Biomarkers of acute kidney injury in anesthesia, intensive care and major surgery: from the bench to clinical research to clinical practice

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ABSTRACT

Acute kidney injury (AKI) is common after major surgery and reportedly occurs in approximately 36% of ICU patients (RIFLE Risk/Injury/Failure categories). It is associated with increased mortality, greater cost, and prolonged Intensive Care Unit (ICU) and hospital stay, despite attempts to develop therapies to prevent or attenuate AKI, which have had limited success. One major reason for this lack of success may be the result of delayed implementation due to the inability to detect AKI early. Traditional biomarkers of AKI (creatinine and urea) do not detect injury early enough. Thus, it is a priority to find reliable, early biomarkers that predict subsequent AKI. Innovative technologies such as functional genomics and proteomics have facilitated detection of several promising early biomarkers of AKI, such as neutrophil gelatinase-associated lipocalin (NGAL), cystatin C (CysC), liver-type fatty acid binding protein (L-FABP), interleukin-18 (IL-18), and kidney injury molecule-1 (KIM-1). These biomarkers have many potential applications during anesthesia and in the ICU. They can be used to evaluate the effect of new techniques and therapies on kidney function, as safety markers to monitor toxicity and as measures of treatment effect. For example, NGAL and cystatin C have been used in a safety monitoring trial of hydroxyethylstarch therapy and to detect AKI early, during or immediately after cardiac surgery. Clinical use beyond research settings is rapidly expanding.

Key words: Kidney failure - Creatinine - Urea - Biological markers - Lipocalins - Cystatin C - Fatty acid-binding proteins - Interleukin-18.

AKI is common with major surgery and critical illness

Acute kidney injury (AKI) is the consensus term now used to describe the continuum of the condition previously called acute renal failure. AKI as classified by the RIFLE criteria (acronym for Risk, Injury, Failure, Loss and Endstage) has been reported to occur in approximately 36% of critically ill patients and is common after major surgery such as open heart surgery. Although the Acute Kidney Injury Network (AKIN) criteria, based on the RIFLE classification system are being used increasingly and are presumed to improve sensitivity in diagnosing AKI, they have not been shown to improve the ability to predict outcomes. The RIFLE criteria have been extensively used and validated to classify renal function in several populations with studies cumulatively involving >250,000 subjects. Furthermore, a
recent study found RIFLE to be more robust, with a higher detection rate of AKI than AKIN in the first 48 hours post-ICU admission. Therefore, we favor the RIFLE classification for AKI.

AKI is independently associated with an increased risk of death and with prolonged length of stay. Severe cases require costly treatment, can result in prolonged kidney dysfunction, and escalate the human and financial costs of care. Therefore, it seems desirable to detect AKI as early as possible in order to develop or implement potentially protective therapy.

Why therapies have been unsuccessful

To date, attempts to develop therapies to prevent or attenuate AKI have failed to show consistently protective effects. The use of diuretics has not proven to be of benefit. Fenoldopam, a vasodilator, has shown promise in certain populations but not in others; natriuretic peptides may be of use in major surgery but have also not been effective in other situations. The capacity of N-acetyl cysteine to prevent AKI after radiocontrast has been tested with unconvincing results, and the benefits of perioperative IV sodium bicarbonate infusion in cardiac surgical patients are yet to be confirmed. The most widely accepted treatment to prevent or treat AKI (although untested in controlled trials) remains prompt fluid resuscitation of circulatory volume and appropriate use of inotropes/vasopressors to maintain adequate cardiac output and perfusion pressure. Beyond these measures, cases unresponsive to fluid resuscitation in the presence of hyperkalemia, metabolic acidosis or fluid overload commonly receive renal replacement therapy/dialysis to support the kidneys.

There are several reasons why no reproducibly and consistently effective treatment for AKI has been found. First, AKI can occur as a result of multiple causes and disease processes. Whereas certain treatments may benefit subgroups, heterogeneity of disease makes finding one treatment for all types of AKI unlikely. Second, understanding of the pathogenesis of AKI is limited. An incomplete knowledge of the mechanisms involved has resulted in difficulties in developing logical approaches to prevention or treatment. Third, interventions are implemented too late. This delay is due to our reliance on conventional biomarkers (creatinine, urea, urine output) to diagnose AKI. Such biomarkers either do not detect injury in real-time and become abnormal many hours later in the course of injury (creatinine or urea) or lack specificity (urine output). To draw a parallel with the treatment for acute myocardial infarction, one can easily imagine how difficult it would be to show the benefits of thrombolysis or stenting in the absence of troponin, electrocardiogram changes or the presence of chest pain to allow early and specific diagnosis within minutes instead of hours or a day later. By analogy, if we could detect AKI early, we would treat it more rapidly and should have a better chance of preventing or reducing injury, as is the case for several other acute syndromes in medicine.

The limitations of traditional biomarkers

The traditional clinical biomarkers for the detection of AKI are creatinine, urea, and urine output. All have serious limitations as early detectors of AKI. Creatinine is the product of the breakdown of creatine to phosphocreatine in skeletal muscle and of the subsequent liver metabolism of creatine to form creatinine. Unfortunately, there are several limitations to serum creatinine (SCr) as a biomarker of AKI. Creatinine is not reabsorbed in the tubules or metabolized by the kidney. If filtering of creatinine is deficient, blood levels rise with an inverse relationship with GFR. Unfortunately, there are several limitations to serum creatinine (SCr) as a biomarker of AKI. First, its release varies with age, gender, diet, muscle mass, drugs, and vigorous exercise. Second, secretion accounts for 10-40% of creatinine clearance with an inverse relationship with GFR. Third, the accuracy of SCr assays can be reduced by artifact. Fourth, creatinine becomes abnormal only when more than 50% of GFR is lost and may require up to 24 hours before sufficient increases in blood concentration are detectable.

Urea is a water-soluble, low molecular weight by-product of protein metabolism. Its level is also inversely related to GFR, but several factors affect its production and clearance, limiting its reliability in estimating GFR. Urea production is incon-
ACUTE RENAL BIOMARKERS

Moore

Urine output is measured routinely in operating rooms and ICUs with indwelling catheters. A trend in urine output is a crude gauge of kidney function and may be a more sensitive indicator of changes in renal hemodynamics than a measure of solute clearance. Nonetheless, many patients with AKI do not have oliguria, and many patients with oliguria in the operating room or ICU do not manifest AKI.

Table I.—Characteristics, function and significance of NGAL, cystatin C and L-FABP.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Molecular weight</th>
<th>Origin</th>
<th>Normal concentration</th>
<th>Physiological function</th>
<th>Significance of rise in level</th>
</tr>
</thead>
<tbody>
<tr>
<td>NGAL</td>
<td>21 kDa</td>
<td>Urine</td>
<td>Adults: 1.0-20.0 ng/mL, Children: 1.0-20.0 ng/mL</td>
<td>Bacteriostatic effect - binds to iron-carrying molecules (siderophores) which are synthesized by specific bacteria to gather iron. By doing this, NGAL reduces bacterial growth.</td>
<td>Tubular stress/injury. There is an earlier rise in urine than serum.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NGAL</td>
<td>Adults: 70-105 ng/mL, Children: 30-80 ng/mL</td>
<td>Antioxidant effect - binds to “human siderophores” to transport iron into target cells thus stopping free and reactive iron from producing oxygen radicals which can cause oxidative stress and cell injury. Scavenges intracellular iron for extracellular export (hypothesis).</td>
<td></td>
</tr>
<tr>
<td>Cystatin C</td>
<td>13.3 kDa</td>
<td>Produced at a constant rate by nucleated cells → filtered by glomerulus → reabsorbed and catabolized (but not secreted) in the proximal tubules</td>
<td>&lt;0.1 mg/L Cr</td>
<td>Potent inhibitor of lysosomal proteases (inhibits breakdown of lysosomal protein) and extracellular inhibitor of cysteine proteases (prevents breakdown of extracellular protein).</td>
<td>Change in GFR is reflected in changes in serum and urine levels of cystatin C - acts as a marker of GFR.</td>
</tr>
<tr>
<td>L-FABP</td>
<td>14.3 kDa</td>
<td>Expressed in the liver, intestine, pancreas, lung, stomach, and kidneys.</td>
<td>Adults: &lt;10 ng/mg Cr/L, Portilla et al.,27 Children: 5-20 µg/g Cr, Nakamura et al.,25</td>
<td>Renal L-FABP helps maintain low levels of free FAs in the cytoplasm by a) binding to them and transporting them to cell components to accelerate FFA metabolism and by b) binding to free FAs and being excreted from the proximal tubule cells into urine for elimination.</td>
<td>Tubular stress/injury, which results in an accumulation of free FAs in the proximal tubules. There is an earlier rise in urine than serum.</td>
</tr>
</tbody>
</table>

stent, and values can be altered by changes in circulatory volume, protein intake, gastrointestinal bleeding, among other parameters. Rate of renal clearance of urea is not constant; 40-50% of filtered urea may be reabsorbed in the tubules. Consequently, urea is a poor measure of GFR, requires time to accumulate, does not reflect real-time changes in GFR and delays diagnosis.
not develop AKI. Finally, many drugs used in the operating room or ICU (i.e., diuretics and vaso-pressors) act as additional confounders.

In response to these problems, innovative technologies such as functional genomics and proteomics have facilitated the detection of several potential earlier biomarkers of AKI. Some of the most promising biomarkers include neutrophil gelatinase-associated lipocalin (NGAL), cystatin C (CyC), and liver-type fatty acid binding protein (L-FABP), which have now been evaluated in several populations.

### NGAL

The expression of NGAL in early, acute tubular injury was identified using functional genomics. NGAL is a measure of tubular stress (Table I); its concentration increases dramatically in response to tubular injury and precedes rises in SCr by >24 hours. NGAL normally exerts protective bacteriostatic and antioxidant effects involving iron transport and is thought to act as an iron scavenger and growth factor. The proposed role of NGAL and its twin molecule, hepcidin (a "master regulator" of iron) are summarized in Figure 1.

NGAL has been intensely investigated in recent years, predominantly in adult cardiac surgery. ELISA techniques have been used to measure NGAL; however, accurate and sensitive point of care tests that reduce costs and the potential for measurement error are now available (Tables II-IV).

<table>
<thead>
<tr>
<th>Year</th>
<th>Authors</th>
<th>Setting</th>
<th>Age</th>
<th>Sex</th>
<th>Existing renal disease excluded</th>
<th>% AKI</th>
<th>AKI Definition (↑ in SCr)</th>
<th>Timing (AKI)</th>
<th>Sample size</th>
</tr>
</thead>
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<tr>
<td>2009</td>
<td>Liangos et al.</td>
<td>CSA</td>
<td>68</td>
<td>28</td>
<td>* † 13</td>
<td>50%</td>
<td>≥50%</td>
<td>72 h</td>
<td>103</td>
</tr>
<tr>
<td>2009</td>
<td>Tuladhar et al.</td>
<td>CSA</td>
<td>67</td>
<td>30</td>
<td>Y 18</td>
<td>&gt;44.2 µmol/L</td>
<td>48 h</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>Han et al.</td>
<td>CSA</td>
<td>NA</td>
<td>NA</td>
<td>NA NA</td>
<td>26.5 µmol/L</td>
<td>72 h</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>Haase-Fielitz et al.</td>
<td>CSA</td>
<td>70</td>
<td>39</td>
<td>Y 23</td>
<td>&gt;50%</td>
<td>5 days</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>2009</td>
<td>Haase-Fielitz et al.</td>
<td>CSA</td>
<td>70</td>
<td>39</td>
<td>* ‡ 39</td>
<td>&gt;25%</td>
<td>5 days</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>Xin et al.</td>
<td>CSA</td>
<td>38</td>
<td>56</td>
<td>N 27</td>
<td>&gt;50% or &gt;26.5 µmol/L or ¥ 48 h</td>
<td>33</td>
<td></td>
<td></td>
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<tr>
<td>2008</td>
<td>Wagener G. et al.</td>
<td>CSA</td>
<td>63</td>
<td>34</td>
<td>$ 20</td>
<td>≥50% or &gt;0 26.5 µmol/L</td>
<td>48 h</td>
<td>426</td>
<td></td>
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<tr>
<td>2008</td>
<td>Koyner et al.</td>
<td>CSA</td>
<td>65</td>
<td>29</td>
<td>Y 47</td>
<td>≥25% or RRT</td>
<td>72 h</td>
<td>72</td>
<td></td>
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<tr>
<td>2006</td>
<td>Wagener G. et al.</td>
<td>CSA</td>
<td>68</td>
<td>35</td>
<td>* 20</td>
<td>≥50%</td>
<td>10 days</td>
<td>81</td>
<td></td>
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<tr>
<td>2009</td>
<td>Makris et al.</td>
<td>Trauma</td>
<td>NA</td>
<td>19</td>
<td>Y N A</td>
<td>N A</td>
<td>5 days</td>
<td>31</td>
<td></td>
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<tr>
<td>2008</td>
<td>Ling W. et al.</td>
<td>CIN</td>
<td>67</td>
<td>40</td>
<td>N NA</td>
<td>≥25% or &gt;44.2 µmol/L</td>
<td>72 h</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>Nickolas et al.</td>
<td>ED</td>
<td>60</td>
<td>49</td>
<td>* 5</td>
<td>≥50% or 25% ↓ in eGFR</td>
<td>Hosp stay</td>
<td>635</td>
<td></td>
</tr>
</tbody>
</table>

CSA: cardiac surgery associated AKI; CIN: contrast induced nephropathy; ED: emergency department; Y: yes; N: no; NA: not available or not applicable; RRT: renal replacement therapy; Hosp: hospital stay; Quantikine® - R&D systems. *: dialysis, †: transplant, ‡: SCr >300 µmol/L; §: endstage renal disease; ¥: urine output <0.5 mL/kg/h for >6 h.

In a landmark study, diagnosis of AKI by SCr occurred 1-3 days after surgery, whereas plasma and urine NGAL levels were powerful independent predictors of AKI within 2 hours of surgery (Table IV). Subsequent studies confirmed these findings. Moreover, plasma NGAL predicted duration of AKI, length of stay and mortality.

Cardiac surgery - adults

The findings in children were broadly confirmed...
### Table 1

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Time from admission (h)</th>
<th>Cutoff</th>
<th>Urine Sensitivity (%)</th>
<th>Urine Specificity (%)</th>
<th>Plasma Sensitivity (%)</th>
<th>Plasma Specificity (%)</th>
<th>AUC-ROC</th>
<th>Urine %</th>
<th>Plasma %</th>
</tr>
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<tr>
<td>Quantikine®</td>
<td>2</td>
<td>166 ng/mg</td>
<td>67</td>
<td>11</td>
<td>80</td>
<td>67</td>
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<tr>
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<td>2</td>
<td>393 ng/mL</td>
<td>93</td>
<td>78</td>
<td>N/A</td>
<td>N/A</td>
<td>59</td>
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<tr>
<td>N/A</td>
<td>0</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
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<td>Triage® Biosite</td>
<td>ICU/6hrs</td>
<td>150 ng/mL</td>
<td>79</td>
<td>78</td>
<td>80</td>
<td>78</td>
<td>80</td>
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</tr>
<tr>
<td>Triage® Biosite</td>
<td>ICU/6hrs</td>
<td>90 ng/mL</td>
<td>91</td>
<td>76</td>
<td>87</td>
<td>68</td>
<td>67</td>
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<td></td>
</tr>
<tr>
<td>Triage® Biosite</td>
<td>ICU/6hrs</td>
<td>145 ng/mL</td>
<td>68</td>
<td>64</td>
<td>67</td>
<td>67</td>
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<td>ELISA kit</td>
<td>2</td>
<td>250 µg/mL</td>
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<td>74</td>
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<td>ELISA kit</td>
<td>3</td>
<td>250 µg/mmol</td>
<td>81</td>
<td>78</td>
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<td>23.5 ng/mL</td>
<td>31</td>
<td>81</td>
<td>57</td>
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<td>ELISA kit</td>
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<td>18.1 ng/mL</td>
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<tr>
<td>ELISA kit</td>
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<td>15.6 ng/mL</td>
<td>39</td>
<td>78</td>
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<tr>
<td>ELISA kit</td>
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<td>300 ng/mg</td>
<td>67</td>
<td>62</td>
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<td>N/A</td>
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<td>53</td>
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<tr>
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<td>ICU +6hrs</td>
<td>300 ng/mg</td>
<td>34</td>
<td>86</td>
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<td>N/A</td>
<td>70</td>
<td>46</td>
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<tr>
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<td>3</td>
<td>213 ng/mL</td>
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<td>65</td>
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<tr>
<td>Immunoblot</td>
<td>18</td>
<td>213 ng/mL</td>
<td>73</td>
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<td>80</td>
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<tr>
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<td>25 ng/mL</td>
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<td>95</td>
<td>98</td>
<td>98</td>
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<td>ELISA kit</td>
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<td>70</td>
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<td>0</td>
<td>130 µg/gCr</td>
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<td>100</td>
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<td>95</td>
<td></td>
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</tr>
</tbody>
</table>

CSA: cardiac surgery associated AKI; CIN: contrast induced nephropathy; ED: emergency department; Y: yes; N: no; NA: not available or not applicable; RRT: renal replacement therapy; Hosp stay: hospital stay; Quantikine® - R&D systems. *: dialysis, †: transplant, ‡: SCr >300 µmol/L; §: endstage renal disease; ¥: urine output <0.5 mL/kg/h for >6 h.

**Figure 1.**—A) Proposed model of NGAL and hepcidin-mediated iron trafficking in the proximal tubule. NGAL bound to siderophore and iron, delivers iron to cells via megalin and NGAL receptors. In the endosome, NGAL releases iron, which promotes expression of iron-dependent genes (e.g., ferritin), stimulates epithelial proliferation and reduces renal damage. Iron moves out of cells via the ferroportin pathway.28 Diagram modified from Schmidt-Ött et al. 2007.30 B) Model representing potential mechanisms and pathways present in renal injury. Local and systemic NGAL expression increases. Tubular injury results in disruption to megalin receptor function, reduced NGAL-mediated intracellular iron uptake, and subsequent increase in urine and serum NGAL concentration. With inflammation, hepcidin is up-regulated and binds to ferroportin, which is internalized, endocytosed and degraded. Absence of the ferroportin pathway for iron removal promotes the accumulation of intracellular iron, which reduces extracellular free reactive iron, thereby, reducing oxidative stress. The association of higher hepcidin levels with those that do not develop AKI vs. those that develop AKI after cardiopulmonary bypass surgery is consistent with this proposed mechanism.31
in adults, albeit with somewhat diminished accuracy (Table II), with an AU C of 0.77-0.96 (Table II). Moreover, plasma NGAL was an independent predictor of AKI duration and severity, of length of ICU stay, and of the need for renal replacement therapy, and of hospital death (Table V). The predictive value of NGAL post cardiac surgery was stronger with a strict definition of AKI (>50% versus >25% increase in SCr) and increased with progressive severity of AKI (Table II).  

Contrast-induced nephropathy (CIN)  
In children with congenital heart disease, Hirsch et al. found that both serum and urine NGAL were excellent predictors for AKI at 2 hours after contrast (Table IV). In adults with normal SCr, NGAL levels were significantly higher in urine at
2 hours and in serum 4 hours post contrast in patients who developed CIN, whereas SCr was significantly higher at 48 hours. Consistent with this, Ling et al.\textsuperscript{42} found urine NGAL performed well in the early diagnosis of CIN (Table II).

Critically ill

Wheeler\textsuperscript{53} found a significant difference in serum NGAL between children who were healthy, critically ill with SIRS, and critically ill with septic shock (medians 80, 108 and 303 ng/mL, respectively). Furthermore, NGAL was significantly elevated in those with AKI compared to those without. In addition, Zappitelli et al.\textsuperscript{55} found that there was a progressive increase in urine NGAL concentration with worsening pRIFLEmax and that urine NGAL was a good diagnostic marker for persistent AKI (Table VI). In critically ill adults,\textsuperscript{54}

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Time from procedure/admission (h)</th>
<th>Cutoff</th>
<th>Urine</th>
<th>Plasma</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>AUC-ROC</th>
</tr>
</thead>
<tbody>
<tr>
<td>PENIA</td>
<td>2</td>
<td>192 ng/mg</td>
<td>42</td>
<td>86</td>
<td>50</td>
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<td></td>
</tr>
<tr>
<td>Nephelometry</td>
<td>ICU/6 h</td>
<td>&gt;1.1 mg/L</td>
<td>77</td>
<td>86</td>
<td>83</td>
<td></td>
<td></td>
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<tr>
<td>Nephelometry</td>
<td>24</td>
<td>&gt;1.2 mg/L</td>
<td>91</td>
<td>64</td>
<td>84</td>
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<td>&gt;1.1 mg/L</td>
<td>75</td>
<td>89</td>
<td>78</td>
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<td>&gt;1.2 mg/L</td>
<td>86</td>
<td>80</td>
<td>84</td>
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<tr>
<td>Nephelometry</td>
<td>ICU</td>
<td>&gt;1.1 mg/L</td>
<td>74</td>
<td>67</td>
<td>76</td>
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<tr>
<td>ELISA kit-CyC</td>
<td>ICU</td>
<td>0.35 mg/g</td>
<td>58</td>
<td>72</td>
<td>69</td>
<td>62</td>
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<tr>
<td>ELISA kit-CyC</td>
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<td>0.11 mg/g</td>
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<td>84</td>
<td>72</td>
<td>63</td>
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<td>ELISA</td>
<td>day 1,4,7</td>
<td>1.57 mg/L</td>
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</table>

CSA: cardiac surgery associated AKI; CIN: contrast induced nephropathy; ED: emergency department; Y: yes; N: no; NA: not available or not applicable; RRT: renal replacement therapy; Hosp stay: hospital stay; Quantikine® - R&D systems. *: dialysis, †: transplant, ‡: SCr >300 µmol/L; §: endstage renal disease; ¥: urine output <0.5 mL/kg/h for >6 h.

<table>
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<th>Instrument</th>
<th>Time from procedure/admission (h)</th>
<th>Cutoff</th>
<th>Urine</th>
<th>Plasma</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>AUC-ROC</th>
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the median urine NGAL at enrolment was significantly higher in those who developed AKI within 48 hours.

**Kidney transplant**

In a multicenter study, urine NGAL measured on the day of transplant predicted delayed graft function and dialysis (AUC 0.9). In addition, Kusaka et al. found that a decrease in NGAL predicted organ recovery before a decrease in SCr or recovery of urine output.

**NGAL and chronic kidney disease (CKD)**

NGAL in serum and urine appears to reflect the presence, severity, and progression of CKD. NGAL seems to be a better predictor of GFR than SCr (and than CyC when GFR <30 mL/min) in patients with CKD.

Urine NGAL was also strongly predictive of AKI in children with diarrhea-associated hemolytic uremic syndrome, in multitrauma and in emergency department patients (Table II).

A recent systematic review and meta-analysis of NGAL studies using standardized data sheets sent to authors, confirmed the value of NGAL as an early predictor of AKI across settings. Urine and plasma/serum NGAL performed similarly well, and the performance of NGAL improved with standardized laboratory platforms versus research-based assays (cutoff >150 ng/mL). NGAL level had prognostic value for renal replacement therapy and mortality.

NGAL is now the most promising novel renal biomarker in urine and plasma. The cutoff values for NGAL range widely, with higher values used for adults versus children (effect of age and co-morbidities) and for cardiac surgical studies versus CIN (lower magnitude of injury in CIN). Therefore, it seems that each clinical setting would require the establishment of a "normal" range and cutoff value. As a general rule, however, a concentration >150 ng/mL can identify patients at high risk for AKI, and a level >350 ng/mL, those at high risk for renal replacement therapy.

If we compare the performance of NGAL in diagnosing AKI to widely used markers like troponin I, which has carried an AUC in the range of 0.7-0.8 for diagnosis of acute myocardial infarction, its performance would seem sufficient for clinical adoption and inclusion into an early diagnostic panel for AKI. Clinical use of NGAL beyond confirmation of its utility is rapidly expanding.

**Cystatin C**

CyC is a low molecular weight cysteine protease inhibitor (Table I, Figure 2). Its serum level is determined by glomerular filtration, in contrast to NGAL, which responds to stimuli and is a measure of tubular stress (Table I, Figure 2). Therefore,
Changes in serum and urine levels of CysC reflect changes in GFR. Given that its levels are not significantly affected by age, gender, race, muscle mass, infection, liver disease or inflammatory disease and that it is not secreted by the tubules, CysC is probably a better measure of glomerular function than SCr.49, 71

Comparison with SCr

In a systematic review of 24 studies, Laterza et al.72 found CysC superior to SCr in detecting “impaired GFR” (0.95 vs. 0.91, P=0.003). Apart from two unfavorable results,46, 73 the superiority of CysC over SCr as a diagnostic marker for AKI is supported by several studies of ICU patients.49, 51
Using the combined results of 11 datasets, Royakkers et al. found the diagnostic accuracy for GFR determination of CyC superior to that of Scr: AUC 0.93 vs. Scr 0.84, and CyC is now often used in this role. The use of CyC as a biomarker for AKI has been investigated in several settings.

Cardiac surgery
In adults, despite an inconclusive result from Heise et al., other studies found that CyC predicted AKI early before Scr but that it was not superior to Ngal (Table II-III). Notably, after excluding those with preoperative renal impairment, Haase-Fielitz et al. found that the predictive performance of CyC for AKI was reduced from 0.83 to AUC 0.78, whereas that of Ngal remained the same (Table II-III). In this setting, the predictive value of CyC appears to be partly as a marker of chronic renal injury rather than acute injury, and, as such, it may be useful as a complementary marker to Ngal. CyC displayed considerable prognostic value in Haase-Fielitz' study; in this study, serum CyC and plasma Ngal were independent predictors of AKI and excellent predictors of the need for renal replacement therapy and of hospital death. In addition, Haase et al. found that CyC was an independent predictor of severity and duration of AKI after adult cardiac surgery.

Post transplant
After kidney transplant, CyC predicted delayed graft function, but the prediction was relatively late (at three days). In contrast, after liver transplants, Hei et al. found that postoperative CyC predicted AKI earlier and more accurately than Scr (within 24 h) and that preoperative serum CyC also predicted postoperative AKI. Ling et al. confirmed these findings (Table III).

L-FABP
L-FABP is expressed in various organs including liver and kidney. Its function in the kidney is presumed to be the same as that in the liver: cellular uptake of fatty acids (FAs) from plasma and promotion of intracellular FA metabolism. Free FAs are easily oxidized, leading to oxidative stress that can induce cellular injury. Through its involvement in regulation of FA metabolism, L-FABP may inhibit the accumulation of intracellular FAs (Table I, Figure 3), thereby preventing oxidation of free FAs. L-FABP may be an important cellular antioxidant during oxidative stress.

L-FABP can be filtered via glomeruli and reabsorbed in the proximal tubule cells due to its small size, which could partly explain the increase of L-FABP in proximal tubular cell injury. However, an experimental study revealed that renal L-FABP expression was up-regulated and that urinary L-FABP excretion was accelerated by accumulation of free FAs. Renal L-FABP may help maintain low levels of free FAs in the cytoplasm by facilitating their intracellular metabolism and their excretion in urine (Table I, Figure 3).

Rickli et al. found that, after contrast application, serum CyC levels increased before Scr. Furthermore, Kimmel et al. found that serum CyC reflected contrast-induced changes better than Scr. Another study found that urine CyC rose significantly at 8 hours (P < 0.05) and at 24 h (P < 0.01) after contrast, with no change in creatinine. Thus, CyC is an earlier and more accurate marker of AKI than Scr, but it is generally preceded by Ngal in detecting AKI; elevated preoperative CyC before liver transplant predicts postoperative AKI; CyC is more sensitive to contrast-induced changes than Scr; and CyC is a better marker of chronic renal impairment and its effect on outcomes, which accounts for part of its diagnostic value. As both rise sequentially, CyC could complement Ngal, which can lose diagnostic accuracy in the presence of co-morbidities. Given that automated, standardized immunonephelometric assays are commercially available and provide results in minutes, CyC represents a feasible and promising biomarker for AKI. Its inclusion in a sequential AKI diagnostic panel with Ngal appears logical.
children.\textsuperscript{27} ELISA techniques have been used to measure L-FABP levels; however, a urine dipstick kit has been developed and requires evaluation.\textsuperscript{26}

In an experimental study, urine L-FABP showed great potential for early and accurate detection of histological and functional decline in both nephrotoxin-induced and ischemia-reperfusion injury in mice.\textsuperscript{86} Dose-response to injury was well-reflect-
ed in L-FABP levels in this study; severity of histological injury increased with ischemia time and cisplatin dose and correlated well with L-FABP levels. Urine L-FABP increased after 1 h, even in mice subjected to only 5 min of ischemia.\textsuperscript{86}

In a clinical study,\textsuperscript{25} 13 of 66 patients had significantly elevated urine L-FABP before non-emergency angiogram; later, all 13 showed contrast-induced nephropathy, whereas no patient with low urine L-FABP showed signs of nephropathy. Furthermore, pre-contrast SCr showed no difference between the AKI/no AKI groups. Urine L-FABP appears to be more sensitive predictor of AKI than SCr and could serve as a clinical predictor of contrast-induced nephropathy. In a further study, higher urine L-FABP levels differentiated patients with septic shock from those with severe sepsis, from those with AKI, and from healthy controls.\textsuperscript{87} Of the septic shock patients, urine L-FABP levels in survivors were reduced by treatment. Non-survivors had higher urine levels with a smaller reduction after treatment compared with survivors. Thus, L-FABP may be useful in treatment evaluation. Urine L-FABP can predict AKI in pediatric cardiopulmonary bypass surgery\textsuperscript{27} with an AUC at 4 h post-surgery of 0.81 (Table IV).

Urine L-FABP shows promise as an early, accurate biomarker of AKI; however, it appears to rise later than NGAL (pediatric bypass surgery, 4 h L-FABP vs. 2 h NGAL). The predictive ability of L-FABP for AKI requires further clinical confirmation in different patient populations.

**Interleukin-18 (IL-18)**

IL-18 is a proinflammatory cytokine and a powerful mediator of ischemia-induced AKI in animal models. It is induced and cleaved in the proximal tubule and is detected in urine following experimental AKI.\textsuperscript{88, 89} In a cross-sectional study, IL-18 levels were significantly greater in patients with established AKI but not in those with urinary tract infections, pre-renal azotemia or CKD.\textsuperscript{90} The AUC for the diagnosis of established AKI (acute tubular necrosis) was 0.95. Consistent with this finding, the AUC of IL-18 as an early predictor of AKI in patients after kidney transplant, in patients after pediatric cardiac surgery, and in those with Acute Respiratory Distress Syndrome\textsuperscript{59, 91, 92} showed good performance (0.70-0.9), with the
strongest predictive value post transplant. However, in critically ill children, IL-18 performed weakly.93 Haase et al. concluded that IL-18 may be a non-specific marker of inflammation but that it did not predict AKI post cardiac surgery.94 In general, IL-18 has displayed low sensitivity and high specificity. There have been weak positive results for the prognostic ability of urine IL-18: at 4 hours after cardiac surgery, IL-18 weakly correlated with number of days with AKI,92 and in non-septic critically ill children, IL-18 predicted severity of AKI and mortality.93 Furthermore, IL-18 predicts mortality in critically ill adults.91 IL-18 is specific to ischemic AKI but may also be a non-specific marker of inflammation and has shown inconsistent results. Its inclusion in urinary panels requires further evaluation.

Kidney injury molecule-1 (KIM-1)

KIM-1 is a transmembrane glycoprotein that is not expressed in normal kidneys but that is upregulated in proximal tubular cells after ischemic or nephrotoxic injury. The ectodomain segment of KIM-1 is shed and is detected in urine.95, 96 In a cross-sectional study,97 KIM-1 was markedly induced in proximal tubules in biopsies from patients with established AKI (largely ischemic), and it differentiated ischemic AKI from pre-renal azotemia and CKD. In another cross-sectional study98 of hospitalized patients, the AUC for KIM-1 for differentiating those with AKI from controls was 0.9. A further case-control study98 found an AUC of 0.83 for KIM-1 for the diagnosis of AKI at 12 hours post-cardiopulmonary bypass. In a recent prospective study of 90 adults undergoing cardiac surgery, urinary KIM-1, N-acetyl-β-D-glucosaminidase (NAG - a lysosomal glucosidase abundant in tubular cells which is excreted in the urine when proximal tubule cells are damaged), and NAG were measured.37 The AUC for KIM-1 to predict AKI immediately post-surgery (0.68), although low, was higher than those for NAG and NAG. Combining the three biomarkers enhanced the sensitivity of early detection of postoperative AKI, and AUCs became 0.75 and 0.78. Furthermore, in a study that examined the relationship between KIM-1 and a composite end-point (dialysis or death) in hospitalized patients, there was a suggestion that elevated urinary KIM-1 levels are associated with adverse outcomes in hospitalized patients who develop AKI.99 The strength of KIM-1 appears to be detection of existing AKI. Its inclusion in a urinary AKI panel requires further investigation.

Conclusions

Given the heterogeneity of AKI and the settings in which it occurs, it is likely that diagnosis and classification of AKI will not be possible using one biomarker alone, and a panel of biomarkers comparable to the panel of cardiac enzymes used to diagnose and assess severity of acute myocardial infarction will be required. Current key renal biomarkers are NGAL and CyC, which 1) show great promise and utility, 2) have commercially available assays to provide immediate results, and 3) assess complementary aspects of renal injury (NGAL - tubular stress; CyC - GFR). In terms of sequence post-cardiac surgery, NGAL and L-FABP have higher predictive accuracy for AKI in urine and/or plasma early, at 2-4 h, which later wanes; in contrast, the predictive accuracy of CyC, IL-18 and KIM-1 increases at 12-24 h.100 Such information can be used to include appropriate biomarkers in sequential predictive panels, which would open the door to a whole new area of research and, perhaps, interventions.

Novel renal biomarkers can be used to evaluate the effect of new techniques and therapies on kidney function and to provide safety markers for monitoring toxicity and AKI associated with established treatments.18, 69, 101 To this end, NGAL, L-FABP and CyC have superior sensitivity and detect AKI earlier than SCR, enhancing the ability to demonstrate benefits and to justify the implementation of therapies or kidney protective techniques in evaluation studies.

Optimum perioperative hemodynamic management and measures such as preoperative hydration for high risk patients could be more effectively explored. In patients undergoing liver transplantation or major surgery, in which AKI is common,48, 102 early detection of AKI with novel biomarkers has great potential. Novel protective therapies or those that have previously been difficult to evaluate or administer in time to prevent or
ameliorate AKI (e.g., N-acetyl cysteine, bicarbonate or fenoldopam) could be more appropriately assessed or administered in a targeted manner or, in patients receiving nephrotoxins (such as calcineurin inhibitors or aminoglycosides), renal injury could be detected much earlier and drug therapy adjusted. The potential benefit and mechanisms of volatile anesthetics for kidney protection could also be investigated using novel biomarkers. The above information is highly relevant to the anesthesiologist; if early point-of-care biomarker measurements (e.g., NGAL) were performed during or immediately after major surgery, this would allow timely implementation and evaluation of potential protective therapies, either intra-operatively or on ICU admission, targeted to those at high risk of AKI. Indeed, the consideration of pre- to postoperative changes in NGAL concentration could also aid medical decision-making, which might lead to improved outcomes. Furthermore, if NGAL reflected the likelihood of timely discharge, it may be useful in surgical case and ICU bed planning.

References


Conflicts of interest. — Elizabeth Moore: None; Rinaldo Bellomo has acted as paid consultant to Abbott Diagnostics and Biosite, Inverness. Both companies are involved in the development of NGAL assays to be applied in clinical practice; Alistair Nichol: None.

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