# **REVIEW ARTICLES**

# Novel biomarkers of acute kidney injury and failure: clinical applicability

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## **Editor's key points**

- Acute kidney injury is common in critical illness and is often diagnosed late.
- Biomarkers indicating kidney injury and function would be beneficial as changes in creatinine are delayed.
- Several substances undergoing evaluation show potential but need further study.
- Carefully designed studies of potential markers of this multifactorial disease are needed.

Summary. Acute kidney injury (AKI) has a number of triggers, including ischaemia, nephrotoxins, radiocontrast, and bacterial endotoxins. It occurs in around one-third of patients treated in intensive care unit (ICU) and is even more prevalent in cardiac surgery patients. There is a higher mortality in patients with AKI compared with non-AKI counterparts, and in severe AKI requiring renal support, the 6 month mortality is >50%. Unlike the progressive development of biomarkers in cardiology, there have been few changes in kidney diagnostic markers. Creatinine is still used as an indicator of kidney function but not of the parenchymal kidney injury. Serum creatinine (sCr) concentration does not change until around 50% of kidney function is lost, and varies with muscle mass, age, sex, medications, and hydration status. The lag time between injury and loss of function, risks missing a therapeutic opportunity, and may explain the high associated mortality. Novel biomarkers of AKI- and failure include neutrophil gelatinase-associated lipocalin, N-acetyl- $\beta$ -D-glucosaminidase, kidney injury molecule-1, interleukin-18, and cystatin C. The pathophysiology associated with accumulation of these markers in plasma and urine is not clear, but a common denominator is inflammation. Some of these new AKI biomarkers may have clinical applicability in anaesthesia and intensive care in the future. It is possible that a 'kidney biomarker panel' will become standard before and after major surgery. If elevated or positive, the anaesthetist must take special care to optimize the patients after operation on the surgical wards or ICU to avoid further nephrotoxic insults and initiate supplementary care.

Keywords: acute kidney injury; biological markers; intensive care; postoperative care

The use of biochemical markers of myocardial injury has undergone profound changes in the past 50 yr. We have moved from the measurement of aspartate amino transferase to the present use of troponins. This progress in diagnostic ability and sensitivity has been a cornerstone in the parallel improvement in treatment and survival after cardiac injury.<sup>1</sup> This stands in stark contrast to clinical practice relating to biochemical markers of kidney function and injury which has remained focused on the measurement of creatinine. Creatinine is an indicator of renal function but not of kidney injury and serum creatinine (sCr) concentration does not change until around 50% of kidney function is lost,<sup>2</sup> and varies with muscle mass, age, sex, medications, and hydration status. The lag time between the injury and the resulting loss of function which finally results in an elevation of sCr is a missed therapeutic opportunity, and this may explain the high mortality associated with acute kidney injury (AKI). AKI is common, it occurs in more than 30% of critically ill patients and patients undergoing major surgery are also at risk.<sup>3 4</sup> A recent study showed

that AKI occurs in as many as 40% of patients after cardiac surgery.  $^{\rm 5}$ 

The Acute Dialysis Quality Initiative (ADQI) proposed a consensus definition, in 2004–5, for AKI using the Risk, Injury, Failure, Loss of kidney function and End-stage kidney disease (RIFLE) criteria (Table 1).<sup>6 7</sup> Before this, AKI (formerly called 'acute renal failure') lacked definition and more than 35 definitions were used.<sup>8</sup> This was beneficial for the research area as we can compare studies, and results, locally and globally. However, we must be aware of the uncertain relationship between sCr and urine output, our 'gold standard', and the pathophysiology behind AKI.

A number of promising biomarkers of kidney injury have been identified during the last decade.

The properties of an ideal biomarker have been defined as:<sup>9</sup>

- (i) It must be generated by the damaged cells and exhibit the organ specificity.
- (ii) Its concentration in the body must be proportional to the extent of damage.

**Table 1** Definition of AKI according to the RIFLE and AKIN criteria. \*Modifications by the AKIN group. <sup>1</sup>When baseline creatinine is unknown, it is recommended to estimate baseline using the modification of diet in renal disease (MDRD) equation assuming a GFR of 75 ml min<sup>-1</sup> 1.73 m<sup>-2</sup>. <sup>‡</sup>Patients with chronic kidney dysfunction reach class Failure when creatinine increases  $\geq$ 44.2  $\mu$ mol litre<sup>-1</sup> from baseline to >354  $\mu$ mol litre<sup>-1</sup>

AKI severity	Serum creatinine criteria	Urine output criteria
Risk	$\geq$ 1.5-fold increase in serum creatinine from baseline^{+} (or an absolute increase in serum creatinine of $\geq$ 26.3 $\mu mol$ litre^{-1} within 48 h*)	$<$ 0.5 ml kg <sup>-1</sup> h <sup>-1</sup> for $\ge$ 6 h
Injury	$\geq$ 2.0-fold increase in serum creatinine from baseline <sup>†</sup>	$<$ 0.5 ml kg $^{-1}$ h $^{-1}$ for $\ge$ 12 h
Failure <sup>‡</sup>	$\geq$ 3.0-fold increase in serum creatinine from baseline <sup>†</sup> (or initiation of renal replacement therapy*)	${<}0.3 \text{ ml kg}^{-1} \text{ h}^{-1}$ for ${\geq}24 \text{ h}$ or an uria ${\geq}12 \text{ h}$
Loss of kidney function	Complete loss of kidney function >4 weeks	
End-stage kidney disease	End-stage kidney disease >3 months	

- (iii) It should be expressed early after the organ damage, when such damage is still potentially reversible.
- (iv) Its concentration should decrease quickly after the acute injury episode to enable its use as a therapeutic monitoring tool.
- (v) It should be rapidly and reliably measurable.

This narrative review aims at describing some novel markers of AKI and kidney failure, including neutrophil gelatinaseassociated lipocalin (NGAL), *N*-acetyl- $\beta$ -D-glucosaminidase (NAG), kidney injury molecule-1 (KIM-1), interleukin-18 (IL-18), and cystatin C. The theory behind the pathophysiological events that leads to accumulation of these markers in plasma and urine will be discussed.

# Pathophysiology of AKI and biomarkers

The current understanding of the pathophysiology of AKI in humans is mainly transferred from animal studies. Irrespective of the type of insult and of the clinical setting [after major surgery or in intensive care unit (ICU) patients], an inflammatory response seems to play a major role in the initiation of AKI (Fig. 1). Triggers of AKI (ischaemia, nephrotoxins, and bacterial endotoxins) induce the release of inflammatory mediators (e.g. cytokines and chemokines) from endothelial and tubular cells in the kidney. Neutrophils and other leucocytes migrate to the site of inflammation and marginate along the peritubular capillary wall very early after the insult.<sup>10</sup> Endothelial inflammatory injury is followed by increased vascular permeability which, within 24 h, facilitates migration of neutrophils into the kidney interstitium and tubular lumen.<sup>10</sup> During transmigration, neutrophils release pro-inflammatory cytokines that further aggravate the tubular injury.<sup>11</sup> Eventually, the tubular response to AKI is characterized by a loss of cytoskeletal integrity leading to desquamation of viable cells and also apoptosis and necrosis.<sup>12</sup> The underlying pathology behind and the timing of decreased glomerular filtration rate (GFR) during this process is yet to be determined. Tubular obstruction from desquamated cells, renal vasoconstriction due to the release of vasoactive mediators, and direct effects on the glomerular filter have been proposed as mechanisms.

During the development of AKI, a number of causes result in biomarkers accumulating in plasma and urine and may represent different pathophysiological events during the process of kidney injury and repair (Fig. 2). Biomarkers accumulate in urine due to an induced tubular epithelial synthesis in different parts of the nephron (NGAL, IL-18, NAG, KIM-1) and as an effect of impaired reabsorption of the filtered load in the proximal tubule (NGAL, cystatin C). Secretion of biomarkers from activated immune cells miaratina into the tubular lumen may also be a source (NGAL, IL-18). Finally, increased synthesis of some biomarkers in extra-renal tissues has been shown in animal AKI models (NGAL, IL-18).<sup>13</sup> This extra-renal production will most certainly increase circulating biomarker levels and a decline in GFR will further amplify this increase. However, the secretion from immune cells and extra-renal tissues into the bloodstream can increase in response to systemic inflammation, for example during sepsis and after major surgery or trauma, even in the absence of AKI. This must be taken into account when elevated biomarker levels are evaluated in critically ill and postoperative patients.

# Markers of kidney injury

#### Neutrophil gelatinase-associated lipocalin

NGAL, also known as human neutrophil lipocalin or Lipocalin 2, was first identified as a 25 kDa protein in the secondary granules of human neutrophils.<sup>14 15</sup> In response to bacterial infection, NGAL is released into the bloodstream. Raised concentrations can be used to distinguish between bacterial and viral infection.<sup>16</sup> Later, NGAL was localized in a number of human tissues, including trachea, lung, stomach, colon, and kidney.<sup>17</sup> NGAL secretion from epithelial cells is induced by several pathological conditions.<sup>18 19</sup> In the search for novel biomarkers of AKI, NGAL was identified as the most rapidly induced protein in murine models of ischaemic and nephrotoxic AKI.<sup>20</sup> Concentrations increased several fold in both serum and urine within hours of the insult. This serendipitous finding shifted the focus on NGAL from a marker of bacterial infection to an early signal for AKI.

Evidence of the biological role of NGAL in different pathological states has recently emerged. By its ability to bind siderophores (small iron-binding molecules) produced by Downloaded from http://bja.oxfordjournals.org/ by John Vogel on November 21, 2012



**Fig 1** Pathophysiological mechanisms of AKI and repair. Adhesion molecules up-regulate on the surface of endothelial cells and facilitate migration of neutrophils into the kidney interstitium and tubular lumen. Inflammatory and vasoactive mediators and reactive oxygen species (ROS) damage the tubular cells. Shedding of the proximal tubule brush border, loss of polarity with mislocation of Na+/K+- ATPase and also apoptosis and necrosis may occur. With severe injury, viable and non-viable cells are desquamated, leaving parts of the basement membrane denuded. Inflammatory and vasoactive substances released from the injured tubular cells worsen the pathophysiological changes. If the repair process is successful, viable cells de-differentiate and spread to cover exposed areas of the basement membrane and restore the functional integrity of the nephron. AC, apoptotic cell; DC, de-differentiating cell; NC, necrotic cell.

eukaryotic cells, NGAL is involved in iron transport to and from cells. NGAL assists in the delivery of iron to kidney tubular cells and may be involved in the injury-repair-process of AKI by inducing differentiation of renal progenitor cells into epithelial tubules.<sup>21 22</sup> Siderophores are also produced by bacteria to acquire iron necessary for bacterial growth from the surrounding tissues. By binding to these siderophores, NGAL blocks the iron supply and may act as an endogenous bacteriostatic agent.<sup>23</sup> Interestingly, tissues in which NGAL is expressed are all frequently exposed to microorganisms, and this supports its role in host defence.

In an early study, elevated NGAL levels were detected in both urine and plasma in adult patients with established AKI.<sup>22</sup> Human kidney biopsies also showed an accumulation of NGAL in cortical tubules in AKI patients. Accumulation was most pronounced in the most injured cells. The first clinical study evaluating NGAL as an AKI predictor was in children at risk of AKI after cardiopulmonary bypass (CPB). Urinary NGAL increased almost 100-fold and serum NGAL 20-fold within 2 h post-CPB in children who later (24–48 h) developed AKI.<sup>24</sup> The area under the receiver-operating characteristic curve (AuROC) using the 2 h urinary NGAL concentration for AKI prediction was almost perfect (0.998).<sup>25</sup> Since these encouraging results, the predictive performance of NGAL has been tested on adult patients in various clinical settings.

#### Cardiac surgery

Studies in adult patients after cardiac surgery show conflicting results with AuROC for AKI prediction within 48 h up to 10 days ranging from 0.50 to 0.98.<sup>26–35</sup> This was different from most paediatric studies where NGAL generally performed better.<sup>25–36</sup> Co-morbidities such as diabetes<sup>37</sup> and preexisting kidney dysfunction<sup>38</sup> may explain the limited ability of NGAL to predict AKI in adult patients. Non-uniform definitions of AKI used are likely to have contributed to the varying predictive performances. The value of NGAL as an AKI predictor with increasing AKI severity has recently been demonstrated.<sup>39</sup> Finally, it was recently shown that extracorporeal



circulation through the CPB circuit *per se* increases NGAL levels several fold in urine in non-AKI patients.<sup>40</sup>

#### Critically ill/emergency department

Evaluating NGAL for AKI prediction in general ICU patients is difficult for several reasons. First, as part of multiorgan failure, AKI is already present at ICU admission in the majority of cases. Even if a 'window' exists on the ICU before AKI is diagnosed, the timing of the kidney insult is often unknown. Secondly, the aetiology of AKI in this setting is often multifactorial including ischaemic insults, nephrotoxic drugs, and bacterial toxins. Thirdly, an ICU population displays a heterogeneous mixture of co-morbidities. Finally, a baseline creatinine is lacking in many patients admitted to the ICU. This clearly increases the risk of misclassification of the AKI diagnosis defined by the RIFLE criteria.

Activated neutrophils release NGAL and raised plasma concentrations have been observed in non-AKI patients with sepsis.<sup>41</sup> NGAL concentrations were shown to be almost 80% higher in septic than in non-septic AKI patients.<sup>42</sup> This may affect the predictive value of plasma NGAL in AKI. In patients with septic shock, the AuROC for AKI prediction within 12 h was not significant in one study,<sup>41</sup> but in studies, where sepsis was over-represented in the AKI patients, AuROC of 0.82 was found for NGAL as a predictor for AKI [44.2  $\mu$ mol litre<sup>-1</sup> increase in sCR or acute renal replacement therapy (RRT)] within 72 h in septic patients admitted to an emergency department (ED), but when a more common AKI classification (RIFLE R) was used, the sensitivity decreased.<sup>46</sup>

Urinary NGAL on admission to ICU reasonably predicted subsequent AKI within 12 h to 7 days.<sup>41 45 47</sup> On ED admission, urinary NGAL was a strong predictor of sustained AKI (>3 days) with an AuROC of 0.95.<sup>48</sup>

The effect of sepsis on urinary NGAL levels is not clear. In non-AKI patients, urinary levels were virtually unaffected by the presence of sepsis in one small study,<sup>41</sup> and predictive values did not change when patients with septic shock were studied exclusively. In contrast, septic AKI patients had urinary concentrations more than five-fold higher than non-septic patients in another study.<sup>42</sup>

Studies have used different platforms for NGAL quantification including western blotting, radio-immuno assay, enzyme-linked immunosorbent assay, and Triage<sup>®</sup> device. It was recently shown that the antibody configuration has an impact on the clinical performance of the assay,<sup>40</sup> and this may explain the variable results for NGAL as an AKI predictor. In addition, different forms of NGAL are secreted by kidney epithelial cells (mainly monomeric NGAL) and neutrophils (mainly dimeric NGAL), respectively.<sup>49</sup> In view of the timescale of pathophysiological changes as AKI develops, monomer-specific assays may improve the early detection of renal cell injury and avoid the confounding effect of urinary tract infection.<sup>50</sup>

#### N-acetyl-β-D-glucosaminidase

NAG is a large (>130 kDa) lysosomal enzyme found in several human cells including the renal tubules. Its size precludes glomerular filtration, and raised urinary concentrations are believed to have a tubular origin. Increased NAG levels reflect tubular injury, but could also be due to increased lysosomal activity without cell damage. NAG catalyses hydrolysis of terminal glucose residues in glycoproteins and is the most active glycosidase found in proximal tubular epithelial cell lysosomes. Urinary NAG activity has been shown to be high during active renal disease.<sup>51</sup>

A cross-sectional study compared nine different urinary markers, including NAG, in a total of 102 patients with AKI, tested at the time of nephrology consultation, and non-AKI patients.<sup>52</sup> An AuROC of 0.83 [95% confidence interval (CI), 0.77–0.88] was found for NAG in the identification of established AKI. NAG was found to predict RRT, mortality, and their composite endpoint in patients with AKI. The median normalized NAG level in patients with AKI who underwent RRT was 0.06 U mg Cr<sup>-1</sup>, compared with 0.02 U in those who did not.

Urinary NAG and KIM-1 were studied in 201 consecutive adult patients with AKI at the time of nephrology consultation.<sup>53</sup> For the composite outcome of RRT requirement or hospital death, NAG had an AUC of 0.71 (95% CI, 0.63–0.78), which was better than that of sCr (0.60, 95% CI, 0.52–0.68) or urine output (0.65, 95% CI, 0.57–0.73).

A study of 73 patients with 'initially non-oliguric acute tubular necrosis (ATN)' had measured urinary excretion of NAG and other biomarkers early in the course of ATN.<sup>54</sup> The urinary excretion of NAG was significantly higher in patients requiring RRT. The AuROC for NAG was 0.81 (95% CI, 0.73–0.88). At a cut-off of 4.5 U mmol  $Cr^{-1}$ , NAG was a sensitive (85%) but non-specific (62%) predictor for RRT. In a study of 635 unselected emergency room patients, urinary NAG

did not predict the composite outcome of ICU admission, need for RRT, nephrology consultation, or mortality.<sup>48</sup>

#### Kidney injury molecule-1

KIM-1 is a type I cell membrane glycoprotein. It has a cleavable ectodomain, localized in the apical membrane of dilated tubules in acute and chronic injury.<sup>55</sup> KIM-1 and its soluble ectodomain in urine (90 kDa) are believed to play a role in the regeneration processes after epithelial injury.

In a recent multisite, preclinical, rat toxicology study, the diagnostic performance of urinary KIM-1 was compared with traditional biomarkers as predictors of kidney tubular histopathological changes. In multiple models of kidney injury, urinary KIM-1 significantly outperformed sCr and BUN. The AuROC for KIM-1 was between 0.91 and 0.99 when compared with 0.79-0.9 for BUN and 0.73-0.85 for sCr.<sup>56</sup> These striking results have not been shown in humans. In the study mentioned earlier,<sup>53</sup> KIM-1 did not perform better than sCr or urine output in the prediction of RRT or death, with an AuROC of 0.61 (95% CI, 0.53-0.61). Patients in the highest KIM-1 quartile had 3.2-fold higher odds (95% CI, 1.4-7.4) for the composite outcome compared with patients with the lowest quartile, but after multivariable analysis, this was not significant. Similar results were found in another study, also mentioned in the NAG section<sup>52</sup> where KIM-1 did not predict the need for RRT, but was a significant predictor for mortality.

#### Interleukin-18

IL-18 is a proinflammatory cytokine with a molecular weight of 18 kDa. It is produced by renal tubular cells and by macrophages. In a number of renal disease processes such as apoptosis, ischaemia/reperfusion, allograft rejection, infection, autoimmune conditions, and malignancy, IL-18 has an active role.

Several novel biomarkers have been compared in a heterogeneous high-risk population as diagnostic and predictive markers of AKI, need for dialysis, and prediction of mortality at 7 days in patients stratified both for time elapsed after renal insult and for GFR at the time of ICU admission.<sup>57</sup> CysC, IL-18, and NGAL were the strongest predictors of dialysis (AuROC > 0.70). In patients without AKI on entry, AP (alkaline phosphatase), NGAL, and IL-18 were the strongest predictors of dialysis. All biomarkers except KIM-1 were moderately predictive of death within 7 days (AuROC > 0.60), especially IL-18 (AuROC=0.68). No biomarker predicted AKI within 48 h. IL-18 was the only one to predict more severe AKI (AuROC > 0.7).

Three studies of patients undergoing coronary artery bypass surgery (CABG)<sup>30 58 59</sup> involving a total of 258 patients showed that urinary IL-18 had a moderate predictive performance for AKI (AuROCs from 0.53 to 0.66) at post-CABG ICU admission. The combination of urinary IL-18 and urinary NGAL was shown to diagnose AKI after CABG much earlier than the increase in sCr.<sup>59</sup> Urinary IL-18 correlated with the duration of CPB, suggesting that IL-18 levels may represent a non-specific marker of bypass-associated systemic inflammation rather than tubular damage.<sup>58</sup>

In a study of patients undergoing coronary angiography, urinary IL-18 and NGAL levels were significantly increased in the CIN group 24 h after the procedure, but not in the control group (P<0.05).<sup>60</sup> IL-18 and NGAL outperformed sCr (P<0.05) for the detection of CIN. Elevated urinary IL-18 levels 24 h post-contrast administration have also been found to be an independent predictive marker for later major cardiac events (relative risk, 2.09; P < 0.01).

#### Urine cystatin C

Cystatin C, a 13 kDa proteinase inhibitor, enters the proximal tubules by glomerular filtration. The protein is reabsorbed and completely broken down by the healthy proximal tubular cells and only minimal concentrations are found in urine under normal conditions. Urinary levels of cystatin C increase when the reabsorptive capacity of proximal tubular cells is impaired. Cystatin C has therefore been proposed as a marker of AKI. Urinary cystatin C was a good predictor of dialysis requirement in ICU patients with established AKI.54 As a predictor of less severe AKI, results are less convincing mainly due to the lack of sensitivity.<sup>28 30 33 61 62</sup>

Low molecular weight proteins such as cystatin C are reabsorbed by the proximal tubular cells by receptormediated endocytosis.<sup>63</sup> Excess albumin in the urine competes with this process and may decrease the tubular uptake of cystatin C.<sup>64</sup> Sepsis alone may be associated with albuminuria<sup>65</sup> and could therefore cause elevated cystatin C levels in urine without AKI being present. Recently, higher cystatin C levels were found in the urine of non-AKI patients with septic shock when compared with non-septic patients.<sup>41</sup> This was supported by a study which found higher urine cystatin C levels in septic than in non-septic patients. This applied to patients with and without AKI. Urinary cystatin C was only predictive of AKI in the subgroup of septic patients.<sup>61</sup>

# Markers of kidney function

#### Plasma cystatin C

Cystatin C is believed to be a more robust endogenous marker of GFR than creatinine as it is:

- (i) thought to be produced at a constant rate by all nucleated cells,
- (ii) freely filtered by the glomeruli,
- (iii) minimally bound to proteins, and
- (iv) not reabsorbed to the systemic circulation after filtration.<sup>24 66</sup>

The cystatin C molecule is more than 100 times larger than creatinine. In theory, narrowing of the glomerular filter could impair filtration of cystatin C but still allow free passage of creatinine. This has led researchers to investigate whether an increase in plasma cystatin C precedes the conventional creatinine-based AKI criteria. In a mixed ICU population, a >50% increase in plasma cystatin C was shown to predict AKI within 24 h, with an AuROC of 0.97.<sup>67</sup> However, these results have not been verified in subsequent studies.<sup>28 68</sup> Recently, cystatin C was shown to be a poor predictor of AKI,<sup>69</sup> and another study showed no benefit of cystatin C over creatinine, serum urea nitrogen, or even urine output as an AKI predictor.<sup>70</sup>

Although the increase in cystatin C seems to coincide with creatinine when GFR decreases acutely, cystatin C might be better than creatinine as a GFR monitor at a later stage in ICU patients. Immobilized and catabolic ICU patients lose muscle mass and hence a gradual decline in sCR is expected. A >20% decline in sCR during the first week in the ICU has been observed in non-AKI patients. During the same time frame, cystatin C significantly increased in these patients.<sup>71 72</sup>

Future studies assessing the agreement between cystatin C- and creatinine-based GFR estimations and gold-standard GFR methods (e.g. using inulin) in the ICU are vital to determine which endogenous marker best reflects GFR.

#### The future

This review has focused on a number of potential biomarkers of kidney function and structural kidney injury that finally are moving from a laboratory setting to the bedside. However, other promising AKI biomarkers, like L-type fatty acidbinding protein, may be of use.<sup>73</sup> In the future, we will hope-fully see physicians in the field of anaesthesia and intensive care having the possibility of detecting, treating, and, hope-fully, preventing AKI. If a kidney biomarker panel is 'positive', the patients will have to be monitored intensively, with control over fluid balance, urine output, electrolytes, and functional kidney markers. Equally important will then be to avoid further harm from hypotension, hypovolaemia, contrast agents, and nephrotoxic medications.

The properties of an ideal biomarker have been described above, but as the pathophysiology behind AKI is multifaceted (Figs 1 and 2), it is unlikely that we will find a single marker that fulfils all criteria. Instead, combinations of different biomarkers with specific characteristics are probably needed. Searching for a future panel of novel biomarkers of AKI needs to address three problems.

- (i) The risks of using creatinine as a 'gold standard' when investigating potential injury markers.<sup>74</sup>
- (ii) Problems of study design. Several studies of AKI biomarkers have evaluated the predictive properties of the substance when the insult already has occurred, but a prospective case-control setting is more appropriate. Studying AKI in a general ICU this can be difficult, as many patients are admitted already with AKI. However, some studies have accounted for this and made an effort to find patients without AKI in the study of predictive biomarkers.<sup>41 57</sup>
- (iii) Problems of patient stratification. In a population undergoing major surgery and for patients in the ICU, it is not uncommon that patients have lower pre-ICU GFR (<60 ml min<sup>-1</sup>). This could lead to poorer performance of a biomarker due to impaired

excretion in chronic kidney disease, or because more variable excretion occurs in CKD. For example, NGAL excretion is increased in CKD.<sup>75</sup> As a consequence, the biomarker may have to be measured against already raised levels.

As cardiology moved from lactate dehydrogenase to troponins for the diagnosis of myocardial infarction, intensive care nephrology will have to evolve from sCr to tissue-specific injury biomarkers. At the same time, it is essential to further investigate the pitfalls of functional markers (measurements of estimated GFR) in order to properly assess dosage of antibiotics and other drugs in the postoperative or intensive care setting. To conduct treatment studies of AKI, it is important that future studies take into account the methodological issues and utilize the differences between the various potential of kidney function (i.e. GFR) and markers for structural kidney injury.

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#### References

- 1 Åberg A. Myocardial infarctions in Sweden 1987–2005. 2008
- 2 Coca SG, Yalavarthy R, Concato J, Parikh CR. Biomarkers for the diagnosis and risk stratification of acute kidney injury: a systematic review. *Kidney Int* 2008; 73: 1008–16
- 3 Bagshaw SM, George C, Dinu I, Bellomo R. A multi-centre evaluation of the RIFLE criteria for early acute kidney injury in critically ill patients. Nephrol Dial Transplant 2008; 23: 1203–10
- 4 Ostermann M, Chang RW. Acute kidney injury in the intensive care unit according to RIFLE. *Crit Care Med* 2007; **35**: 1837–43; quiz 52
- 5 Mariscalco G, Lorusso R, Dominici C, Renzulli A, Sala A. Acute kidney injury: a relevant complication after cardiac surgery. *Ann Thorac Surg* 2011; **92**: 1539–47
- 6 Bellomo R, Ronco C, Kellum JA, Mehta RL, Palevsky P. Acute renal failure—definition, outcome measures, animal models, fluid therapy and information technology needs: the Second International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI) Group. Crit Care 2004; 8: R204-12
- 7 Kellum JA, Bellomo R, Ronco C, Mehta R, Clark W, Levin NW. The 3rd International Consensus Conference of the Acute Dialysis Quality Initiative (ADQI). *Int J Artif Organs* 2005; **28**: 441–4
- 8 Mehta RL, Chertow GM. Acute renal failure definitions and classification: time for change? J Am Soc Nephrol 2003; **14**: 2178–87
- 9 Cruz DN, Goh CY, Haase-Fielitz A, Ronco C, Haase M. Early biomarkers of renal injury. Congest Heart Fail 2010; 16 (Suppl. 1): S25–31
- 10 Awad AS, Rouse M, Huang L, et al. Compartmentalization of neutrophils in the kidney and lung following acute ischemic kidney injury. Kidney Int 2009; 75: 689–98
- 11 Akcay A, Nguyen Q, Edelstein CL. Mediators of inflammation in acute kidney injury. *Mediat Inflamm* 2009; **2009**: 137072

- 12 Abuelo JG. Normotensive ischemic acute renal failure. N Engl J Med 2007; **357**: 797–805
- 13 Grigoryev DN, Liu M, Hassoun HT, Cheadle C, Barnes KC, Rabb H. The local and systemic inflammatory transcriptome after acute kidney injury. J Am Soc Nephrol 2008; **19**: 547–58
- 14 Kjeldsen L, Johnsen AH, Sengelov H, Borregaard N. Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J Biol Chem 1993; **268**: 10425-32
- 15 Xu SY, Carlson M, Engstrom A, Garcia R, Peterson CG, Venge P. Purification and characterization of a human neutrophil lipocalin (HNL) from the secondary granules of human neutrophils. *Scand J Clin Lab Invest* 1994; **54**: 365–76
- 16 Xu SY, Pauksen K, Venge P. Serum measurements of human neutrophil lipocalin (HNL) discriminate between acute bacterial and viral infections. Scand J Clin Lab Invest 1995; 55: 125–31
- 17 Cowland JB, Borregaard N. Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinaseassociated lipocalin from humans. *Genomics* 1997; **45**: 17–23
- 18 Cowland JB, Sorensen OE, Sehested M, Borregaard N. Neutrophil gelatinase-associated lipocalin is up-regulated in human epithelial cells by IL-1 beta, but not by TNF-alpha. J Immunol 2003; 171: 6630–9
- 19 Xu S, Venge P. Lipocalins as biochemical markers of disease. Biochim Biophys Acta 2000; **1482**: 298–307
- 20 Mishra J, Ma Q, Prada A, et al. Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol 2003; 14: 2534–43
- 21 Yang J, Goetz D, Li JY, et al. An iron delivery pathway mediated by a lipocalin. *Mol Cell* 2002; **10**: 1045–56
- 22 Mori K, Lee HT, Rapoport D, *et al.* Endocytic delivery of lipocalinsiderophore-iron complex rescues the kidney from ischemiareperfusion injury. *J Clin Invest* 2005; **115**: 610–21
- 23 Goetz DH, Holmes MA, Borregaard N, Bluhm ME, Raymond KN, Strong RK. The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. *Mol Cell* 2002; **10**: 1033–43
- 24 Abrahamson M, Olafsson I, Palsdottir A, et al. Structure and expression of the human cystatin C gene. *Biochem J* 1990; **268**: 287–94
- 25 Mishra J, Dent C, Tarabishi R, et al. Neutrophil gelatinaseassociated lipocalin (NGAL) as a biomarker for acute renal injury after cardiac surgery. Lancet 2005; 365: 1231–8
- 26 Wagener G, Jan M, Kim M, et al. Association between increases in urinary neutrophil gelatinase-associated lipocalin and acute renal dysfunction after adult cardiac surgery. Anesthesiology 2006; 105: 485–91
- 27 Wagener G, Gubitosa G, Wang S, Borregaard N, Kim M, Lee HT. Urinary neutrophil gelatinase-associated lipocalin and acute kidney injury after cardiac surgery. Am J Kidney Dis 2008; 52: 425–33
- 28 Koyner JL, Bennett MR, Worcester EM, et al. Urinary cystatin C as an early biomarker of acute kidney injury following adult cardiothoracic surgery. Kidney Int 2008; 74: 1059–69
- 29 Xin C, Yulong X, Yu C, Changchun C, Feng Z, Xinwei M. Urine neutrophil gelatinase-associated lipocalin and interleukin-18 predict acute kidney injury after cardiac surgery. *Ren Fail* 2008; **30**: 904–13
- 30 Liangos O, Tighiouart H, Perianayagam MC, et al. Comparative analysis of urinary biomarkers for early detection of acute kidney injury following cardiopulmonary bypass. Biomarkers 2009; 14: 423–31
- 31 Tuladhar SM, Puntmann VO, Soni M, Punjabi PP, Bogle RG. Rapid detection of acute kidney injury by plasma and urinary neutrophil

gelatinase-associated lipocalin after cardiopulmonary bypass. J Cardiovasc Pharmacol 2009; **53**: 261–6

- 32 Han WK, Wagener G, Zhu Y, Wang S, Lee HT. Urinary biomarkers in the early detection of acute kidney injury after cardiac surgery. *Clin J Am Soc Nephrol* 2009; **4**: 873–82
- 33 Koyner JL, Vaidya VS, Bennett MR, et al. Urinary biomarkers in the clinical prognosis and early detection of acute kidney injury. Clin J Am Soc Nephrol 2010; 5: 2154–65
- 34 Che M, Xie B, Xue S, *et al.* Clinical usefulness of novel biomarkers for the detection of acute kidney injury following elective cardiac surgery. *Nephron Clin Pract* 2010; **115**: c66–72
- 35 Haase M, Bellomo R, Devarajan P, et al. Novel biomarkers early predict the severity of acute kidney injury after cardiac surgery in adults. *Ann Thorac Surg* 2009; **88**: 124–30
- 36 Bennett M, Dent CL, Ma Q, et al. Urine NGAL predicts severity of acute kidney injury after cardiac surgery: a prospective study. Clin J Am Soc Nephrol 2008; 3: 665–73
- 37 Nauta FL, Boertien WE, Bakker SJ, et al. Glomerular and tubular damage markers are elevated in patients with diabetes. *Diabetes Care* 2011; **34**: 975–81
- 38 McIlroy DR, Wagener G, Lee HT. Neutrophil gelatinase-associated lipocalin and acute kidney injury after cardiac surgery: the effect of baseline renal function on diagnostic performance. Clin J Am Soc Nephrol 2010; 5: 211–9
- 39 Haase-Fielitz A, Bellomo R, Devarajan P, et al. The predictive performance of plasma neutrophil gelatinase-associated lipocalin (NGAL) increases with grade of acute kidney injury. Nephrol Dial Transplant 2009; 24: 3349–54
- 40 Cai L, Borowiec J, Xu S, Han W, Venge P. Assays of urine levels of HNL/NGAL in patients undergoing cardiac surgery and the impact of antibody configuration on their clinical performances. *Clin Chim Acta* 2009; **403**: 121–5
- 41 Martensson J, Bell M, Oldner A, Xu S, Venge P, Martling CR. Neutrophil gelatinase-associated lipocalin in adult septic patients with and without acute kidney injury. *Intensive Care Med* 2010; 36: 1333–40
- 42 Bagshaw SM, Bennett M, Haase M, et al. Plasma and urine neutrophil gelatinase-associated lipocalin in septic versus non-septic acute kidney injury in critical illness. *Intensive Care Med* 2010; 36: 452–61
- 43 Cruz DN, de Cal M, Garzotto F, et al. Plasma neutrophil gelatinase-associated lipocalin is an early biomarker for acute kidney injury in an adult ICU population. *Intensive Care Med* 2010; **36**: 444–51
- 44 Constantin JM, Futier E, Perbet S, et al. Plasma neutrophil gelatinase-associated lipocalin is an early marker of acute kidney injury in adult critically ill patients: a prospective study. J Crit Care 2010; **25**: 176 e1–6
- 45 de Geus HR, Bakker J, Lesaffre EM, le Noble JL. Neutrophil gelatinase-associated lipocalin at ICU admission predicts for acute kidney injury in adult patients. *Am J Respir Crit Care Med* 2011; **183**: 907–14
- 46 Shapiro NI, Trzeciak S, Hollander JE, *et al.* The diagnostic accuracy of plasma neutrophil gelatinase-associated lipocalin in the prediction of acute kidney injury in emergency department patients with suspected sepsis. *Ann Emerg Med* 2010; **56**: 52–9.e1
- 47 Siew ED, Ware LB, Gebretsadik T, *et al.* Urine neutrophil gelatinase-associated lipocalin moderately predicts acute kidney injury in critically ill adults. *J Am Soc Nephrol* 2009; **20**: 1823–32
- 48 Nickolas TL, O'Rourke MJ, Yang J, et al. Sensitivity and specificity of a single emergency department measurement of urinary

neutrophil gelatinase-associated lipocalin for diagnosing acute kidney injury. Ann Intern Med 2008; **148**: 810–9

- 49 Cai L, Rubin J, Han W, Venge P, Xu S. The origin of multiple molecular forms in urine of HNL/NGAL. *Clin J Am Soc Nephrol* 2010; 5: 2229-35
- 50 Yilmaz A, Sevketoglu E, Gedikbasi A, et al. Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin. *Pediatr Nephrol* 2009; **24**: 2387–92
- 51 Price RG. The role of NAG (*N*-acetyl-beta-D-glucosaminidase) in the diagnosis of kidney disease including the monitoring of nephrotoxicity. *Clin Nephrol* 1992; **38** (Suppl. 1): S14–9
- 52 Vaidya VS, Waikar SS, Ferguson MA, *et al.* Urinary biomarkers for sensitive and specific detection of acute kidney injury in humans. *Clin Transl Sci* 2008; **1**: 200–8
- 53 Liangos O, Perianayagam MC, Vaidya VS, et al. Urinary N-acetyl-beta-(p)-glucosaminidase activity and kidney injury molecule-1 level are associated with adverse outcomes in acute renal failure. J Am Soc Nephrol 2007; 18: 904–12
- 54 Herget-Rosenthal S, Poppen D, Husing J, et al. Prognostic value of tubular proteinuria and enzymuria in nonoliguric acute tubular necrosis. Clin Chem 2004; 50: 552–8
- 55 Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV. Kidney injury molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. *Kidney Int* 2002; **62**: 237–44
- 56 Vaidya VS, Ozer JS, Dieterle F, et al. Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. Nat Biotechnol 2010; 28: 478-85
- 57 Endre ZH, Pickering JW, Walker RJ, et al. Improved performance of urinary biomarkers of acute kidney injury in the critically ill by stratification for injury duration and baseline renal function. *Kidney Int* 2011; **79**: 1119–30
- 58 Haase M, Bellomo R, Story D, Davenport P, Haase-Fielitz A. Urinary interleukin-18 does not predict acute kidney injury after adult cardiac surgery: a prospective observational cohort study. Crit Care 2008; 12: R96
- 59 Parikh CR, Mishra J, Thiessen-Philbrook H, et al. Urinary IL-18 is an early predictive biomarker of acute kidney injury after cardiac surgery. Kidney Int 2006; 70: 199–203
- 60 Ling W, Zhaohui N, Ben H, et al. Urinary IL-18 and NGAL as early predictive biomarkers in contrast-induced nephropathy after coronary angiography. *Nephron Clin Pract* 2008; **108**: c176–81
- 61 Nejat M, Pickering JW, Walker RJ, *et al.* Urinary cystatin C is diagnostic of acute kidney injury and sepsis, and predicts mortality in the intensive care unit. *Crit Care* 2010; **14**: R85

- 62 Soto K, Coelho S, Rodrigues B, et al. Cystatin C as a marker of acute kidney injury in the emergency department. *Clin J Am Soc Nephrol* 2010; **5**: 1745–54
- 63 Saito A, Sato H, Iino N, Takeda T. Molecular mechanisms of receptor-mediated endocytosis in the renal proximal tubular epithelium. *J Biomed Biotechnol* 2010; **2010**: 403272
- 64 Thielemans N, Lauwerys R, Bernard A. Competition between albumin and low-molecular-weight proteins for renal tubular uptake in experimental nephropathies. *Nephron* 1994; **66**: 453-8
- 65 Richmond JM, Sibbald WJ, Linton AM, Linton AL. Patterns of urinary protein excretion in patients with sepsis. *Nephron* 1982; 31: 219–23
- 66 Tenstad O, Roald AB, Grubb A, Aukland K. Renal handling of radiolabelled human cystatin C in the rat. Scand J Clin Lab Invest 1996; 56: 409–14
- 67 Herget-Rosenthal S, Marggraf G, Husing J, *et al.* Early detection of acute renal failure by serum cystatin C. *Kidney Int* 2004; **66**: 1115–22
- 68 Haase-Fielitz A, Bellomo R, Devarajan P, et al. Novel and conventional serum biomarkers predicting acute kidney injury in adult cardiac surgery—a prospective cohort study. Crit Care Med 2009; 37: 553–60
- 69 Royakkers AA, Korevaar JC, van Suijlen JD, *et al.* Serum and urine cystatin C are poor biomarkers for acute kidney injury and renal replacement therapy. *Intensive Care Med* 2011; **37**: 493-501
- 70 Perianayagam MC, Seabra VF, Tighiouart H, Liangos O, Jaber BL. Serum cystatin C for prediction of dialysis requirement or death in acute kidney injury: a comparative study. Am J Kidney Dis 2009; 54: 1025–33
- 71 Nejat M, Pickering JW, Walker RJ, Endre ZH. Rapid detection of acute kidney injury by plasma cystatin C in the intensive care unit. Nephrol Dial Transplant 2010; 25: 3283–9
- 72 Martensson J, Martling CR, Oldner A, Bell M. Impact of sepsis on levels of plasma cystatin C in AKI and non-AKI patients. Nephrol Dial Transplant 2012; **27**: 576–81
- 73 Doi K, Negishi K, Ishizu T, *et al.* Evaluation of new acute kidney injury biomarkers in a mixed intensive care unit. *Crit Care Med* 2011; **39**: 2464–9
- 74 Waikar SS, Betensky RA, Bonventre JV. Creatinine as the gold standard for kidney injury biomarker studies? *Nephrol Dial Transplant* 2009; 24: 3263–5
- 75 Bolignano D, Lacquaniti A, Coppolino G, Campo S, Arena A, Buemi M. Neutrophil gelatinase-associated lipocalin reflects the severity of renal impairment in subjects affected by chronic kidney disease. *Kidney Blood Press Res* 2008; **31**: 255-8

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