnosed by traditional criteria and the preliminary results are very promising.[26-28] While it is likely that several such studies will take place in the near future and confirm the importance of these biomarkers, a more logical version

of the KDIGO criteria for AKI can already be proposed (Table 1) and is easily justified on current knowledge.

Conclusion

The data are clear that novel biomarkers, such as cell cycle arrest biomarkers and NGAL, represent a separate tubular form of AKI. Moreover, it is clear that they are able to identify such AKI earlier than traditional GFR-related biomarkers (e.g. creatinine and/or urinary output). Finally, it is clear they identify patients at higher risk of adverse renal and clinical outcomes. Such observations imply the need to incorporate these biomarkers into future consensus definitions of AKI. These observations also generate impetus for increased clinical use to gain experience and to help design controlled interventional studies using these biomarkers as tools and triggers for patient identification, stratification, and for the assessment of targeted interventions.

Table 1: Proposed modification of AKI diagnosis and staging in adults

Novel Biomarker Informed AKI definition

1. Increase in serum creatinine >26.5 µmol/L within 48 hours or increase in serum creatinine >1.5 times baseline known or presumed to have occurred in previous 7 days or urine output <0.5 ml/kg/hr for 6 hours or elevated combined cell cycle arrest biomarkers value [≥2 $(ng/ml)^2/1000$][13] or elevated urinary NGAL value (≥100 ng/mL)[25]

Novel Biomarker Informed Staging

1. **Stage 1**: Increase in serum creatinine >26.5 µmol/L within 48 hours or increase in serum creatinine of 1.5-1.9 times baseline or urine output <0.5 ml/kg/hr for 6 to 12 hours or elevated combined cell cycle arrest biomarkers value (2 to 2.49 $(ng/ml)^2/1000$)[13] or elevated urinary NGAL value (from 100 to 149 ng/mL)[25]
2. **Stage 2**: Increase in serum creatinine of 2 to 2.9 times baseline or urine output <0.5 ml/kg/hr for ≥12 hours or elevated combined cell cycle arrest biomarkers value [from 2.5 to 3.49 $(ng/ml)^2/1000$][13] or elevated urinary NGAL value (from 150 to 249 ng/mL)[25]
3. **Stage 3**: Increase in serum creatinine >3 times baseline or urine output <0.3 ml/kg/hr for ≥24 hours or anuria or increase in serum creatinine to>353.6 µmol/L or initiation of renal replacement therapy or elevated combined cell cycle arrest biomarkers value [≥3.5 $(ng/ml)^2/1000$][13] or elevated urinary NGAL value (≥250 ng/mL)[25]

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